Population Genomics
Editor-in-Chief: Om P. Rajora

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Om P. Rajora *Editors*

Population Genomics: Marine Organisms

Springer

Population Genomics

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This pioneering *Population Genomics Series* deals with the concepts and approaches of population genomics and their applications in addressing fundamental and applied topics in a wide variety of organisms. Population genomics is a fast emerging discipline, which has created a paradigm shift in many fields of life and medical sciences, including population biology, ecology, evolution, conservation, agriculture, horticulture, forestry, fisheries, human health and medicine.

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Population genomics has provided novel conceptual approaches, and is tremendously advancing our understanding the roles of evolutionary processes, such as mutation, genetic drift, gene flow, and natural selection, in shaping up genetic variation at individual loci and across the genome and populations, disentangling the locus-specific effects from the genome-wide effects, detecting and localizing the functional genomic elements, improving the assessment of population genetic parameters or processes such as adaptive evolution, effective population size, gene flow, admixture, inbreeding and outbreeding depression, demography, and biogeography, and resolving evolutionary histories and phylogenetic relationships of extant and extinct species. Population genomics research is also providing key insights into the genomic basis of fitness, local adaptation, ecological and climate acclimation and adaptation, speciation, complex ecologically and economically important traits, and disease and insect resistance in plants, animals and/or humans. In fact, population genomics research has enabled the identification of genes and genetic variants associated with many disease conditions in humans, and it is facilitating genetic medicine and pharmacology. Furthermore, application of population genomics concepts and approaches facilitates plant and animal breeding, forensics, delineation of conservation genetic units, understanding evolutionary and genetic impacts of resource management practices and climate and environmental change, and conservation and sustainable management of plant and animal genetic resources.

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Population Genomics: Marine Organisms

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Population Genomics Book Series
This Population Genomics book series is dedicated with love to my wife Malti and children Apoorva, Anu, and Maneesha.

Om P. Rajora

Population Genomics: Marine Organisms
This book is dedicated with love to my granddaughter Ashna Persaud, whose early passion for marine life/biology is both inspiring and captivating.

Om P. Rajora

This book is dedicated with love to my parents Robert and Melinda, my husband Douglas, and my daughter Samantha.

Marjorie F. Oleksiak

Preface

Genomics has revolutionized biology and is providing powerful approaches and impetus to address common questions across diverse biological fields. Although this commonality has existed previously, now, genomics can be used across biological disciplines and fields to define and more accurately address common questions about the genetic basis underlying a biological trait or function and the evolutionary history, adaptive trajectory, conservation, and management of a population or species. Never before the recent advances in genomics has it been possible to address such common inquires with such power and accuracy, and never before has it been so easy to generate and incorporate genomic information to answer diverse and intractable biological questions. Thus, even though marine sciences encompass the vast breadth of biology, genomics has the potential to provide key insights into diverse biological topics relevant to life in the world's oceans. This potential and the insights gained from genomics approaches are expanding the biological questions being addressed in the marine sciences and driving research in new directions.

For populations of marine organisms, many of which have characteristics that make them quite difficult to study, population genomics approaches provide an exciting potential to generate and integrate knowledge at different levels of organization, from the molecular up through to the ecosystem, to gain a greater understanding of how life in our oceans exists and works. Understanding and integrating this knowledge across different levels of organization and within complex marine environments is also one of the greatest challenges of marine population genomics. Multidisciplinary approaches, already common in the marine sciences, will be integral to this data analysis and integration.

Population genomics approaches are making significant, unprecedented advances in both basic and applied research. Many population genomics studies of marine organisms focus on figuring out which species are present in water columns and gaining some understanding of those species at both the individual and population levels. In the world's oceans, where organisms are difficult to observe and sample, this information is still not known for many species and ecosystems. Undoubtedly, these studies build upon each other, and as we gain a greater understanding of life

forms and organisms present in oceans, we next can ask: When are they there and what are they doing? Population genomics approaches, similar to previous molecular genetic studies, are also being used to figure out population demography and basic population and evolutionary genetic parameters of different marine populations. These basic population parameters include such attributes as genetic diversity and structure, effective population size (N_e), inbreeding and outbreeding depression, historic bottlenecks, natural selection, and gene flow. Historically, these population genetic parameters have been studied using molecular genetic markers (e.g., allozymes, microsatellites, or a few hundred SNPs). New population genomics approaches, which enable easy and inexpensive sequencing of genomes, transcriptomes, and proteomes for any species, often provide similar information but with much greater resolution and are particularly useful for the many extant marine species, which lack prior genetic and genomic data.

In addition to basic research questions to better understand marine species and their populations, population genomics approaches also have practical uses for marine studies, particularly when it comes to understanding local adaptation of marine species and populations and managing and protecting marine resources. Basic needs for marine species management that are well addressed with population genomic data include defining populations and population connectivity because managing fishery species effectively and defining management units depend on knowing what constitutes a population. Population genomics approaches also provide powerful tools for monitoring resources, which are important for tracing and regulating fishery resources as well as detecting and monitoring invasive species that can devastate natural resources. Changing environments also pose a critical challenge for protecting marine resources, and population genomics can be used to provide metrics on the potential of marine populations to respond to climate change as well as how marine populations have already responded. How to integrate this genomics knowledge into management and regulatory decisions remains an ongoing challenge.

This volume provides an overview of how population genomics approaches are being used to address different questions important for marine populations. These questions address the baselines of marine diversity, population structure, their evolutionary histories and potential, biogeography, adaptive divergence, seascape genomics, speciation, biological invasions, environmental epigenomics, conservation, protection, and management of marine resources. The different chapters highlight a variety of species and incorporate different concepts and approaches to better understand marine population genomics.

The book is organized into six parts. The first part provides an overview of challenges, opportunities, and future perspectives of population genomics research in marine organisms. A solo chapter in the second part deals with coral microbiomes as bioindicators for coral reef health. The third part includes three chapters discussing the genetic diversity, population structure, and biogeography of marine organisms. The fourth part discusses seascape genomics. The fifth part includes five chapters focused on various aspects of adaptation, acclimation and speciation, and epigenomics. The sixth and the last part includes three chapters addressing various

aspects of monitoring, managing, and protecting marine resources. With such diverse topics, the book is envisioned for a wide readership, including undergraduate and graduate students, research scholars, and professionals and experts in the field.

This is an exciting time to study population genomics in general and is specifically exciting for species in marine environments where so much remains unexplored. We thank all of the authors who have contributed their time and expertise to this volume to illustrate the challenges and opportunities, as well as the groundbreaking work that has already been accomplished. As leaders in their field, they provide an important perspective as population genomics approaches begin to permeate the oceans' depths.

Miami, FL, USA Marjorie F. Oleksiak
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Contents

Part I Introduction

Marine Population Genomics: Challenges and Opportunities 3
Marjorie F. Oleksiak and Om P. Rajora

Part II Marine Microbiomes

Coral Microbiomes as Bioindicators of Reef Health 39
Sofia Roitman, F. Joseph Pollock, and Mónica Medina

Part III Genetic Diversity, Population Structure, and Biogeography

Population Genomics of Marine Zooplankton 61
Ann Bucklin, Kate R. DiVito, Irina Smolina, Marvin Choquet, Jennifer M. Questel, Galice Hoarau, and Rachel J. O'Neill

Population Genomics of Early-Splitting Lineages of Metazoans 103
Rocío Pérez-Portela and Ana Riesgo

Population Genomics and Biogeography of the Northern Acorn Barnacle (*Semibalanus balanoides*) Using Pooled Sequencing Approaches 139
Joaquin C. B. Nunez, Rebecca G. Elyanow, David A. Ferranti, and David M. Rand

Part IV Seascape Genomics

Seascape Genomics: Contextualizing Adaptive and Neutral Genomic Variation in the Ocean Environment 171
Libby Liggins, Eric A. Treml, and Cynthia Riginos

Part V Adaptation, Acclimation, and Speciation

Clinal Adaptation in the Marine Environment 221
David I. Dayan

The Population Genomics of Parallel Adaptation: Lessons from Threespine Stickleback 249
Paul A. Hohenlohe and Isabel Santos Magalhaes

Mechanisms of Adaptive Divergence and Speciation in *Littorina saxatilis*: Integrating Knowledge from Ecology and Genetics with New Data Emerging from Genomic Studies 277
Kerstin Johannesson, Roger K. Butlin, Marina Panova, and Anja M. Westram

Ecological Speciation in Corals 303
Ana M. González, Carlos A. Prada, Viridiana Ávila, and Mónica Medina

Environmental Epigenomics and Its Applications in Marine Organisms 325
Andria Beal, Javier Rodriguez-Casariego, Ciro Rivera-Casas, Victoria Suarez-Ulloa, and Jose M. Eirin-Lopez

Part VI Protection, Conservation, and Management of Marine Organisms

Marine Invasion Genomics: Revealing Ecological and Evolutionary Consequences of Biological Invasions 363
S. D. Bourne, J. Hudson, L. E. Holman, and M. Rius

Population Genomics Applied to Fishery Management and Conservation 399
Laura Benestan

Marine Conservation and Marine Protected Areas 423
Amanda Xuereb, Cassidy C. D'Aloia, Rémi M. Daigle, Marco Andrello, Alicia Dalongeville, Stéphanie Manel, David Mouillot, Frédéric Guichard, Isabelle M. Côté, Janelle M. R. Curtis, Louis Bernatchez, and Marie-Josée Fortin

Index 447

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Part I
Introduction

Marine Population Genomics: Challenges and Opportunities

Marjorie F. Oleksiak and Om P. Rajora

Abstract Population genomics is revolutionizing biology and stimulating new research questions and directions. While human health has driven many of the genomics tools and approaches, all other biological fields have benefitted. This is certainly true in the world's oceans, which encompass a large diversity of species and ecosystems. In the world's oceans, population genomics approaches are giving us an unprecedented ability to gain a better understanding of the organisms inhabiting these ecosystems. While population genomics approaches are improving our understanding of genetic diversity and population genetic parameters in marine organisms, they also are providing unexpected insights into marine invasions, population connectivity, and how marine organisms are responding to different stimuli and environments. Some examples include identification of connectivity among populations that is not predicted by geography as well as identification of genes and genetic variants under natural selection in response to environment and climate conditions as indicators of genes and pathways responsible for adaption. This knowledge is important because so much of the world's oceans is understudied. This knowledge also is critical for understanding how marine organisms will respond to environmental change and thus how we can better protect marine biodiversity and marine resources. That is, we can better predict the effects of enhanced migration on mitigating anthropogenic stressors affecting marine populations and whether outcrossing will enhance population survival or result in outbreeding depression. Simply put, population genomics provides the genetic resolution to make better predictions about how environmental change is affecting populations and thus provides insights into how we might address environmental change's deleterious effects on important marine resources. In this chapter, we provide an overview of the challenges and opportunities for marine population

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genomics, addressing how population genomics can be used to understand marine biodiversity, population demographics and connectivity, and response to environmental changes as well as assist sustainable management, protection, and conservation of marine biodiversity.

Keywords Acclimation · Adaptation · Environmental DNA (eDNA) · Epigenomics · Fisheries · Invasive species · Marine protected area (MPA) · Metagenomics · Natural selection · Population genomics · Seascape genomics · Speciation · Sustainable management · Zooplankton

1 Introduction

Population genomics has provided unprecedented power and accuracy to address novel and long-standing questions in population biology. These questions are relevant to diverse fields including ecology, evolution, conservation biology, agriculture, forestry, fisheries, and human health (Luikart et al. 2019; Rajora 2019). Population genomics has similarly enhanced our understanding of marine ecosystems. One reason is that genomics provides an approach that requires little prior genetic, molecular, or biochemical information about the diverse marine life that occurs in our oceans and marine ecosystems. Population genomics approaches may be more important for marine systems than for many other ecosystems because many marine species are hard to study simply due to sampling problems: the oceans are huge, covering about 70% of the world, and many areas are hard to access. Thus, while shallow coastal zones make up only a small proportion of the world's oceans, they account for the largest proportion of the collected data and publications on marine species while the vast majority (~85%) of the oceans is undersampled (Webb et al. 2010). However, because most genomics approaches can be relatively easily used regardless of species, genomics approaches are now permeating studies of marine environments, even where the majority of species are hard to study.

With respect to population biology studies of marine species, genomics approaches are building on and expanding the population genetics approaches that dominated the literature for approximately 50 years, starting with the use of allozyme markers in the late 1960s (Table 1). Population genetics studies have typically used genetic markers evolving under neutral expectations. In the marine sciences, common uses of these genetic markers have been to identify morphologically cryptic sister species; to identify population structure and reconstruct demographic history; to identify temporal genetic changes, especially with respect to recruitment; and to reconstruct phylogenies and phylogeographic patterns, which are important for management and conservation of marine organisms (Hedgecock 2019). Population genomics approaches have led to a huge increase in the number of readily available neutral genetic markers, which has increased our ability to resolve fine-scale and cryptic genetic structure and, as well, has given us improved accuracy to estimate some demographic parameters (Cammen et al. 2016).

Table 1 Characteristics of common genetic markers for diploid organisms and experimental studies in marine species using these markers

Characteristics	Allozymes	RFLPs	AFLPs	Microsatellites	SNPs
Codominance (genotypic data)	Yes	Yes	No	Yes	Yes
Selective neutrality	Some	Yes/no depending upon the sequence source	Mostly presumed neutral	Presumed neutral	Transcriptome and exome-capture based: uncertain Genomic based: mostly
Number of variable loci	10–50	10–50	100–1,000	5–20	100s–100,000s
Number of alleles/locus	1–5	1–5	2	1–50	2

In addition to selectively neutral markers, population genomics approaches can also reveal genes and genomic regions evolving by natural selection or other non-neutral processes to give insights into adaptive variation within and between populations and species.

The promise of marine population genomics is the ability to identify genotypes that contribute to successful recruitment, survive disasters, or populate marginal environments. While these are some of the promises of marine genomics, currently, population genomics provides powerful approaches to uncover population connectivity, which can be especially difficult to study in marine ecosystems where organisms are hidden from view and difficult to follow through time. This information is critical for managing and conserving marine species.

The large number of polymorphic loci enabled by population genomics approaches provides insight into marine population connectivity and further has identified population divergence on small ecological, temporal, and spatial scales due to sequence divergence most likely associated with natural selection (Crawford and Oleksiak 2016). This may be the most important impact of population genomics inquiries: identification of the rate and spatial scale at which populations are responding to environmental variation such as seen with global climate change. Examples include significant divergence among sailfin molly (*Poecilia latipinna*) populations less than 10 km apart (Nunez et al. 2015), Baltic Sea herring (*Clupea harengus*) populations (Corander et al. 2013), and Pacific lamprey (*Entosphenus tridentatus*) populations (Hess et al. 2013). Some extreme examples of the sensitivity of population genomics approaches are revelation of the rapid and adaptive divergence of the estuarine minnow (*Fundulus*) due to thermal pollution or its divergences among microhabitats only a few hundreds of meters apart (Wagner et al. 2017; Dayan et al. 2019).

In addition to identifying population structure and adaptive divergence, population genomics approaches are now being used to census marine populations (Bravington et al. 2016). That is, the density of polymorphic loci provided by

genomics approaches allows the identification of parent-offspring pairs (close-kin mark-recapture) even among organisms that are difficult to observe. Similar to classical mark-recapture studies, close-kin mark-recapture provides the means to calculate *N*, population size. Simply put, the ratio of offspring to parents relative to total sample size is a function of *N*. This approach has been used on southern bluefin tuna (*Thunnus maccoyii*), an internationally valuable fisheries species that is widely distributed, highly mobile, and difficult to observe. The close-kin mark-recapture approach gave more precise abundance estimates than those based on fishery catch and effort data or traditional tag release programs and has the potential to revolutionize the conservation and management of previously intractable marine species (Bravington et al. 2016).

Overall, while most marine species are hidden from view under the world's oceans, population genomics approaches are providing an increasingly diverse set of accessible genomics tools with which to study a wide range of research questions. In the oceans, these questions are diverse and match the diversity of the marine environments and the organisms that inhabit them.

In this chapter, we first discuss different population genomics approaches that have been used with marine species. We then illustrate how these approaches are being used to better understand marine biodiversity as well as population genetic diversity, population structure, and biogeography. Next, we discuss different ways organisms can respond to environmental change and introduce some of the species being used to understand population and species responses. Finally, we touch on three important conservation issues for marine resources: invasive species, fisheries management, and marine protected areas. We conclude with future opportunities and challenges.

2 Population Genomics Approaches

In the most basic sense, genomics approaches (Table 2) interrogate genomes to gain insights about species and populations and the diversity of organisms. The genotyping and sequencing approaches applied to population genomics are described in Holliday et al. (2019). The most comprehensive genomics approach is to sequence the whole genome. Whole-genome sequencing is becoming increasingly affordable as sequencing technologies improve, both with respect to error rates and throughput. However, as well as requiring DNA samples, preferably high-quality and high-quantity DNA samples, whole-genome sequencing requires bioinformatics expertise, computational power, and computational memory. While these additional resources exist for important fisheries species (Williams et al. 2008; Nielsen et al. 2009; Davidson et al. 2010; Roberts et al. 2012; Zhang et al. 2012), they are not as readily available for less economically valued species (though they are continually developing: see Nunez et al. for an outline for whole-genome sequencing in a non-model barnacle species (Nunez et al. 2018) using Pool-seq, whole-genome sequencing of pools of individuals that yields genome-wide

Table 2 Common genomics approaches for marine organisms

Term	Description
Whole-genome sequencing	Sequences that capture nearly all of the genome and are assembled into large (10s–100 kb) scaffolds. Because whole genomes can be 0.1–10 Gbps, whole-genome approaches require much more sequencing than GBS, RNA-Seq, or exon-capture. Thus, few individuals are typically analyzed.
Reduced representation sequencing (RAD, RAD-seq, GBS)	Genome sequencing that only sequences a selected "reduced" portion of the genome. Typically this approach sequences 0.1–1% of the genome derived from specific restriction sites. The power of this approach is that the same loci in the genome of approximately 50–100 bp are sequenced in many (100s) of individuals.
Transcriptomics and RNA-Seq	Transcriptomics is the quantitative and qualitative analysis of RNA expression. RNA-Seq is a transcriptomics approach that uses sequences of expressed RNA and provides both quantitative measures of expression and identifies nucleotide variation in expressed DNA (i.e., in RNA).
Capture sequencing, exon-capture, exomic sequencing, targeted sequencing	Capture sequencing, as the name implies, is sequencing selected (captured) genomic DNA targets. The most general approach is exon-capture or exomic sequencing that uses PCR, hybridization probes, or cDNAs to capture exons or DNA that is expressed as RNA. Yet, the selected targets can be any parts of the genome where there are primers or probes.
Chip-seq and DNAse-seq	Chip-seq and DNAse-seq approaches are used to sequence DNA regulatory regions. Chip-seq uses antibodies to capture protein-bound DNA for sequencing. Similarly, DNAse-seq can be used to sequence protected (protein-bound) DNA fragments.

After Crawford and Oleksiak (2016)

polymorphism data at much lower costs than sequencing individuals (Schlotterer et al. 2014)). Thus, depending on the questions being addressed, whole-genome sequencing may be the best approach, but other, simpler genomics approaches also might be able to provide similar information with lower computational load.

Alternatives to whole-genome sequencing entail either sequencing only a portion of the genome or sequencing genomic products (e.g., gene transcripts or proteins) (Crawford and Oleksiak 2016). For population genomics studies, especially those of non-model species, sequencing only a portion of the genome, also known as sequencing a reduced representation of the genome, has been widely used. Two well-known approaches for reduced representation sequencing are genotyping by sequencing (GBS, Elshire et al. 2011) and Rad-Seq (Baird et al. 2008) and varieties thereof. These two approaches use some combination of restriction enzymes, random shearing, and size selection to subset a portion of an organism's genome

(see Andrews et al. (2016) for a review of various methods) prior to sequencing. The big advantage of these reduced representation sequencing approaches results from the fact that only a portion of the genome is sequenced in many (10s–100s) of individuals. These approaches take advantage of being able to identify each individual's genome sequence with the use of sequence tags (barcodes). Thus, the combination of only sequencing a small part of the genome with sequences from many barcoded individuals provides genomic information about populations with minimal expense. The resulting population-level data on many thousands of polymorphic loci can then be used to determine connectivity among populations, information on population history, and the role of evolutionary adaptation in shaping a species' genome.

One disadvantage of GBS and Rad-Seq approaches is that the genomic DNA fragments that are sequenced can be from anywhere in the genome, i.e., they are random genomic DNA fragments. While these random fragments often capture regions that could regulate gene expression, a vast majority is likely to have no function. If one is interested in a higher density of functional portions of a genome, other approaches for reduced representation sequencing can be used (Mamanova et al. 2010). Although many of these approaches targeting functional genomic regions require prior genomic knowledge, RNA-Seq, which targets transcribed genes (the transcriptome), does not require prior knowledge (Wang et al. 2009). Thus, RNA-Seq has been widely used in marine species from bacteria (Croucher and Thomson 2010) to fish (Porteus et al. 2018), to whales (Cammen et al. 2016). On an individual level, RNA-Seq can be used to quantify gene expression and gives insight into the organism's physiological state or response to a stimulus. Many examples of this can be found in the marine literature (Libro et al. 2013; Bilyk and Cheng 2014; Zhang et al. 2017). On a community level, such as in a microbial or viral community, RNA-Seq can be used to quantify in situ microbial responses to environmental fluctuations (Frias-Lopez et al. 2008).

Additional targeted sequencing approaches include Capture-seq, Chip-seq, and DNAse-seq. Capture-seq approaches rely on pre-designed DNA or RNA probes or "baits" to pull out specific portions of the genome using either solid phase or solution-based hybridization (Gnirke et al. 2009). Capture-seq approaches have been used in a variety of model species to pull out coding portions of the genome, i.e., exome sequencing. Because prior genomic information is necessary to design the baits, exome sequencing has been less often used in non-model species, including most marine species. However, cross-species strategies to capture full-length coding sequences up to 200 million years divergent are being developed that enable functional comparative studies (Schott et al. 2017). In addition, expressed messages have been successfully used to create cDNA capture probes to capture exonic regions from genomic DNA (Puritz and Lotterhos 2018). While this approach was developed in eastern oyster, *Crassostrea virginica*, it is suitable for use with any organism.

To sequence DNA regulatory regions, Chip-seq and DNAse-seq approaches can be used. Chip-seq uses antibodies against specific DNA binding proteins to pull out protein-bound DNA fragments. This chromatin immunoprecipitation is

combined with high-throughput sequencing to identify the genomic regulatory elements. Similarly, DNAse-seq uses enzymes to reveal DNA protected by bound proteins. In marine species, approaches to sequence DNA regulatory regions have been more typically used for evolutionary developmental studies in specific species such as sea urchins, *Echinoidea* spp. (Cary et al. 2017). Yet, they highlight a trend in genomics research, which is to correlate DNA variation with downstream, functional effects. These functional effects might be at the level of mRNA or protein expression, tissue or organ traits, or whole organism physiology. Even broader questions that remain to be answered are how functional effects of DNA variation affect populations and, further, how they affect ecosystem interactions.

3 Understanding Marine Biodiversity

3.1 Marine Metagenomics

Some basic questions that are still being asked for marine organisms include: who is there, when are they there, and what are they doing? The ability to use population genomics approaches to answer these sorts of questions became apparent when Venter et al. used whole-genome shotgun sequencing to sequence microbial populations bulk collected from the Sargasso Sea (Venter et al. 2004). This metagenomics approach provided a relatively unbiased way to identify the biodiversity of organisms that had not been or could not be cultured. In addition, by identifying a variety of photosystem genes, this approach also provided insights into microbial photosynthesis in the Sargasso. Thus, we began to gain insights into who and what for species we previously knew little if anything about.

Since the Venter et al. (2004) publication, numerous metagenomics studies have examined both marine microbe and marine virus populations. These studies provide insights not only into the diversity of marine archaea, bacteria, and viruses but also into the ecological processes and biogeochemical cycles involving these organisms. Population genomics approaches provide ecological insights because they identify environmentally-relevant genes (e.g., genes involved in nitrate reduction, sulfur metabolism, or photosynthesis). The world's oceans, with their huge volumes ($\sim 2 \times 10^{18}$ m^3) and where microbe densities can reach up to 10^{12} cells/mL, are thought to host the largest microbe population on earth (Ferrer et al. 2019). Yet with genomics approaches, we can peer into oceans and examine the vastly different environments, from nutrient-rich coastal waters to deep, cold, and dark abyssal depths. We can examine organismal diversity from these different environments without the difficult (and sometime impossible) steps of culturing the organisms inhabiting these environments; this makes population genomics approaches a powerful tool to understand the diversity and function of the microorganisms that inhabit diverse ocean waters. Indeed, metagenomics approaches, coupled with physical, chemical, and biological measurements of the world's oceans through space and time, provide copious data with which to gain a deeper understanding of marine microbial ecosystems (Biller et al. 2019).

For example, as marine metagenomic databases continue to expand, marine metagenomics approaches are giving insights into the ocean's biogeochemical cycles (Mineta and Gojobori 2016). Of pressing concern is the global carbon cycle. In the global carbon cycle, the ocean "carbon pump" is "primed" by photosynthesizing phytoplankton that fixes carbon into carbohydrates. This primary production keeps the surface water carbon dioxide concentrations low so that carbon dioxide transfers into the oceans from the atmosphere. Although much of this fixed carbon is consumed and recycled throughout the ocean's ecosystems, some of the carbon sinks to the seabed making the deep ocean a carbon sink. Metagenomic analyses have shown that integrated plankton networks, which include eukaryotes, prokaryotes, and viruses, drive carbon sequestration in oligotrophic waters (Guidi et al. 2016). Also relevant to carbon sinking into the deep oceans are sinking particles made of both organic and inorganic matter. Surprisingly, genomic analyses showed that the bacterial assemblages on these particles were simple and homogeneous and dominated by deep-sea bacteria across a 9-month sampling period. In contrast, the eukaryotic assemblages associated with these particles were complex and more variable, reflecting their more variable origins from surface waters, mid-depth waters, and the deep sea (Boeuf et al. 2019). Both bacterial and eukaryotic assemblages are critical for the biogeochemical transformation of sinking organic particles in the deep oceans (Boeuf et al. 2019), and metagenomics allow us to better understand how they impact the global carbon cycle.

In addition to identifying microorganisms' functions, genome comparisons among marine microorganisms have given insights into microorganisms' genomes. For instance, marine cyanobacteria of the genus *Synechococcus* enriched from coastal Californian seawater had genomic regions that greatly diverged from the genomes of model *Synechococcus* strains. These divergent genomic regions likely result from extensive horizontal gene transfer where different strains transferred genetic material among individuals instead of the heritable transfer between parent and child (Palenik et al. 2009). Furthermore, the authors also identified three plasmid families, which had not been found previously, and suggest that the plasmids might have a role in horizontal gene transfer in the coastal *Synechococcus* species (Palenik et al. 2009). Overall, metagenomics approaches yield insights not only into who is there and what they are doing but also into the evolution of genomic architecture in different taxa that influences their ecological role and ecosystem function.

Finally, because microbes have fast generation times, microbiomes can respond quickly to changing conditions. This trait, coupled with the ability to do metagenomics quickly, results in microbiomes increasingly being used to monitor organismal health. In mammals, the gut microbiota is being recognized for its health effects on its host (Wang et al. 2015). Similarly, the gut microbiomes of different fishes are being explored for health effects on fish, especially with the expansion of the aquaculture industry (Egerton et al. 2018). As ocean waters' temperatures, acidities, and salinities change with our changing climate, different marine microbiomes are likely to be affected and can be monitored to identify climate change effects. For instance, kelp (*Ecklonia radiata*), an important

foundation species, was treated under future climate scenarios of increased temperature and acidity in experimental mesocosms. During this treatment, the kelp's associated microbiome changed prior to detrimental physiological effects that occurred in the kelp (Qiu et al. 2019) raising the potential that the microbiome is directly affecting the kelp's health. Roitman et al. (2018) explore the idea of monitoring microbiomes with respect to another group of foundation species, stony corals. They discuss the use of microbiomes as a metric to monitor coral reef health. In fact, the Global Coral Microbiome Project (http://coralmicrobes.org/) seeks to catalogue microbial diversity and metabolic capabilities across all major reef-building coral groups to better understand how coral microbiomes affect coral health and disease.

While whole-genome microbial sequencing can be used to gain insight into microbial function, amplicon-based approaches that target specific genes (e.g., 16S rRNA genes for bacteria and 18S rRNA genes for eukaryotic microbes) are widely used to identify species and community structure from diverse marine environments. Many of these amplicon-based approaches use conserved primers to target different taxa. This allows researchers to quickly and inexpensively generate many rRNA gene fragments that can be bulk sequenced on high-throughput sequencing platforms to identify various taxa. This ease of amplifying and sequencing rRNA genes has made amplicon-based approaches the most extensively used approach in marine metagenomics studies (Zhou et al. 2015).

Zooplankton constitutes another group of marine organisms that have benefitted and will continue to benefit from metagenomics approaches. Similar to microbes, zooplankton often is bulk sampled from ocean waters, making metagenomics an efficient way to identify the taxa present in the samples. Zooplankton, which inhabits all of the world's oceans, is also undersampled and has multiple examples of sibling species swarms or flocks that make them difficult to study. Sibling species are two cryptic or phenotypically similar species that are each other's closest relative, and species swarms or flocks are groups of closely related species living in the same habitat. Bucklin et al. (2017) provide a comprehensive review of the genomic and transcriptomic resources for marine zooplankton (this book). They discuss the applications of population genomics research to study population genetic diversity and structure as well as biogeography and connectivity. There is a great diversity of zooplankton, and many zooplanktons have large population sizes; together with short generation times, these characteristics allow zooplankton to rapidly respond to environmental change. Thus, zooplankton species provide powerful systems with which to understand genomic responses to environmental change. Bucklin et al. (2017) summarize knowledge on important zooplankton groups and conclude with the challenges and future opportunities of using genomics to study zooplankton. Overall, population genomics approaches provide important tools to gain new insights into zooplankton diversity, ecology, and ecosystem function.

3.2 Environmental DNA

The ease of amplicon-based sequencing approaches to identify species and taxa coupled with next-generation sequencing throughput has also led to a large increase in environmental DNA or eDNA studies. Environmental DNA is DNA left in the environment by different organisms that persists for different periods of time dependent on the environment. For multicellular organisms, DNA sources include shed cellular material, such as shed skin and blood, and metabolic waste. The use of eDNA first started with DNA purified from soil; 16S rRNA genes were shotgun cloned using DNA purified from soil and then individually sequenced as an approach to identify the diversity of soil microbes, many of which could not be cultured (Pace et al. 1986). Environmental DNA samples have since expanded to include DNA extracted from glaciers, permafrost, aquatic sediments, lakes, ponds, and streams, terrestrial habitats, and the oceans (Thomsen and Willerslev 2015).

The first study to use eDNA to detect an aquatic vertebrate tracked American bullfrogs (*Lithobates catesbeianus*) using water samples from controlled environments and natural wetlands (Ficetola et al. 2008). However, it was not until 2012 that eDNA collected from the marine environment was used to detect marine macroorganisms, marine fish (Thomsen et al. 2012). Using water samples collected from a temperate marine ecosystem, the authors were able to identify 15 fish species in 9 orders and 11 families (Thomsen et al. 2012). Compared to nine other common methods to monitor fish species, which involved capturing and visualizing fish, eDNA proved to be an easier and more cost-effective method to survey marine fishes. This method has the added benefit in that it can be performed in virtually any marine habitat where water samples can be collected. Importantly, the authors also determined that the eDNA fragments in marine waters degrade beyond detection within days. This is in contrast to other eDNA samples, such as those found in terrestrial sediments, which have contained DNA from extinct organisms (Thomsen and Willerslev 2015), and means that marine eDNA collections represent a real-time snapshot of the species present in the environment. This knowledge is important for management and policy decisions based on eDNA analyses (Kelly et al. 2014).

Biomonitoring is one of the major uses of eDNA, especially in marine ecosystems that are difficult to observe. Biomonitoring might be done simply to figure out which organisms are present in an ecosystem, including cryptic species, but it also might target indicator species of concern, perhaps endangered or invasive species (see Bourne et al. (2018), this book, for a discussion on the use of environmental DNA to study marine invasion genomics). The biomonitoring approach with eDNA is similar in principle to DNA barcoding approaches; the premise of both approaches is to amplify and sequence a specific DNA target in order to identify species. In DNA barcoding, some portion of the target sequence is conserved enough across species to be able to design primers that will amplify the target across taxa, while another portion of the target sequence is variable enough to distinguish taxa (Bucklin et al. 2011). This is also true for eDNA studies. However, given that eDNA is often degraded, the amplified targets are typically smaller than those used for barcoding.

Additionally, for both barcoding and metagenomics approaches, which organisms are detected is dependent on the primers used since primers can amplify different species with higher or lower efficiency. Often, multiple primer sets need to be used to identify known target species. For example, Miya et al. (2015) used universal primers based on aligned, whole mitochondrial sequences to identify 93% of the species (168 species) contained in four tanks in the Okinawa Churaumi Aquarium (Miya et al. 2015). However, their initial experiments were not as successful. Initially, they used one pair of universal primers to amplify eDNA collected from the aquarium tanks and sequenced the products on a high-throughput sequencing platform. Surprisingly, although the primers were designed using a number of sequences from elasmobranchs (sharks and rays), these experiments showed that elasmobranchs were represented by only a few assembled reads despite there being more than 100 large-sized elasmobranchs swimming in the tanks. The authors attributed the paucity of elasmobranch sequences to PCR bias derived from primer-template mismatches and ended up designing another set of universal primers specific for elasmobranchs. These results illustrate the importance of primer design and amplification conditions in using eDNA to assess species richness. The authors identified two more challenges for their work with eDNA. One was the presence of false positives in their analyses from unknown sources. The second was that the ability to identify the sequenced species depends on a comprehensive reference sequence database. If species are missing from such a database, they will not be identified in eDNA analyses.

On an even broader scale than monitoring particular groups of organisms (e.g., fishes), an exciting use for eDNA is whole ecosystem monitoring. This was the approach that Stat et al. (2017) took to monitor a tropical marine ecosystem. They compared two methods for ecosystem monitoring, a shotgun sequencing approach (directly sequencing DNA extracted from an environmental sample similar to approaches used with marine microbes) and a metabarcoding approach using a suite of ten metabarcoding assays that target different organisms. Using the shotgun sequencing approach, only 14.1% of the sequencing reads could be assigned to taxa. In contrast, using the metabarcoding approach, 79.7% of the sequence reads could be assigned to taxa. Further, while only 0.34% of the shotgun sequencing reads were assigned to eukaryotes, using metabarcoding, the authors were able to detect 287 families across the major eukaryotic divisions despite having 20-fold fewer sequencing reads (Stat et al. 2017). This study highlights the potential of using eDNA to monitor entire ecosystems in an easy, sensitive, and cost-effective manner. This ability should positively impact natural resource management and ecological studies assessed on different spatial and temporal scales.

Of primary interest to many involved in monitoring marine ecosystems is whether eDNA can be used to quantify species abundances as well as their spatial and temporal distributions. This information is critical for managing species. However, to be able to estimate abundance from eDNA, one needs to know how quickly DNA is shed from the organism and how quickly the DNA lasts in the environment. The rate an organism sheds DNA into the environment is species dependent. For example, an organism that sheds copious amount of mucus, like

hagfish (Myxinidae), may leave a greater quantitative abundance of eDNA than species that shed little. Additionally, the shedding rate is dependent on other biological factors such as size and age (including life stage). Likewise, in the oceans, how long eDNA lasts in the environment is dependent on abiotic factors such as water characteristics (e.g., temperature and salinity, pH, water currents, and mixing) as well as biotic factors, such as surrounding microorganisms, and how long the source organism stays in the area. To better understand how both biotic and abiotic factors affect eDNA and abundance estimates from eDNA, Sassoubre et al. used northern anchovy (*Engraulis mordax*), sardine (*Sardinops sagax*), and chub mackerel (*Scomber japonicus*)-specific qPCR analyses to measure eDNA shedding and decay rates in seawater ecosystems for these three commercially important fish species (Sassoubre et al. 2016). Using this information, they derived a mass-balance model to estimate fish abundance from eDNA concentrations with the hope that such models will be able to provide useful information on biomass and abundance in natural ecosystems (Sassoubre et al. 2016).

Finally, for species that are hard to find or difficult to sample, eDNA has the potential to be used for population genetics and genomics studies. For instance, Parsons et al. (2018) used eDNA to characterize genetic differentiation in the harbour porpoise (*Phocoena phocoena*), one of the smallest cetaceans in the Northern Hemisphere, and found significant genetic differentiation within a currently recognized management stock (Parsons et al. 2018). Similarly, Sigsgaard et al. (2016) used eDNA to characterize mitochondrial haplotype frequencies in a large whale shark aggregation (Sigsgaard et al. 2016). Adams et al. (2019) explored the challenges and potential of using eDNA for population genetics, which would greatly enhance population genetics for otherwise inaccessible marine species (Adams et al. 2019).

4 Genetic Diversity and Marine Population Structure

4.1 Marine Genetic Diversity

Population genomics approaches, by virtue of the large number of polymorphic loci they could reveal, are likely to identify important patterns of genetic diversity and are improving our understanding of genetic diversity and population genetic parameters in marine organisms (Oleksiak 2019). Besides quantifying genetic diversity, numerous molecular markers that are readily available through genomics approaches can be used to quantify such parameters as effective population size, inbreeding, outbreeding, and bottlenecks, in addition to other evolutionarily and ecologically relevant parameters. In population genomics, effective population size (N_e), the number of individuals needed in an idealized population to replicate the genetic change due to genetic drift or inbreeding that is found in the real population (Hartl and Clark 1997), is a critical parameter for conserving species and determining how readily populations will be able to adapt to environmental change. Low N_e/N (where N is the census size) ratios in marine species have been

suggested to be due to "sweepstakes" reproductive success especially of species with type III survivorship curves (high fecundity, high juvenile mortality), where only a few families win the "sweepstakes" and produce offspring that survive to reproduce (Hedgecock and Pudovkin 2011). Early mortality in such high fecundity species has often been thought to be due to environmental factors. However, a recent genome-wide survey of full-sib offspring of wild-caught Pacific oysters (*Crassostrea gigas*) revealed widespread, genotype-dependent mortality where detrimental alleles resulted in 97.9–99.8% offspring mortality (Plough et al. 2016). While these were hatchery-based experiments, the results have broad implications for understanding low and variable recruitment in highly fecund marine species.

4.2 Marine Population Structure

Most marine species spend all (holoplankton) or a portion (meroplankton) of their lives in the plankton. Because ocean currents can move organisms vast distances, this life history trait makes species population connectivity and biogeography of critical importance in understanding and managing marine populations, species, and ecosystems. Population genomics approaches have enhanced studies of connectivity and biogeography throughout marine organisms because they enable researchers to quickly and easily develop molecular markers for any species, regardless of whether or not prior genomic resources exist. Further, population genomics approaches can result in hundreds to thousands of molecular markers that often reveal fine-scale and cryptic genetic structure previously not seen with fewer markers (Crawford and Oleksiak 2016; Drury et al. 2016). For example, microsatellites and allozymes with limited numbers of loci missed the significant divergence among corals species along the Florida Reef Tract (FRT) that was identified using population genomics approaches involving sequencing a reduced representation of the genome (less than 1% of genome) (Drury et al. 2016). This reduced representative sequencing approach not only found significant divergence along the FRT but surprisingly found that corals in the northern FRT were more similar to those in the southern FRT than to geographically closer central FRT corals (Drury et al. 2016). Population genomics approaches have been used with many other marine species to identify population structure. For example, the vast majority of SNPs among red abalone (*Haliotis rufescens*) had little genetic differentiation among populations (De Wit and Palumbi 2013). Yet, ~3% (691/~22,000) of loci had significantly higher F_{ST} values (a measure of genetic differentiation) that readily distinguish populations along the California Coast (De Wit and Palumbi 2013). Similarly, for Atlantic herring (*Clupea harengus*) in the Baltic, 2.0% of SNPs (117/5,985) are indicative of significant population structure having F_{ST} values = 0.128 (Corander et al. 2013). Similar data is found in temporal isolation of pink salmon (*Oncorhynchus gorbuscha*) and Atlantic salmon (*Salmo salar*) where a few out of 1,000s of SNPs are associated with migration and reproduction timing (Johnston et al. 2014; Seeb et al. 2014). While analyses of neutral variation often do not identify population structure, analyses

of adaptive variation (loci with outlier F_{ST} values that are indicative of natural selection), which constitute only a small percentage of the analyzed loci (1–5%), can identify a much finer genetic structure (Fig. 1). Moreover, the number of candidate loci under adaptive selection suggests that adaptive evolution acting on many loci is common.

In this book, Pérez-Portela and Riesgo (2018) focus on the population genetics and genomics of early-splitting metazoan lineages: Porifera, Ctenophora, and Cnidaria, and within Cnidaria, particularly the Medusozoa. In general, they conclude that species with short dispersal potential tend to have strong genetic structure. However, among species with large dispersal potential, generalities about population connectivity cannot be made. Species with large dispersal potential have a lot of variety in genetic patterns. Indeed, for species with large dispersal potential due to a pelagic larval stage, average pelagic larval duration is not well correlated with genetic differentiation (Weersing and Toonen 2009). While initially this finding was paradoxical to the idea of highly connected marine populations, the number of exceptions to large, homogeneous marine populations has led to a paradigm shift with implications for marine species demography, management, and conservation (Hauser and Carvalho 2008). Thus, new questions arise as to what drives marine demographic patterns. To better understand the role of biotic and abiotic factors driving demographic patterns in marine metazoans, Pérez-Portela and Riesgo (2018) advocate for a combination of genome-wide scans, techniques to measure larval dispersal, and oceanographic modeling. The integration of this information will enhance predictions about genetic trends with implications for conservation and management. Indeed, integrating spatial and environmental data to understand genomic variation drives the field of seascape genomics.

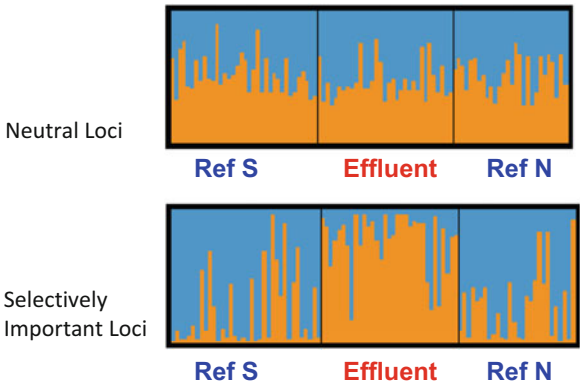

Fig. 1 Analyses of adaptive variation (loci with F_{ST} values that are indicative of natural selection) to identify population structure. Neutral loci cannot distinguish populations collected from a power plant effluent site from reference populations north and south (Ref N and Ref S) of the effluent population. This suggests few demographic differences among these populations. In contrast, selectively important loci distinguish the effluent individuals. The fact that effluent genotypes can be found within the flanking reference populations suggests that the effluent genotype is selected from standing genetic variation

5 Seascape Genomics

The underlying idea behind seascape genomics is to provide a better understanding of both the biotic and abiotic factors that affect population structure. Seascape genomics combines population genomic patterns with seascape ecology to better understand the processes affecting marine populations. While seascape genomics can be considered a subset of landscape genomics, features particularly important for marine species include ocean currents and strong environmental gradients (Riginos et al. 2016). Other features particular to the oceans are the large spatial scales of seascape features and the high dispersal ability of many marine organisms (Riginos and Liggins 2013). This high dispersal means that many marine organisms will experience spatially as well as temporally dynamic habitats. Liggins et al. (2019, this book) discuss the challenges (e.g., the need to sample through both space and time at appropriate scales) and potential of seascape genomics studies. They outline the important considerations for designing effective seascape genomics studies and discuss recently-used methods in seascape genomics appropriate for these challenges. Seascape genomics enhance the ability to detect genetic-environment associations, thereby strengthening population genomics studies and the resulting inferences, including inferences of adaptive genomic variation. A better understanding of adaptive genomic variation in marine populations will inform us about whether these populations are equipped to cope with environmental change, information that is critical with increasing global climate change.

6 Responding to Change: Acclimation, Adaptation, Epigenomics, and Speciation

Multiple mechanisms exist by which organisms and populations can respond to environmental change. One response is to shift range or timing in development, reproduction, or migration. Indeed, time series data are revealing significant phenological variability of marine plankton (Edwards and Richardson 2004), which can have sometimes devastating effects on animals higher in the food chain. For instance, in the Mediterranean, the onset of water stratification results in increases in phytoplankton, zooplankton, and ichthyoplankton abundance. For a small, long-lived seabird, the Mediterranean storm petrel (*Hydrobates pelagicus melitensis*), which depends on these planktonic food sources, delayed reproduction with respect to the onset of water stratification resulted in both hatching and breeding failure (Ramirez et al. 2016). Another response to environmental change is to acclimate; acclimation is a reversible physiological response that alters mRNA expression or protein activity that modulates metabolism, growth, or homeostasis. Alternatively, there are heritable expression pattern changes that have evolved by natural selection and thus are adaptive. Population genomics approaches can be used to identify adaptive genetic changes and thus identify genes that are biologically important

because they affect fitness. In contrast to adaptations which involve genetic changes in DNA that are passed down through many generations, epigenetic changes are changes in the genome that do not alter the DNA sequence but may be passed down between one or a few generations (Deans and Maggert 2015; Moler et al. 2019). Epigenetic changes provide organisms with alternative ways to deal with changing environments (Moler et al. 2019).

6.1 Acclimation

Transcriptomics, the comprehensive study of an organism's RNA transcripts, is widely used to understand how organisms respond to changes, both biotic changes, such as life stage or sex, and abiotic changes, such as changing temperatures or salinities. The general idea behind transcriptomics is that knowledge of which tissue or cell expresses a gene, when the gene is expressed, and under what conditions a gene or set of genes is induced or repressed will give insight into biological and physiological processes (Oleksiak 2010). Transcriptomic responses can occur on different time scales as well and can be acute (minutes to hours), acclimatory (occurring over days or weeks), or adaptive (occurring over generations). Interestingly, the genes that show an acclimation response are often different from those with an adaptive response (Dayan et al. 2015). The two major ways to study transcriptomics are microarrays and RNA-Seq, and both have been used to quantify gene expression in a variety of marine organisms (Hofmann and Place 2007; Caron et al. 2016; Akbar et al. 2018).

The original method for quantifying many 1,000s of mRNAs used microarrays (Brown and Botstein 1999). Microarrays are made up of thousands of DNA probes – 150–250 μm spots of DNA – bound to microscope slides in a specific pattern (Ramsay 1998; Schena et al. 1998). For transcriptomic studies, these DNA spots represent expressed transcripts, and each DNA spot quantitatively hybridizes to a specific mRNA so that expression of thousands of genes can be measured simultaneously. Transcriptomics of natural populations began with microarrays, and these first studies revealed population differences likely due to natural selection (i.e., an adaptive response) despite high levels of individual variation (Oleksiak et al. 2002). Subsequently, microarrays have been used to understand a variety of responses such as responses to salinity (Kalujnaia et al. 2007), hypoxia (Sussarellu et al. 2010; Everett et al. 2012), and pollution (Fisher and Oleksiak 2007; Bozinovic and Oleksiak 2010; Oleksiak et al. 2011; Bozinovic et al. 2013). Microarrays require prior genomic knowledge-gene sequences to print on the microarray, which is a barrier for non-model species. In contrast, RNA-Seq approaches do not require such prior knowledge.

RNA-Seq uses high-throughput sequencing to sequence all expressed transcripts. Because RNA-Seq does not require prior knowledge of the genome, RNA-Seq can be easily and quickly used to quantify gene expression in organisms without genomic resources. In combination with barcoding individual samples, RNA-Seq

approaches also allow for high throughput, making population-level studies feasible. In addition to quantifying gene expression, RNA-Seq can be used to identify alternatively spliced transcripts and SNPs. The ease, throughput, and flexibility of RNA-Seq are making RNA-Seq the favored choice for transcriptomic studies, especially for non-model species. For example, RNA-Seq has been used to better understand the molecular mechanisms underlying the biology and ecology of octocorals (*Heliopora coerulea*) (Guzman et al. 2018), seagrass' (*Zostera marina*) adaptive response to global warming (Franssen et al. 2011), and rock oyster's (*Saccostrea glomerata*) adaptive response to ocean acidification (Goncalves et al. 2017).

While transcriptomic measures can explain biologically important functions such as cardiac metabolism (Oleksiak et al. 2005), transcriptomics are not a direct measure of proteins. Transcriptomics also ignore other processes that affect protein activity such as allosteric activators, phosphorylation, or other posttranslational modifications. In contrast, proteomics is the study of protein abundance and post-translational modifications, often in response to some sort of change. Proteomic approaches have been used to study such topics as the effects of environmental stress, pollutants, infection, symbiosis, and development on marine organisms (Tomanek 2011). By bridging the gap between genotype and phenotype, proteomics integrate gene expression changes with other processes that affect protein activity.

However, unlike many of the other genomics approaches, proteomics require genomic resources to identify the proteins, and the lack of available annotated genomes and proteomes for most marine organisms is a major limitation for marine proteomic studies (Slattery et al. 2012). Nevertheless, proteomics is being used with a variety of marine species and is giving insights into their physiological responses. For instance, proteomics approaches were used to characterize the Pacific oyster's (*Crassostrea gigas*) response to natural environmental differences in pH, dissolved oxygen content, salinity, and temperature at five different estuarine sites in Washington State, USA. Among sibling Pacific oysters placed at these five sites, oysters from one of the sites had higher abundances of seven proteins – antioxidant enzymes and molecular chaperones (Venkataraman et al. 2019). These differences potentially reflect environmental factors at this site, such as higher average temperature. Another proteomics study used *Crassostrea gigas* to predict how larvae might be affected by future climate change. This study examined metamorphosing larvae under decreased pH (pH 7.4), increased temperature (30°C), and reduced salinity (15 parts per thousand) conditions and identified significant protein expression changes in response to both single stressors and interacting stressors (Dineshram et al. 2016). Proteomics approaches have also been used to study tissue regeneration in the stony coral *Montastraea cavernosa* (Horricks et al. 2019) and response to thermal stress in the marine kelp forest gastropod, Kellet's whelk (*Kelletia kelletii*), across a naturally existing thermal gradient across its range (Vasquez et al. 2019). Overall, proteomics studies provide information on protein targets for future studies related to biotic and abiotic changes in marine organisms and should contribute to understanding the physiological responses of marine organisms to future ocean conditions.

6.2 Adaptation

Population genomics approaches provide hundreds to thousands of neutral markers to explore processes affecting population structure. In addition, with the ability to interrogate loci across the genome, population genomics approaches can also reveal genomic regions evolving via non-neutral processes. Typically, the divergence in DNA sequence variation or single nucleotide polymorphisms (SNPs) are compared across the genome, and SNPs with larger changes in allele frequencies than expected among populations (or F_{ST} values) are identified. These outlier SNPs have such large and unexpected differences in allele frequencies that they are thought to contribute to adaptive differences. These analyses give an insight into adaptive variation within and between populations and species. Adaptive variation and the adaptive potential of species are becoming increasingly important for species' survival given current rates of global change. Global change, driven in large part by human activities, is dramatically affecting the world's oceans and subsequently the organisms that live in them. Ocean waters are warming and becoming more acidic. Changing weather patterns are changing ocean currents and stratification. Hypoxic zones are expanding. Pollutants – plastics, nutrients, and chemicals – are not bounded once they reach the oceans and are increasing. All of these factors are changing ocean ecosystems on a global scale. Not surprisingly, whether and how organisms cope with these changes (adapt) as well as their potential to cope with changes are the subject of many marine population genomics studies. Three systems used to understand how species and populations cope with environmental change are discussed: barnacles, a marine snail, and stickleback fish.

6.2.1 Barnacles

The northern acorn barnacle (*Semibalanus balanoides*) is a quintessential marine organism with high fecundities (and large effective population size, N_e), planktonic larval duration that can last weeks, and a benthic adult phase. During the adult phase, these barnacles inhabit the rocky intertidal habitat where they are exposed to both aerial and oceanic environments and experience extremes in environmental variation, particularly with respect to oxygen and temperature. Thus, *S. balanoides*' life history provides multiple stages at which selection can occur, and this organism provides a useful model to study genetic variation and adaptation to thermal stress in natural populations in the context of ample gene flow. Nunez et al. (2018) review the state of the *S. balanoides* genomic resources, analyze genome-wide levels of variation and population structure across the North Atlantic using pooled sequencing approaches, and describe current and future improvements for barnacle genomic resources (this book). One of their main points is that their approach to build genomic resources for *S. balanoides* can be easily applied to most natural populations.

6.2.2 Marine Snail

The marine snail (*Littorina saxatilis*) is also an intertidal species. In contrast to *S. balanoides*, *L. saxatilis* does not have a free-floating larval stage but instead is a direct developer (Johannesson 2016). Thus, *L. saxatilis* disperse only a few meters each generation, on the same spatial scale as the environmental variation that occurs in its intertidal habitat. Further, semi-isolated *L. saxatilis* ecotypes, with differences in size, shell shape, color, behavior, and physiology, have repeatedly evolved in these heterogeneous intertidal habitats (Johannesson 2016). For these reasons, *L. saxatilis* is an outstanding model to explore the interactions between selection, gene flow, and genetic drift during local adaptation and speciation. Previously, *L. saxatilis* has been extensively studied using classic genetic markers (Johannesson et al. 1995; Johannesson and Tatarenkov 1997), and population genomics approaches are now expanding our understanding of the underlying evolutionary processes involved in *L. saxatilis*' adaptation and speciation. Johannesson et al. (2017) review the mechanisms of adaptive divergence and speciation in *L. saxatilis* and the new information and opportunities that genomic resources are providing (this book). Knowledge from *L. saxatilis* studies has broad implications for understanding both barriers to gene flow and reproductive isolation in the face of gene flow.

6.2.3 Stickleback

A final example of a species used to investigate genetic mechanisms of rapid divergence and evolution in natural systems is the three-spined stickleback, *Gasterosteus aculeatus*. Originally known as a nuisance fish in managed fisheries, the first international stickleback conference took place in 1984 (Barber and Nettleship 2010). This meeting was focused almost entirely on behavioral research. Stickleback fish are such useful behavioral models because they can be easily raised in aquaria and exhibit many of their behaviors in an aquarium setting (Barber and Nettleship 2010; Norton and Gutiérrez 2019). In addition to attributes useful for behavioral studies, the three-spined stickleback displays immense intraspecific variation, especially the freshwater populations (Bell and Foster 1994), making it a powerful model also for ecological and evolutionary research. Finally, three-spined stickleback has repeatedly adapted from oceanic to freshwater habitats with resulting morphological, physiological, and behavioral adaptations. These features, combined with genomics, have transformed the three-spined stickleback into a "supermodel" (Gibson 2005). In stickleback, population genomics approaches have been most notoriously used to study repeated evolution from saltwater to freshwater and have shown that the resulting ecological change (fewer predators) has resulted in concordant changes in genes that alter spines and armor plates (Hohenlohe et al. 2010). Hohenlohe and Magalhaes (2019) describe the primary population genomics approaches that have been taken to understand the genetics of adaptation in stickleback, particularly parallel phenotypic evolution, and discuss the demographic and genomic conditions that can facilitate repeated, rapid adaptation (this book).

6.2.4 Clinal Adaptation in the Marine Environment

Both the intertidal coast with contrasts between aerial and oceanic environments and the differences between freshwater and saltwater environments provide clear examples of contrasting environments that can define populations, shape gene flow, and drive natural selection with different selective forces. Yet the vast majority of the world's ocean environments are not coastal, and the selective forces that separate populations and species are less clear. Thus, on the surface, the oceans appear to be without clear boundaries. However, ocean habitats do harbor boundaries in the form of gradients or clines. Major clinal gradients in the oceans include temperature, salinity, oxygen, and depth. Dayan (2018) discusses how genomic approaches are being used to understand the genetic basis of clinal adaptation to environmental gradients in the oceans (this book). Understanding clinal adaptation is difficult because clines are often correlated with demography. Thus, Dayan presents other explanations for allele frequency changes along clines that are not necessarily adaptive and then describes the approaches for identifying adaptive variants. Using recent examples of clinal adaptation in the oceans, he makes some general conclusions about the genetic basis of clinal adaptation and then proposes future directions. Notably, Dayan (2018), identifies a lack of understanding across many genomic analyses about the mechanisms by which DNA sequence variations are related to adaptively important trait variation.

6.3 Epigenomics

In addition to adaptation, organisms can respond to environmental change via epigenetic mechanisms. Epigenetics has been defined to include the study of heritable (typically for one or a few generations, but see Moler et al. 2019) mechanisms that change gene expression independent of DNA sequence changes (Deans and Maggert 2015). Yet there is a broader definition of epigenetics that includes mechanisms that change gene expression independent of DNA sequence changes that are not necessarily heritable (Metzger and Schulte 2016; Moler et al. 2019). Regardless of the breadth of the definition, epigenetics is another way that populations can respond to environmental change. However, the question remains as to whether epigenetic changes provide a buffer that allows enough time for populations to adapt to environmental change, or conversely, by providing a temporary fix to the need to respond to environmental change, do they hinder acclimation and adaptation (Bonduriansky and Day 2009)? Indeed, models show that epigenetic changes can both increase and decrease the adaptive rate, resulting in populations with higher and lower fitness, respectively: small effect epigenetic changes generally help adaptation, while larger effect epigenetic changes hinder adaptation (Kronholm and Collins 2016). However, empirical data on how epigenetic changes affect adaption are scarce in natural populations.

Environmental epigenomics explores the relationships between environmental changes, epigenetic changes, and subsequent phenotypic consequences. This is a growing field, and a variety of different approaches exist to quantify epigenetic changes (see Moler et al. 2019). These approaches provide different levels of resolution from low-resolution global signals of methylation relative to total DNA to a complete picture of methylation with single base pair resolution. Some techniques such as those based on HPLC or ELISA, which quantify methylation relative to total DNA, are relatively easy and inexpensive (Moler et al. 2019). The higher resolution techniques tend to be more costly with varying levels of complexity (see Eirin-Lopez and Putnam (2019) for a list of techniques to study epigenetic signals as well as references to studies using said techniques to study epigenetics in marine organisms). In this book, Beal et al. (2018) discuss environmental epigenomics, first defining epigenetics and the different mechanisms involved in epigenetic regulation of gene expression and then discussing potential applications of epigenomics research in marine organisms (Beal et al. 2018, this book). They provide many examples of epigenomics studies in marine organisms, yet population-level epigenetic analyses are still rare for marine species. Thus, there remains much potential to assess epigenetic variation between populations experiencing different environments to better understand how populations respond to their surrounding environment.

7 Speciation

Evolutionary and demographic processes that affect population structure, genomics approaches can be used to discern mechanisms affecting speciation, divergence of closely related taxa, and species richness (Grunwald et al. 2016). Unlike in terrestrial systems, in the oceans, there are very few absolute dispersal barriers. Additionally, the large number of marine species that spend at least some portion of their life in the plankton means that many marine species have the potential for extensive gene flow across large distances. This gene flow minimizes population differentiation and consequently should inhibit speciation. Yet the world's oceans have high levels of species diversity – they contain approximately 2.2 million eukaryotic marine species (Mora et al. 2011). How do so many species evolve with so much potential gene flow? A potential mechanism for speciation with gene flow results from the ecological hypothesis of speciation in which reproductive isolation results from divergent or disruptive natural selection (Puebla 2009). González et al. (2018) review studies of ecological speciation in marine environments with a focus on coral systems (this book). They explore how adaptation to depth, an important selective force for many marine species, has the potential to reduce gene flow and eventually lead to reproductive isolation. They go on to discuss mechanisms of reproductive isolation among populations living in different habitats. Finally, they illustrate how these studies have been enhanced by population genomics tools.

8 Protection, Conservation, and Sustainable Management

For management of ecologically important species, conservation, and protection of rare or endangered species, genomic data provide detailed information on marine populations. Specifically, population genomics approaches that identify neutral and selectively advantageous DNA sequence variation can define the forces that shaped populations and their distributions. Genomic DNA analyses, by providing a finer-scale description of population structure, provide a richer understanding of effective population size, migration rates, and the importance of local adaptations and thus inform conservation or management decisions of diverse populations. It is the depth and breadth of information across the genome that provides information that was not possible a decade ago. Three important considerations for protecting marine resources include invasive species, fisheries management, and marine protected areas.

8.1 Invasive Species

Marine invasive species pose a major threat to global biodiversity and can have significant ecological and economic impacts on the ecosystems upon which humans depend. A meta-analysis of more than 350 databases shows that only 16% of marine eco-regions have no reported marine invasions (Molnar et al. 2008). Marine invasions are a growing problem due to ocean connectivity paired with faster and more frequent trans-oceanic transport. Indeed, invasive species introductions are largely driven by international shipping, followed by aquaculture practices (Molnar et al. 2008). Additionally, durable rafting materials, such as plastics, are facilitating marine invasive species. Plastics, unlike most natural products, last much longer in the oceans and thus serve as long-lasting vehicles for trans-ocean transport. For example, stalked barnacles (*Lepas* spp.) have colonized buoyant plastics floating in the Pacific Ocean, where they act as a foundation species, and as foundation species on plastic debris, they have facilitated the survival of coastal species from opposite sides of the Pacific cohabiting the same plastic debris (Gil and Pfaller 2016).

Population genomics approaches are providing new methods for both detecting and understanding the processes involved in successful invasions. In addition, population genomics approaches are providing new opportunities to understand the ecological and evolutionary consequences of biological invasions. Bourne et al. (2018) detail how population genomics approaches have been used to study invasive marine species (this book). They first focus on some key mechanisms driving biological invasions including pre-invasion adaptation, the role of propagule pressure (the number of individuals introduced and the total number of introduction events), and post-invasion hybridization and adaptation. Next, they review the genomics methods used to study marine invasions. Finally, they outline the future

potential of population genomics approaches for studying marine invasive species. These approaches, which allow researchers to address a wider set of research questions using a broader range of taxa, are important for protecting marine ecosystems from the threats posed by invasive species.

8.2 Fisheries Management

Fisheries sciences seek to maintain sustainable fisheries, thus understanding population structure is critical for effectively managing fisheries stocks. Population genomics approaches can provide thousands of genetic markers with which to determine population structure, both connectivity or conversely, adaptive divergence. For instance, ~8,000 SNPs were used to define fine-scale genetic structure in American lobster (*Homarus americanus*), a valuable fishery in North America (Benestan et al. 2015). Interestingly, the defined genetic structuring generally matched the defined lobster fishing areas. Knowledge of population structure is important because different populations are unlikely to have the same response to different stressors such as environmental change or fishing pressures. Consequently, these different populations probably should be managed separately.

In addition to defining genetic structure in American lobster, SNPs also were used to assess population assignment success; the most differentiated SNPs improved population assignment success even in the face of only weak genetic structure. Population assignment allows inferences about the lobsters' migration rates (Benestan et al. 2015) and can be used to improve fisheries management. SNPs that differentiate between populations also can be used for mixed-stock analyses to identify the origins of the species in mixed fisheries such as Pacific salmon (Ackerman et al. 2011), another important consideration for fisheries management. Finally, given their ability to identify origin, differentiating SNPs also can be used to trace illegal fishing and identify fish mislabeling (Nielsen et al. 2012). Illegal, unreported, and unregulated (IUU) fishing is a global problem that affects most of the world's fisheries, and being able to identify IUU fishing is critical to even begin to address the problem (Martinsohn and Ogden 2009). Moreover, SNPs are especially useful for collaborative efforts among agencies or laboratories, which are necessary for such applications as genetic stock identification or tracing illegal fishing, because these bi-allelic markers enable rapid, automated, and reproducible genotyping results (Ackerman et al. 2011).

Benestan (2019) discusses the use of population genomics approaches for fisheries sciences and management (this book). Fisheries population genomics is expanding not only the number of taxa that are being studied but also the fishery management issues that can be addressed. Benestan (2019) gives insight into how population genomics approaches can be integrated with fisheries management and conservation (this book). She focuses on four themes: (1) stock structure and defining population boundaries; (2) the implications of climate change for fisheries and how climate change is influencing species' distributions and adaptation patterns;

(3) the identification of the geographic origin of harvested fishery species, which is integral to regulatory enforcement; and (4) fishery-induced evolution, which can impact population productivity. Of critical importance overall is how population genomics results should be best integrated into management practices.

8.3 Marine Protected Areas

How population genomics results should be best integrated into management practices is also important for designing marine protected areas (MPAs). MPAs are designated zones in which human access and activities are restricted in order to protect the organisms living in the protected area from local anthropogenic stresses. MPAs vary in how much they restrict human activities, from allowing minimal activities (i.e., a no-take marine reserve) to being much more permissive, and they serve as an important strategy for both conservation and fisheries management goals (Lester et al. 2009). Encouragingly, a meta-analysis of 124 different marine reserves located in 29 countries showed that the marine reserves positively affected species richness as well as biomass, number, and size of the organisms within their boundaries (Lester et al. 2009). Similar to fisheries management, being able to define populations is critical to protecting the organisms that make up the marine populations of interest. Thus, in designing MPAs, important considerations concern population connectivity and whether particular populations are source or sink populations for other areas in the ocean. However, unlike fisheries management, MPAs need to consider not just one fishery species at a time. Instead, their design needs to take into consideration multiple organisms inhabiting the protected area as well as multiple stakeholders – from fishers to conservationists – with a vested interest in the designated area. All of these considerations make designing successful MPAs a complex endeavor. Although population genomics approaches can provide valuable information relevant to designing MPAs, that information often is not incorporated. Xuereb et al. (2018) discuss both the benefits of population genomics approaches and also the challenges of effectively incorporating the results of genomic analyses into MPA design (this book). Effective use of genomic analyses for MPA design is becoming more important with increasing global change and resulting stresses on marine environments because MPAs will need to play an increasing role in preserving and maintaining ocean ecosystems.

9 Conclusions and Future Directions

Population genomics approaches are giving us unprecedented insight into life in the world's oceans. This insight results because many population genomics approaches can be used on any organism, regardless of whether or not prior genomic information or resources for that organism exist. Thus, population genomics approaches are

especially germane for marine organisms, which often are hard to sample, hard to maintain in captivity, and hard to culture (i.e., raise through different life stages). By using population genomics approaches, we are learning who, what, where, and when even for marine species we previously knew nothing or almost nothing about. Yet because we still cannot do controlled laboratory experiments on many marine species, genomics approaches often only give us discrete bits of information beyond who, what, where, and when, e.g., gene expression levels under certain conditions, insight into population connectivity, and lists of putatively adaptive loci.

Thus, one of the major challenges that remains for marine population genomics is to expand these bits or snapshots of genomic information to gain a cohesive understanding of marine ecosystems (Fig. 2). Gaining a cohesive understanding of marine ecosystems, while laudable, is a tall order! However, this challenge does not have to be solved all at once, making it a much more manageable although still

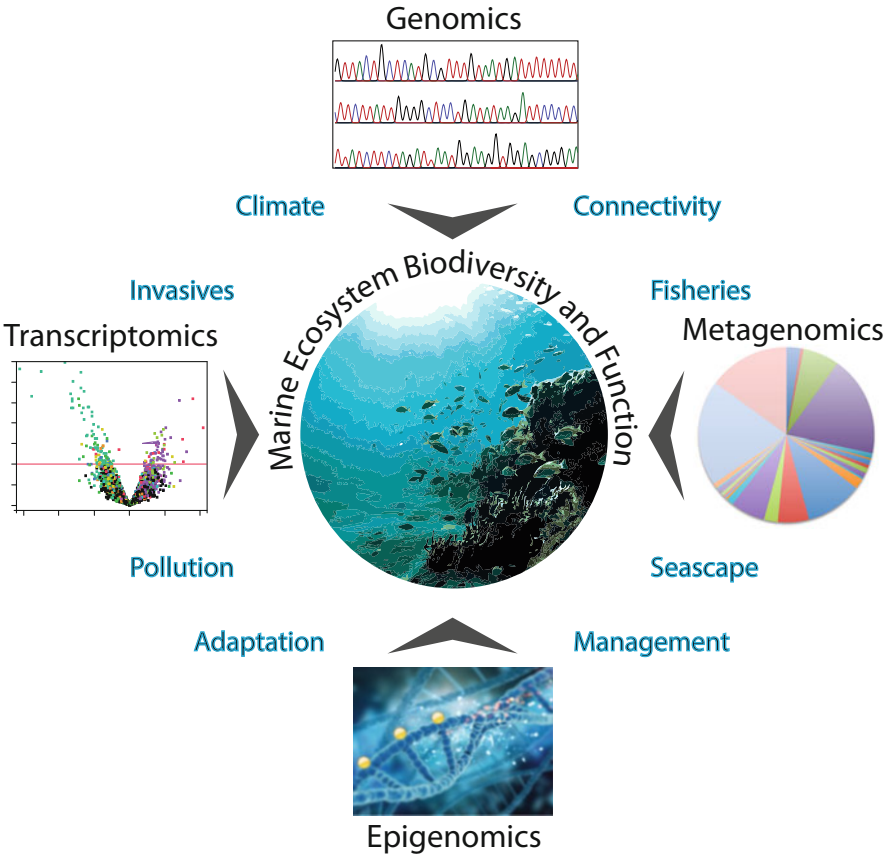

Fig. 2 Genomics informs our understanding of marine ecosystems. Genomics approaches are providing novel insights into marine ecosystem diversity and function. Both biotic and abiotic factors impact these insights, necessitating an integrated analysis. Epigenomic image based on a design by Kjpargeter/Freepik. Reef image adapted from an image by rsoler616 from Pixabay

formidable challenge. Part of the answer to this challenge involves understanding how genotype and phenotype are related. Another part involves understanding which DNA sequence variations are related to adaptively important trait variation. Yet another part involves understanding environmental effects on genotypes and phenotypes at both the individual and population levels. All of these parts are complicated by species interactions and changing environments. Thus, for example, even when we know the genetic reasons for the phenotypic mechanisms that enhance fitness with warmer climates, we may not understand how altered phenotypes will alter species interactions and how altered species interactions will modulate ecosystem diversity. Yet the hope is that with the application of ecological principles, we can integrate all of these parts. Thus, we should be able to better define the relationships from genotype to phenotype to population function to ecosystem function and so gain a greater understanding of how life in our oceans works.

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References

Ackerman MW, Habicht C, Seeb LW. Single-nucleotide polymorphisms (SNPs) under diversifying selection provide increased accuracy and precision in mixed-stock analyses of sockeye Salmon from the Copper River, Alaska. Trans Am Fish Soc. 2011;140(3):865–81.

Adams CIM, Knapp M, Gemmell NJ, Jeunen GJ, Bunce M, Lamare MD, Taylor HR. Beyond biodiversity: can environmental DNA (eDNA) cut it as a population genetics tool? Genes. 2019;10(3):192.

Akbar MA, Ahmad A, Usup G, Bunawan H. Current knowledge and recent advances in marine dinoflagellate transcriptomic research. J Mar Sci Eng. 2018;6(1):13.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. Harnessing the power of RADseq for ecological and evolutionary genomics. Nat Rev Genet. 2016;17:81.

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One. 2008;3(10):e3376.

Barber I, Nettleship S. From 'trash fish' to supermodel: the rise and rise of the three-spined stickleback in evolution and ecology. Biologist. 2010;57:15–21.

Beal A, Rodriguez-Casariego J, Rivera-Casas C, Suarez-Ulloa V, Eirin-Lopez JM. Environmental epigenomics and its applications in marine organisms. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_28.

Bell M, Foster S. The evolutionary biology of the threespine stickleback. Oxford: Oxford University Press; 1994.

Benestan L. Population genomics applied to fishery management and conservation. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2019. https://doi.org/10.1007/13836_2019_66.

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). Mol Ecol. 2015;24(13):3299–315.

Biller SJ, Berube PM, Dooley K, Williams M, Satinsky BM, Hackl T, Hogle SL, Coe A, Bergauer K, Bouman HA, Browning TJ, De Corte D, Hassler C, Hulston D, Jacquot JE, Maas EW, Reinthaler T, Sintes E, Yokokawa T, Chisholm SW. Marine microbial metagenomes sampled across space and time (vol 5, 180176, 2018). Scientific Data. 2019; 6.

Bilyk KT, Cheng CH. RNA-seq analyses of cellular responses to elevated body temperature in the high Antarctic cryopelagic nototheniid fish *Pagothenia borchgrevinki*. Mar Genomics. 2014;18(Pt B):163–71.

Boeuf D, Edwards BR, Eppley JM, Hu SK, Poff KE, Romano AE, Caron DA, Karl DM, DeLong EF. Biological composition and microbial dynamics of sinking particulate organic matter at abyssal depths in the oligotrophic open ocean. Proc Natl Acad Sci U S A. 2019;116(24):11824–32.

Bonduriansky R, Day T. Nongenetic inheritance and its evolutionary implications. Annu Rev Ecol Evol Syst. 2009;40:103–25.

Bourne SD, Hudson J, Holman LE, Rius M. Marine invasion genomics: revealing ecological and evolutionary consequences of biological invasions. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_21.

Bozinovic G, Oleksiak MF. Embryonic gene expression among pollutant resistant and sensitive *Fundulus heteroclitus* populations. Aquat Toxicol. 2010;98(3):221–9.

Bozinovic G, Sit TL, Di Giulio R, Wills LF, Oleksiak MF. Genomic and physiological responses to strong selective pressure during late organogenesis: few gene expression changes found despite striking morphological differences. BMC Genomics. 2013;14:779.

Bravington MV, Grewe PM, Davies CR. Absolute abundance of southern bluefin tuna estimated by close-kin mark-recapture. Nat Commun. 2016;7(1):13162.

Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. Nat Genet. 1999;21(Suppl S):33–7.

Bucklin A, Steinke D, Blanco-Bercial L. DNA barcoding of marine metazoa. Annu Rev Mar Sci. 2011;3(1):471–508.

Bucklin A, DiVito K, Smolina I, Choquet M, Questel JM, Hoarau G, O'Neill RJ. Population genomics of marine zooplankton. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2017. https://doi.org/10.1007/13836_2017_9.

Cammen KM, Andrews KR, Carroll EL, Foote AD, Humble E, Khudyakov JI, Louis M, McGowen MR, Olsen MT, Van Cise AM. Genomic methods take the plunge: recent advances in high-throughput sequencing of marine mammals. J Hered. 2016;107(6):481–95.

Caron DA, Alexander H, Allen AE, Archibald JM, Armbrust EV, Bachy C, Bell CJ, Bharti A, Dyhrman ST, Guida SM, Heidelberg KB, Kaye JZ, Metzner J, Smith SR, Worden AZ. Probing the evolution, ecology and physiology of marine protists using transcriptomics. Nat Rev Microbiol. 2016;15:6.

Cary GA, Cheatle Jarvela AM, Francolini RD, Hinman VF. Genome-wide use of high- and low-affinity Tbrain transcription factor binding sites during echinoderm development. Proc Natl Acad Sci U S A. 2017;114(23):5854–61.

Corander J, Majander KK, Cheng L, Merila J. High degree of cryptic population differentiation in the Baltic Sea herring *Clupea harengus*. Mol Ecol. 2013;22(11):2931–40.

Crawford DL, Oleksiak MF. Ecological population genomics in the marine environment. Brief Funct Genomics. 2016;15(5):342–51.

Croucher NJ, Thomson NR. Studying bacterial transcriptomes using RNA-seq. Curr Opin Microbiol. 2010;13(5):619–24.

Davidson WS, Koop BF, Jones SJ, Iturra P, Vidal R, Maass A, Jonassen I, Lien S, Omholt SW. Sequencing the genome of the Atlantic salmon (*Salmo salar*). Genome Biol. 2010;11(9):403.

Dayan DI. Clinal adaptation in the marine environment. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_62.

Dayan DI, Crawford DL, Oleksiak MF. Phenotypic plasticity in gene expression contributes to divergence of locally adapted populations of *Fundulus heteroclitus*. Mol Ecol. 2015;24(13):3345–59.

Dayan DI, Du X, Baris TZ, Wagner DN, Crawford DL, Oleksiak MF. Population genomics of rapid evolution in natural populations: polygenic selection in response to power station thermal effluents. BMC Evol Biol. 2019;19(1):61.

De Wit P, Palumbi SR. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Mol Ecol. 2013;22(11):2884–97.

Deans C, Maggert KA. What do you mean, "epigenetic"? Genetics. 2015;199(4):887–96.

Dineshram R, Chandramouli K, Ko GW, Zhang H, Qian PY, Ravasi T, Thiyagarajan V. Quantitative analysis of oyster larval proteome provides new insights into the effects of multiple climate change stressors. Glob Chang Biol. 2016;22(6):2054–68.

Drury C, Dale KE, Panlilio JM, Miller SV, Lirman D, Larson EA, Bartels E, Crawford DL, Oleksiak MF. Genomic variation among populations of threatened coral: *Acropora cervicornis*. BMC Genomics. 2016;17(1):286.

Edwards M, Richardson AJ. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature. 2004;430(7002):881–4.

Egerton S, Culloty S, Whooley J, Stanton C, Ross RP. The gut microbiota of marine fish. Front Microbiol. 2018;9:873.

Eirin-Lopez JM, Putnam HM. Marine environmental epigenetics. Annu Rev Mar Sci. 2019;11:335–68.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One. 2011;6(5):e19379.

Everett MV, Antal CE, Crawford DL. The effect of short-term hypoxic exposure on metabolic gene expression. J Exp Zool A Ecol Genet Physiol. 2012;317A(1):9–23.

Ferrer M, Mendez-Garcia C, Bargiela R, Chow J, Alonso S, Garcia-Moyano A, Bjerga GEK, Steen IH, Schwabe T, Blom C, Vester J, Weckbecker A, Shahgaldian P, de Carvalho C, Meskys R, Zanaroli G, Glockner FO, Fernandez-Guerra A, Thambisetty S, de la Calle F, Golyshina OV, Yakimov MM, Jaeger KE, Yakunin AF, Streit WR, McMeel O, Calewaert JB, Tonne N, Golyshin PN, I. Consortium. Decoding the ocean's microbiological secrets for marine enzyme biodiscovery. FEMS Microbiol Lett. 2019;366(1). https://doi.org/10.1093/femsle/fny285.

Ficetola GF, Miaud C, Pompanon F, Taberlet P. Species detection using environmental DNA from water samples. Biol Lett. 2008;4(4):423–5.

Fisher MA, Oleksiak MF. Convergence and divergence in gene expression among natural populations exposed to pollution. BMC Genomics. 2007;8:108.

Franssen SU, Gu J, Bergmann N, Winters G, Klostermeier UC, Rosenstiel P, Bornberg-Bauer E, Reusch TB. Transcriptomic resilience to global warming in the seagrass *Zostera marina*, a marine foundation species. Proc Natl Acad Sci U S A. 2011;108(48):19276–81.

Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Chisholm SW, Delong EF. Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci U S A. 2008;105(10):3805–10.

Gibson G. The synthesis and evolution of a supermodel. Science. 2005;307(5717):1890.

Gil MA, Pfaller JB. Oceanic barnacles act as foundation species on plastic debris: implications for marine dispersal. Sci Rep. 2016;6:19987.

Gnirke A, Melnikov A, Maguire J, Rogov P, LeProust EM, Brockman W, Fennell T, Giannoukos G, Fisher S, Russ C, Gabriel S, Jaffe DB, Lander ES, Nusbaum C. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol. 2009;27(2):182–9.

Goncalves P, Jones DB, Thompson EL, Parker LM, Ross PM, Raftos DA. Transcriptomic profiling of adaptive responses to ocean acidification. Mol Ecol. 2017;26(21):5974–88.

González AM, Prada CA, Ávila V, Medina M. Ecological speciation in corals. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_35.

Grunwald NJ, McDonald BA, Milgroom MG. Population genomics of fungal and oomycete pathogens. Annu Rev Phytopathol. 2016;54:323–46.

Guidi L, Chaffron S, Bittner L, Eveillard D, Larhlimi A, Roux S, Darzi Y, Audic S, Berline L, Brum J, Coelho LP, Espinoza JCI, Malviya S, Sunagawa S, Dimier C, Kandels-Lewis S, Picheral M, Poulain J, Searson S, Tara Oceans C, Stemmann L, Not F, Hingamp P, Speich S, Follows M, Karp-Boss L, Boss E, Ogata H, Pesant S, Weissenbach J, Wincker P, Acinas SG, Bork P, de Vargas C, Iudicone D, Sullivan MB, Raes J, Karsenti E, Bowler C, Gorsky G. Plankton networks driving carbon export in the oligotrophic ocean. Nature. 2016;532(7600):465–70.

Guzman C, Shinzato C, Lu TM, Conaco C. Transcriptome analysis of the reef-building octocoral, *Heliopora coerulea*. Sci Rep. 2018;8(1):8397.

Hartl DL, Clark AG. Principles of population genetics. Sunderland: Sinauer Associates; 1997.

Hauser L, Carvalho GR. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 2008;9(4):333–62.

Hedgecock D. Population genetics of marine organisms. In: Cochran JK, Bokuniewicz HJ, Yager PL, editors. Encyclopedia of ocean sciences, vol. 1. 3rd ed. Oxford: Elsevier; 2019. p. 778–83.

Hedgecock D, Pudovkin AI. Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. Bull Mar Sci. 2011;87(4):971–1002.

Hess JE, Campbell NR, Close DA, Docker MF, Narum SR. Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. Mol Ecol. 2013;22(11):2898–916.

Hofmann GE, Place SP. Genomics-enabled research in marine ecology: challenges, risks and pay-offs. Mar Ecol Prog Ser. 2007;332:249–55.

Hohenlohe PA, Magalhaes IS. The population genomics of parallel adaptation: lessons from threespine stickleback. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2019. https://doi.org/10.1007/13836_2019_67.

Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genet. 2010;6(2):e1000862.

Holliday JA, Hallerman EM, Haak DC. Genotyping and sequencing technologies in population genetics and genomics. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 83–126.

Horricks RA, Herbinger CM, Lillie BN, Taylor P, Lumsden JS. Differential protein abundance during the first month of regeneration of the Caribbean star coral *Montastraea cavernosa*. Coral Reefs. 2019;38(1):45–61.

Johannesson K. What can be learnt from a snail? Evol Appl. 2016;9(1):153–65.

Johannesson K, Tatarenkov A. Allozyme variation in a snail (*Littorina saxatilis*) – deconfounding the effects of microhabitat and gene flow. Evolution. 1997;51(2):402–9.

Johannesson K, Johannesson B, Lundgren U. Strong natural selection causes microscale allozyme variation in a marine snail. Proc Natl Acad Sci U S A. 1995;92(7):2602–6.

Johannesson K, Butlin RK, Panova M, Anja M, Westram AM. Mechanisms of adaptive divergence and speciation in *Littorina saxatilis*: integrating knowledge from ecology and genetics with new data emerging from genomic studies. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2017. https://doi.org/10.1007/13836_2017_6.

Johnston SE, Orell P, Pritchard VL, Kent MP, Lien S, Niemela E, Erkinaro J, Primmer CR. Genome-wide SNP analysis reveals a genetic basis for sea-age variation in a wild population of Atlantic salmon (*Salmo salar*). Mol Ecol. 2014;23(14):3452–68.

Kalujnaia S, McWilliam IS, Zaguinaiko VA, Feilen AL, Nicholson J, Hazon N, Cutler CP, Balment RJ, Cossins AR, Hughes M, Cramb G. Salinity adaptation and gene profiling analysis in the European eel (*Anguilla anguilla*) using microarray technology. Gen Comp Endocrinol. 2007;152(2–3):274–80.

Kelly RP, Port JA, Yamahara KM, Martone RG, Lowell N, Thomsen PF, Mach ME, Bennett M, Prahler E, Caldwell MR, Crowder LB. Environmental monitoring. Harnessing DNA to improve environmental management. Science. 2014;344(6191):1455–6.

Kronholm I, Collins S. Epigenetic mutations can both help and hinder adaptive evolution. Mol Ecol. 2016;25(8):1856–68.

Lester SE, Halpern BS, Grorud-Colvert K, Lubchenco J, Ruttenberg BI, Gaines SD, Airamé S, Warner RR. Biological effects within no-take marine reserves: a global synthesis. Mar Ecol Prog Ser. 2009;384:33–46.

Libro S, Kaluziak ST, Vollmer SV. RNA-seq profiles of immune related genes in the staghorn coral *Acropora cervicornis* infected with white band disease. PLoS One. 2013;8(11):e81821.

Liggins L, Treml EA, Riginos C. Seascape genomics: contextualizing adaptive and neutral genomic variation in the ocean environment. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2019. https://doi.org/10.1007/13836_2019_68.

Luikart G, Kardos M, Hand B, Rajora OP, Aitken S, Hohenlohe PA. Population genomics: advancing understanding of nature. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 3–79.

Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, Howard E, Shendure J, Turner DJ. Target-enrichment strategies for next-generation sequencing. Nat Methods. 2010;7(2):111–8.

Martinsohn JT, Ogden R. FishPopTrace – developing SNP-based population genetic assignment methods to investigate illegal fishing. Forensic Sci Int. 2009;2(1):294–6.

Metzger DCH, Schulte PM. Epigenomics in marine fishes. Mar Genomics. 2016;30:43–54.

Mineta K, Gojobori T. Databases of the marine metagenomics. Gene. 2016;576(2, Part 1):724–8.

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Minamoto T, Yamamoto S, Yamanaka H, Araki H, Kondoh M, Iwasaki W. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. R Soc Open Sci. 2015;2(7):150088.

Moler ERV, Abakir A, Eleftheriou M, Johnson JS, Krutovsky KV, Lewis LC, Ruzov A, Whipple AV, Rajora OP. Population epigenomics: advancing understanding of phenotypic plasticity, acclimation, adaptation and diseases. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 179–260.

Molnar JL, Gamboa RL, Revenga C, Spalding MD. Assessing the global threat of invasive species to marine biodiversity. Front Ecol Environ. 2008;6(9):485–92.

Mora C, Tittensor DP, Adl S, Simpson AG, Worm B. How many species are there on earth and in the ocean? PLoS Biol. 2011;9(8):e1001127.

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D. Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol. 2009;18(15):3128–50.

Nielsen EE, Cariani A, Aoidh EM, Maes GE, Milano I, Ogden R, Taylor M, Hemmer-Hansen J, Babbucci M, Bargelloni L, Bekkevold D, Diopere E, Grenfell L, Helyar S, Limborg MT, Martinsohn JT, McEwing R, Panitz F, Patarnello T, Tinti F, Van Houdt JKJ, Volckaert FAM, Waples RS, C. FishPopTrace, Albin JEJ, Vieites Baptista JM, Barmintsev V, Bautista JM, Bendixen C, Bergé J-P, Blohm D, Cardazzo B, Diez A, Espiñeira M, Geffen AJ, Gonzalez E, González-Lavín N, Guarniero I, Jeráme M, Kochzius M, Krey G, Mouchel O, Negrisolo E, Piccinetti C, Puyet A, Rastorguev S, Smith JP, Trentini M, Verrez-Bagnis V, Volkov A, Zanzi A, Carvalho GR. Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. Nat Commun. 2012;3:851.

Norton WHJ, Gutiérrez HC. The three-spined stickleback as a model for behavioural neuroscience. PLoS One. 2019;14(3):e0213320.

Nunez JC, Seale TP, Fraser MA, Burton TL, Fortson TN, Hoover D, Travis J, Oleksiak MF, Crawford DL. Population genomics of the euryhaline teleost *Poecilia latipinna*. PLoS One. 2015;10(9):e0137077.

Nunez JCB, Elyanow RG, Ferranti DA, Rand DM. Population genomics and biogeography of the northern acorn barnacle (*Semibalanus balanoides*) using pooled sequencing approaches. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_58.

Oleksiak MF. Genomic approaches with natural fish populations. J Fish Biol. 2010;76(5):1067–93.

Oleksiak MF. Adaptation without boundaries: population genomics in marine systems. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 587–612.

Oleksiak MF, Churchill GA, Crawford DL. Variation in gene expression within and among natural populations. Nat Genet. 2002;32(2):261–6.

Oleksiak MF, Roach JL, Crawford DL. Natural variation in cardiac metabolism and gene expression in *Fundulus heteroclitus*. Nat Genet. 2005;37(1):67–72.

Oleksiak MF, Karchner SI, Jenny MJ, Franks DG, Welch DB, Hahn ME. Transcriptomic assessment of resistance to effects of an aryl hydrocarbon receptor (AHR) agonist in embryos of Atlantic killifish (*Fundulus heteroclitus*) from a marine Superfund site. BMC Genomics. 2011;12:263.

Pace NR, Stahl DA, Lane DJ, Olsen GJ. The analysis of natural microbial-populations by ribosomal-RNA sequences. Adv Microb Ecol. 1986;9:1–55.

Palenik B, Ren Q, Tai V, Paulsen IT. Coastal Synechococcus metagenome reveals major roles for horizontal gene transfer and plasmids in population diversity. Environ Microbiol. 2009;11(2):349–59.

Parsons KM, Everett M, Dahlheim M, Park L. Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. R Soc Open Sci. 2018;5(8):180537.

Pérez-Portela R, Riesgo A. Population genomics of early-splitting lineages of metazoans. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_13.

Plough LV, Shin G, Hedgecock D. Genetic inviability is a major driver of type III survivorship in experimental families of a highly fecund marine bivalve. Mol Ecol. 2016;25(4):895–910.

Porteus CS, Hubbard PC, Uren Webster TM, van Aerle R, Canário AVM, Santos EM, Wilson RW. Near-future CO2 levels impair the olfactory system of a marine fish. Nat Clim Chang. 2018;8(8):737–43.

Puebla O. Ecological speciation in marine v. freshwater fishes. J Fish Biol. 2009;75(5):960–96.

Puritz JB, Lotterhos KE. Expressed exome capture sequencing: a method for cost-effective exome sequencing for all organisms. Mol Ecol Resour. 2018;18(6):1209–22.

Qiu ZG, Coleman MA, Provost E, Campbell AH, Kelaher BP, Dalton SJ, Thomas T, Steinberg PD, Marzinelli EM. Future climate change is predicted to affect the microbiome and condition of habitat-forming kelp. Proc R Soc B Biol Sci. 2019;286(1896):20181887.

Rajora OP, editor. Population genomics: concepts, approaches and application. Cham: Springer Nature Switzerland AG; 2019. 823 pp. ISBN 978-3-030-04587-6; ISBN 978-3-030-04589-0 (eBook).

Ramirez F, Afan I, Tavecchia G, Catalan IA, Oro D, Sanz-Aguilar A. Oceanographic drivers and mistiming processes shape breeding success in a seabird. Proc R Soc B Biol Sci. 2016;283(1826):20152287.

Ramsay G. DNA chips: state-of-the art. Nat Biotechnol. 1998;16(1):40–4.

Riginos C, Liggins L. Seascape genetics: populations, individuals, and genes marooned and adrift. Geogr Compass. 2013;7(3):197–216.

Riginos C, Crandall ED, Liggins L, Bongaerts P, Treml EA. Navigating the currents of seascape genomics: how spatial analyses can augment population genomic studies. Curr Zool. 2016;62(6):581–601.

Roberts SB, Hauser L, Seeb LW, Seeb JE. Development of genomic resources for Pacific herring through targeted transcriptome pyrosequencing. PLoS One. 2012;7(2):e30908.

Roitman S, Pollock FJ, Medina M. Coral microbiomes as bioindicators of reef health. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_29.

Sassoubre LM, Yamahara KM, Gardner LD, Block BA, Boehm AB. Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. Environ Sci Technol. 2016;50(19):10456–64.

Schena M, Heller RA, Theriault TP, Konrad K, Lachenmeier E, Davis RW. Microarrays: biotechnology's discovery platform for functional genomics. Trends Biotechnol. 1998;16(7):301–6.

Schlotterer C, Tobler R, Kofler R, Nolte V. Sequencing pools of individuals – mining genome-wide polymorphism data without big funding. Nat Rev Genet. 2014;15(11):749–63.

Schott RK, Panesar B, Card DC, Preston M, Castoe TA, Chang BS. Targeted capture of complete coding regions across divergent species. Genome Biol Evol. 2017;9(2):398–414.

Seeb LW, Waples RK, Limborg MT, Warheit KI, Pascal CE, Seeb JE. Parallel signatures of selection in temporally isolated lineages of pink salmon. Mol Ecol. 2014;23(10):2473–85.

Sigsgaard EE, Nielsen IB, Bach SS, Lorenzen ED, Robinson DP, Knudsen SW, Pedersen MW, Jaidah MA, Orlando L, Willerslev E, Møller PR, Thomsen PF. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. Nat Ecol Evol. 2016;1:0004.

Slattery M, Ankisetty S, Corrales J, Marsh-Hunkin KE, Gochfeld DJ, Willett KL, Rimoldi JM. Marine proteomics: a critical assessment of an emerging technology. J Nat Prod. 2012;75(10):1833–77.

Stat M, Huggett MJ, Bernasconi R, DiBattista JD, Berry TE, Newman SJ, Harvey ES, Bunce M. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. Sci Rep. 2017;7(1):12240.

Sussarellu R, Fabioux C, Le Moullac G, Fleury E, Moraga D. Transcriptomic response of the Pacific oyster *Crassostrea gigas* to hypoxia. Mar Genomics. 2010;3(3–4):133–43.

Thomsen PF, Willerslev E. Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv. 2015;183:4–18.

Thomsen PF, Kielgast J, Iversen LL, Moller PR, Rasmussen M, Willerslev E. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One. 2012;7(8):e41732.

Tomanek L. Environmental proteomics: changes in the proteome of marine organisms in response to environmental stress, pollutants, infection, symbiosis, and development. Annu Rev Mar Sci. 2011;3:373–99.

Vasquez MC, Lippert MR, White C, Walter RK, Tomanek L. Proteomic changes across a natural temperature gradient in a marine gastropod. Mar Environ Res. 2019;149:137–47.

Venkataraman YR, Timmins-Schiffman E, Horwith MJ, Lowe AT, Nunn B, Vadopalas B, Spencer LH, Roberts SB. Characterization of Pacific oyster *Crassostrea gigas* proteomic response to natural environmental differences. Mar Ecol Prog Ser. 2019;610:65–81.

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu D, Paulsen I, Nelson KE, Nelson W, Fouts DE, Levy S, Knap AH, Lomas MW, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers YH, Smith HO. Environmental genome shotgun sequencing of the Sargasso Sea. Science. 2004;304(5667):66–74.

Wagner DN, Baris TZ, Dayan DI, Du X, Oleksiak MF, Crawford DL. Fine-scale genetic structure due to adaptive divergence among microhabitats. Heredity (Edinb). 2017;118(6):594–604.

Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009;10(1):57–63.

Wang WL, Xu SY, Ren ZG, Tao L, Jiang JW, Zheng SS. Application of metagenomics in the human gut microbiome. World J Gastroenterol. 2015;21(3):803–14.

Webb TJ, Vanden Berghe E, O'Dor R. Biodiversity's big wet secret: the global distribution of marine biological records reveals chronic under-exploration of the deep pelagic ocean. PLoS One. 2010;5(8):e10223.

Weersing K, Toonen RJ. Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser. 2009;393:1–12.

Williams DR, Li W, Hughes MA, Gonzalez SF, Vernon C, Vidal MC, Jeney Z, Jeney G, Dixon P, McAndrew B, Bartfai R, Orban L, Trudeau V, Rogers J, Matthews L, Fraser EJ, Gracey AY, Cossins AR. Genomic resources and microarrays for the common carp *Cyprinus carpio* L. J Fish Biol. 2008;72(9):2095–117.

Xuereb A, D'Aloia CC, Daigle RM, Andrello M, Dalongeville A, Manel S, Mouillot D, Guichard F, Côté IM, Curtis JMR, Bernatchez L, Fortin M-J. Marine conservation and marine protected areas. In: Oleksiak MF, Rajora OP, editors. Population genomics: marine organisms. Cham: Springer Nature Switzerland AG; 2018. https://doi.org/10.1007/13836_2018_63.

Zhang G, Fang X, Guo X, Li L, Luo R, Xu F, Yang P, Zhang L, Wang X, Qi H, Xiong Z, Que H, Xie Y, Holland PW, Paps J, Zhu Y, Wu F, Chen Y, Wang J, Peng C, Meng J, Yang L, Liu J, Wen B, Zhang N, Huang Z, Zhu Q, Feng Y, Mount A, Hedgecock D, Xu Z, Liu Y, Domazet-Loso T, Du Y, Sun X, Zhang S, Liu B, Cheng P, Jiang X, Li J, Fan D, Wang W, Fu W, Wang T, Wang B, Zhang J, Peng Z, Li Y, Li N, Wang J, Chen M, He Y, Tan F, Song X, Zheng Q, Huang R, Yang H, Du X, Chen L, Yang M, Gaffney PM, Wang S, Luo L, She Z, Ming Y, Huang W, Zhang S, Huang B, Zhang Y, Qu T, Ni P, Miao G, Wang J, Wang Q, Steinberg CE, Wang H, Li N, Qian L, Zhang G, Li Y, Yang H, Liu X, Wang J, Yin Y, Wang J. The oyster genome reveals stress adaptation and complexity of shell formation. Nature. 2012;490(7418):49–54.

Zhang X, Wen H, Wang H, Ren Y, Zhao J, Li Y. RNA-Seq analysis of salinity stress-responsive transcriptome in the liver of spotted sea bass (*Lateolabrax maculatus*). PLoS One. 2017;12(3): e0173238.

Zhou J, He Z, Yang Y, Deng Y, Tringe SG, Alvarez-Cohen L. High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. MBio. 2015;6(1):e02288–14.

Part II
Marine Microbiomes

Coral Microbiomes as Bioindicators of Reef Health

Sofia Roitman, F. Joseph Pollock, and Mónica Medina

Abstract Coral reefs are currently in steep decline worldwide due to changes in climate and anthropogenic activity. Given reefs' key roles as centers of biodiversity and the variety of services they provide for humans, it is imperative that we develop reef management strategies that are sensitive to environmental changes and that allow timely interventions in response to specific threats. The use of bioindicators has been demonstrated as an effective way to monitor a broad range of ecosystems, and coral microbiomes show immense potential as bioindicators for coral reefs. Given the decline of coral reefs worldwide, and the diversity of species that are currently under threat, coral microbiomes can provide much-needed insights and information for the purposes of reef conservation and protection.

Keywords 16S · Bacteria · Bioindicator · Conservation · Coral reef · Ecosystem management · Metagenomics · Metatranscriptomics · Microbiome · Sequencing

1 Introduction

Coral reefs are often compared to tropical rainforest ecosystems for their high species richness and complexity (Mulhall 2008). Containing one-quarter to one-third of all marine species, coral reefs provide a milieu of economic and ecological services to humans, including fisheries and coastal protection (Hoegh-Guldberg et al. 2007; Plaisance et al. 2011). Due to a combination of overexploitation, pollution, and global climate change, these ecosystems have undergone significant degradation in recent decades (Parry et al. 2007). Given their immense value and threatened status, conservation of coral reefs has emerged globally as a pressing management concern. Monitoring potential stressors (e.g., increased temperature, sedimentation, nutrients)

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and assessing their influence on ecosystem status and function form the basis of effective adaptive natural resource management (Holling 1978). However, the sporadic and heterogeneous nature of anthropogenic pressures combined with the remoteness of many reef systems presents a significant challenge to traditional monitoring methods. Bioindicators hold the promise of providing highly sensitive, time-integrated measures linking changes in water quality to the condition and function of reef ecosystems (Cooper et al. 2009; Fabricius et al. 2012; Leite et al. 2018). In this chapter, we highlight the potential of coral microbiomes as novel bioindicators of reef health.

Many reef monitoring programs aim to document changes in environmental condition and quantify the impact of these changes on the diversity, health, and function of reef-dwelling organisms (Rogers et al. 1994). This information is critical for understanding the role of specific human activities in reef declines and informing the effective management of ongoing and proposed projects. Reef monitoring programs often employ direct measurement of fish, coral, and/or non-coral invertebrate health and abundance and collect data on environmental parameters (e.g., temperature, salinity, and turbidity). These methods require highly trained SCUBA divers to conduct repeated underwater surveys often at multiple reef locations, which can be time-consuming and extremely costly. For example, the Australian Institute of Marine Science's Long-Term Monitoring Program has cost an estimated AUD$50 million over 27 years (AIMS 2013). This detailed monitoring provides invaluable documentation of coral decline on the Great Barrier Reef. It also serves as a unique resource to assess the causes of this decline and assess the effectiveness of protection measures. However, direct measurements of environmental conditions are limited in their ability to provide holistic information on reef health. Direct environmental measurements, for example, can only capture the state of an ecosystem at the time of sampling. The significant costs and logistical challenges (e.g., extreme weather) associated with delivering teams of specialist divers to often remote reefs limit the frequency of in situ monitoring (Holt and Miller 2011). Sporadic, infrequent sampling is likely to miss rare or highly variable impacts and/or responses and limits the utility of such monitoring as early-warning management triggers (Fabricius et al. 2012). Inferences of ecosystem or organismal health based on direct environmental measurements are currently limited by an imperfect understanding of the complex relationships between environmental and biotic variability (Fabricius et al. 2012). It is worth noting that these monitoring methods are not able to determine the quality of the reef ecosystem as a whole or to fully assess the presence or extent of changes in the surrounding environment. In order to continue protecting and conserving coral reefs in the future, it is imperative to develop monitoring methods that are more sensitive to changes in reef ecosystems and organismal responses to these changes.

2 Bioindicators of Coral Reef Ecosystem Health

In contrast to traditional environmental monitoring, which often relies upon direct measurement of environment parameters (e.g., temperature, salinity, nutrients, pollutants, turbidity), bioindicator-based monitoring quantifies specific biological processes, species, and/or communities as proxies for environmental condition. Embracing the view that the biota itself is the best predictor of how ecosystems respond to disturbance, researchers and managers working in terrestrial, freshwater, and marine ecosystems increasingly employ bioindicators for environmental monitoring (Bispo et al. 2009; Phillips and Rainbow 2013; Thakur et al. 2013). While monitoring whole-community dynamics – rather than a subset of bioindicators – can be informative, it is simply not feasible in high diversity systems like coral reefs (Bouchet 2006). Focusing on a subset of species satisfies the established criteria for effective bioindicators – specificity, monotonicity, variability, practicality, and ecological relevance (see Box 1) – maximizing sensitivity while reducing costs.

Box 1 Microbiomes Meet the Criteria for Useful Bioindicators

While the criteria for adequate bioindicators can vary by environment and need, universal standards for useful bioindicators have been prescribed. These include an organism's ability to exhibit biological responses that are specific to one stressor or environmental change, termed *specificity*; its *monotonicity*, which dictates that the size of an organism's response to a stressor or change should be proportional to both the intensity and the duration of said stressor or change; an organism's (or community of organisms') *variability*, that is, its ability to remain relatively consistent in the absence of changes or stressors; the *practicality* of a bioindicator, which hinges on the feasibility, cost-effectiveness, and the ease of measurement of an organism's biological responses; and finally that a bioindicator must be *relevant* and exhibit stress responses that are both ecologically relevant as well as relevant to the general public in order to facilitate the communication of results (Cooper et al. 2009).

Microbiomes meet these criteria for useful bioindicators. Microbial responses have been shown to be specific to certain changes in the environment, particularly with regard to environmental pollution. For example, studies on soil microbiomes have shown them to be highly responsive to lead pollution even at low concentrations, as well as to copper and zinc (Nwuche and Ugoji 2008; Sobolev and Begonia 2008). Shifts in microbial diversity have also been observed in aquatic environments in response to increased pCO_2, suggesting that microorganisms could be used to detect increases in the acidification of the water column (Liu et al. 2010). A study conducted by Cooper et al. (2009) on potential bioindicators also found that microbes ranked well in both monotonicity and variability as bioindicators, indicating that their response to disturbance is proportional to the strength of the disturbance and

(continued)

Box 1 (continued)
that they show low variability in the absence of stress (Cooper et al. 2009). Microorganisms are also a quite practical option: they tend to be abundant in all environments and are easily accessible and testable, which allows for cost- and time-effective sampling that can yield quick results for the purposes of environmental assessment (Parmar et al. 2016). Finally, microbial responses are relevant at ecological scales, and associated changes are relevant to the public. This is because microorganisms are key players in a variety of different biogeochemical cycles, such as the carbon and nitrogen cycles, that are integral for the continued survival of an ecosystem (Bloem and Breure 2003). As aggregate microbiomes, they can also have large impacts on the health and function of their host, which makes them particularly important in ecosystems such as coral reefs in which their hosts are also keystone species. Responses to changes in their ecosystem can take the form of changes in community diversity and overall function, which can have serious implications for the natural cycles of an ecosystem as well as for organisms with which they have symbiotic relationships.

Despite their vast potential, only a handful of studies have explored the potential of bioindicators in reef monitoring (Cooper et al. 2009; Leite et al. 2018). Studies examining the suitability of different marine fauna (e.g., invertebrates, fishes, and marine plants and algae) as bioindicators of water quality and reef health have consistently highlighted coral physiology, health, and benthic cover as promising options (Cooper et al. 2009; Fabricius et al. 2012). Measurements of coral health, function, and diversity have been employed as bioindicators, providing information on environmental changes at different temporal scales and impact intensities (Fabricius et al. 2012). As the backbone of reef ecosystems, corals are intrinsically tied to the fluxes and changes in reef health, and as a result they display a range of measureable responses to changes in the environment. Measurements of coral symbiont photophysiology, for example, provide information on environmental disturbances at the colony level within a short time frame (with responses ranging from seconds to days) (Cooper et al. 2009; Jones et al. 2003). Coral disease, another common bioindicator, provides information at intermediate time scales, with diseases becoming visually evident days to weeks after disturbance (Cooper et al. 2009). Coral diseases have been tied to changes in a multitude of environmental conditions, including increases in temperature, algal contact, nutrient enrichment, sedimentation, and turbidity (Bruno et al. 2007; Harvell et al. 2007; Nugues et al. 2004; Vega Thurber et al. 2014; Pollock et al. 2014). Changes in hard coral taxonomic richness and cover have been employed to demonstrate prolonged impacts over time frames ranging from months to years and serve as community-level bioindicators for chronic declines in water quality (Cooper et al. 2009; De'ath and Fabricius 2010).

Despite their immense value, several constraints limit the utility of coral-related factors as effective bioindicators. Direct reef monitoring methods, such as coral disease detection and observance of community-level changes, lack the sensitivity to detect sublethal levels of stress and/or identify stress prior to the macroscopic manifestation of bleaching, disease, or population declines. One of the main goals of reef monitoring is to detect harmful changes in ecosystem health before they cause damage to the health of keystone reef organisms. Since the visible signs of coral diseases and bleaching can take days to weeks to appear, by the time they are observed, the most effective window for adaptive management interventions may have passed (Gil-Agudelo et al. 2004; Gladfelter 1982). While coral disease and taxonomic richness can be useful indicators of chronic and/or extreme water quality changes, their potential for rapid adaptive management is limited. Ultimately, an adequate reef bioindicator must be both intimately affiliated with the coral species that form the basis of these ecosystems and provide broad-range responses that span time scales and impact levels.

3 Microorganisms as Bioindicators

The abundance, diversity, and sensitivity of coral-associated microbial communities and their intimate ties with the water column and keystone reef species make coral microbiomes ideal targets for bioindicator development. Microorganisms have been successfully utilized as bioindicators in diverse ecosystems, including soils, rivers, and even the human body, but the potential application of microbes as reef bioindicators has only just begun to be explored (Glasl et al. 2017; Leite et al. 2018; Peixoto et al. 2017). The extensive literature on microbial bioindicators and the responses of coral microbiomes to environmental change provides an ideal base for the establishment of coral-associated microbes as bioindicators in reef ecosystems (see Box 2 and Table 1).

Box 2 Microorganisms and Microbiomes Have Been Used as Bioindicators in Diverse Ecosystems

Microorganisms are well-established bioindicators in both aquatic and terrestrial ecosystems and have proven successful for the purposes of monitoring environmental health. Microbial bioindicators are particularly common in studies focusing on soil health, as soil microbial communities are often good indicators of changes in soil quality. This is because of their involvement in many key cycling processes in the soil as well as their sensitivity to alterations in the ecosystem, which results in quick responses and even responses that precede environmental changes, further establishing their potential to act as early-warning systems of soil changes (Nielsen et al. 2002). Researchers seeking to identify the best indicators for soil health consistently include

(continued)

Box 2 (continued)
microorganisms, and their functions, near the top of their lists. For example, studies using diversity analyses have found that bacteria and archaea in soil were the best indicators for the monitoring of carbon, nitrogen, and phosphorus cycling, as well as for soil biodiversity and the availability of appropriate habitats for sustaining biodiversity (Bispo et al. 2009; Mendes et al. 2016; Stone et al. 2016). Microbial parameters have also been used to detect and monitor soil pollution as an alternative to more expensive and long-term field experiments, due to the fact that microorganisms can be highly responsive to certain pollutants even at low concentrations (Brookes 1995; Nwuche and Ugoji 2008; Sobolev and Begonia 2008).

Monitoring efforts focused on aquatic environments have also benefitted from the use of microbial bioindicators. Though microbial bioindicators are more established in certain ecosystems and monitoring situations than others, their sensitivity to changes in water quality has led researchers to call for their use in broad contexts, from wetland monitoring to the detection of mining pollutants in rivers (Sims et al. 2013; Yergeau et al. 2012). Organisms such as ciliates, with over 300 species having established roles in river and lake monitoring efforts, are an excellent example of the variety of microorganisms available for the purposes of indicating changes in an aquatic environment (Foissner and Berger 1996).

The use of microbial bioindicators to determine organismal health has also been growing in popularity, particularly in the context of human health. Studies on the gut microbiome have found that it can be affected by a variety of factors including diet, and the microbiome's responses can be used as indicators for changes in the health of an organism. This concept was highlighted by a study on gut microbiota that found that structural shifts in the microbial composition of mice's guts can be used as bioindicators to monitor the health of organisms exposed to carcinogens (Wei et al. 2010). Microbial bioindicators are also beginning to gain traction in the realm of disease and preventative care: a recent study on leukemia patients found that microbiome diversity before chemotherapy treatment is linked to risk of infection during the treatment (Galloway-Peña et al. 2016). The authors suggest that the composition of the microbiome could therefore be used as an indicator of infection risk and help to mitigate infections in leukemia patients undergoing chemotherapy (Galloway-Peña et al. 2016).

Microorganisms are widely applicable as biological indicators for different environmental and even organismal changes. With continued study of their function and roles in ecosystem health, microorganisms will be able to be integrated with common monitoring systems for the purposes of providing a wider and more accurate range of information on environmental shifts and ecosystem response.

Table 1 Specific microbial responses have been detected within coral hosts as the result of environmental stress

Stressor	Coral species	Microbial response	Source
Thermal changes	*Acropora muricata*	Shift towards *Verrucomicrobiae- and α-Proteobacteria*-dominated community	Lee et al. (2015)
Pollution/ proximity to shore	*Orbicella faveolata, Porites astreoides; Orbicella annularis*	Increase in bacterial diversity	Morrow et al. (2012); Klaus et al. (2007)
Pathogens	*Diploria strigosa, Siderastrea siderea; Orbicella faveolata*	Increase in *α-Proteobacteria,* decrease in β- and *γ-proteobacteria;* increase in diversity and *Rhodobacterales*	Cárdenas et. al. (2012); Sunagawa et al. (2009)
Eutrophication	*Acropora hemprichii*	Increase in diversity	Jessen et al. (2013)
Salinity	*Fungia granulosa*	Increase in abundance of *Rhodobacteraceae*	Röthig et al. (2016)

Microorganisms' sensitivity and ability to show marked shifts in community composition and abundance in response to specific stressors highlight their potential as indicators of changes in reef ecosystems as well as in coral host health

Microorganisms and microbiomes meet the established criteria for ideal environmental indicators (see Box 1). In the context of reef health, using microorganisms as bioindicators can provide valuable insights into both biological and environmental changes. Microorganisms are present not only in the sediment and water column but also in association with keystone organisms such as sponges or corals (Bourne and Webster 2013). These intimate connections could allow for a more thorough analysis of the state of a coral reef than would coral health assessments on their own. Several microbe-focused monitoring techniques currently employed on reefs rely on microorganisms residing in the water column and benthos and can provide information on changes in water quality and temperature. Tools such as Autonomous Reef Monitoring Structures (ARMS) capitalize on the wealth of bioindicators present in the water column and the reef ecosystem, serving as long-term collection devices for a wide variety of marine species, including microbial ones. ARMS allow for the monitoring of marine diversity in reef environments by analyzing the communities of invertebrates and algae that colonize three-dimensional structures deployed on the benthic substrate, which are used as proxies for overall biodiversity (Knowlton et al. 2010). Coupled with molecular tools, ARMS can also be useful for determining overall reef bacterial diversity by focusing on colonizing bacteria and, in turn, determining fluxes in microbial diversity over time and with changes in the environment. Colonizing bacteria tend to form biofilms on the structure. Biofilms, microbial assemblages that are attached to surfaces and enclosed in extracellular polymeric substance matrices, often serve as bioindicators in aquatic environments, providing information on water quality due to their ability to absorb heavy metals and their sensitivity to changes in their environment (Burns and Ryder 2001; Donlan 2002; Mages et al. 2004). Biofilms have been implemented successfully in reef

systems as well, showing changes in the diversity of the biofilm's microbial community alongside a water quality gradient as well as in correlation to terrestrial runoff (Kriwy and Uthicke 2011; Witt et al. 2012). Though biofilms collected by ARMS can serve as useful bioindicators for the purposes of assessing reef water quality as well as determining coral health if they respond to similar disease-causing or coral health-altering factors, their functional disconnect from corals themselves puts them as a disadvantage and limits their ability and scope as bioindicators.

Foraminifera, a group of eukaryotic microbes that are highly responsive to fluxes in the nutrient content of the water column, provide a good example of microorganisms successfully used as bioindicators for reef health (Hallock et al. 2003). Foraminifers are particularly suitable for reef monitoring since their water quality requirements are similar to those of reef-building corals (Hallock et al. 2003). Hallock et al. (2003) developed the FORAM Index, a comprehensive and easily implemented procedure allowing resource managers to collect foraminifers and use simple abundance and diversity analyses to detect and quantify environmental change and assess the water column's suitability for reef growth (Hallock et al. 2003). While this method remains limited due to its focus on one phylum, the FORAM Index is an excellent example of the immense potential of microbial species as bioindicators. For this reason, it serves as a stepping stone toward similar systems and indexes that are able to integrate information from a variety of different microorganisms and provide a more holistic picture of reef health.

While both biofilms collected by ARMS and the FORAM Index have been relatively successful, there is still a need for a bioindicator that can be used as a proxy for both coral and reef ecosystem-wide health. In this context, coral microbiomes show substantial promise in providing more accurate measures for environmental changes and health. Coral microbiota are assemblages of bacteria, archaea, and microscopic eukaryotes that sustain long-term symbioses with corals, collectively comprising the coral holobiont (Ainsworth et al. 2010; Knowlton and Rohwer 2003; Rohwer et al. 2002). Coral-associated microorganisms provide a variety of services for corals, ranging from antibiotic production to the metabolism of coral waste products (Ainsworth et al. 2010; Glasl et al. 2017; Rohwer et al. 2002). One important criterion of an effective bioindicator is *specificity* in its response to changes and stressors in the environment. Microorganisms satisfy this criterion well. Many studies have shown that the microorganisms that inhabit corals are sensitive and have specific reactions to environmental changes; the diversity and community composition of a reef's microbiome will often change in response to stress (Fig. 1). Common stressors such as increases in temperature, variations in the water's nutrient content, and contact with algae have been shown to increase microbial diversity within corals (see Table 1) (Jessen et al. 2013; Morrow et al. 2012; Zaneveld et al. 2016). Environmental changes can also alter microbial community structure in the coral holobiont, usually eliciting an increase in beta diversity (variability) and affecting the abundance of specific microbial taxa (Klaus et al. 2007; Lee et al. 2016; Röthig et al. 2016; Zaneveld et al. 2017). *Endozoicomonas*, a bacterium thought to be beneficial to corals, appears particularly sensitive to environmental changes, decreasing in abundance during periods of stress

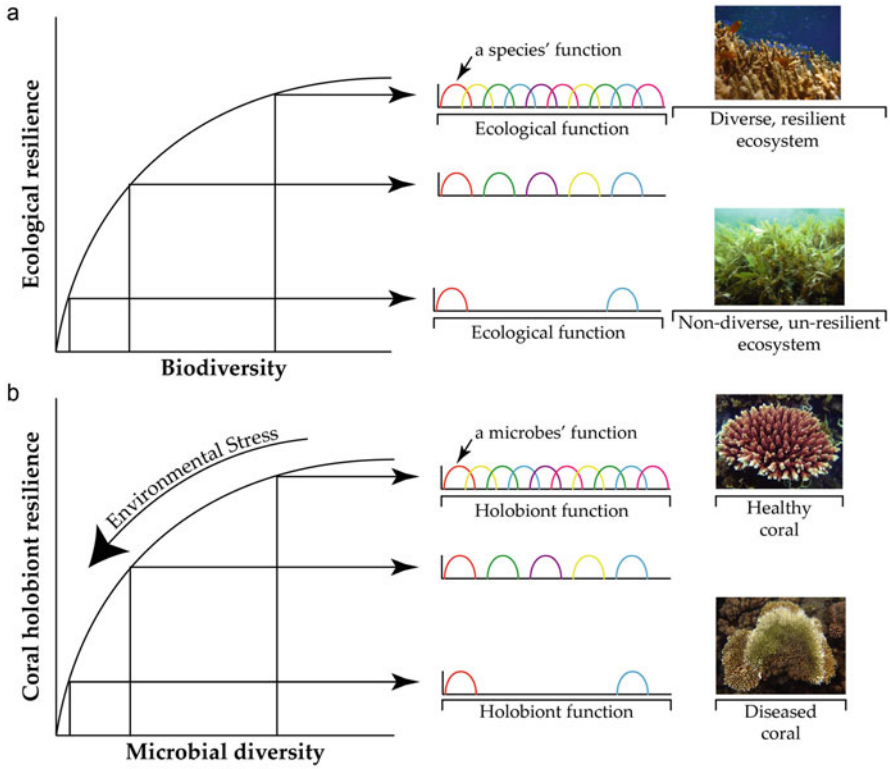

Fig. 1 (**a**) The "rivet hypothesis" is an ecological theory positing that biodiversity in an ecosystem creates functional redundancy and complementarity due to the limited number of niches available (Ehrlich and Ehrlich 1981). As a result of this functional overlap, biodiverse ecosystems tend to be more resilient to change, given that the loss of one or two species will not affect the ecosystem severely. (**b**) The structure and function of microorganisms and the microbial community within the coral holobiont could potentially mirror that of individual species within a biodiverse system, with the multitude of species and consequent overlap in function making the system more resilient. However, coral microbiomes can also be subjected to and respond to environmental stressors, potentially in ways that can reduce diversity and, in turn, coral host resiliency

(McDevitt-Irwin et al. 2017). Studies have also shown that despite their ability to form long-term stable symbioses with corals, microbial communities vary in their resistance and resilience based on the length and impact of environmental disturbances, thereby meeting the criteria for both *variability* and *monotonicity* (Glasl et al. 2017). It is worth noting that not all coral microbiomes can serve as adequate bioindicators. Coral microbiomes vary by species, regions, temperatures, and depths (Apprill et al. 2016). To serve as suitable indicators for changes in reef ecosystems, specific coral-associated microorganisms (and/or the combined microbiome) must have intermediate resiliency: too much sensitivity or too much resistance will not provide useful data on water quality and reef changes.

Using coral microbiomes as proxies for reef health has many advantages over traditional monitoring methods and the use of corals themselves in the context of *practicality*, including ease of sampling, availability, and an abundance of data generated. Coral microbiomes can be sampled simply by extracting a small section of a coral, in the form of tissue, mucus, skeleton, or all three, which is a relatively cheap and rapid process. Mucus in particular can be sampled without inflicting significant harm on the coral, and mucus-associated bacteria show greater responses to environmental changes than those associated with the skeleton or tissue (Pollock et al., in review). Each sample yields a wealth of data, providing information on microbial diversity, community structure, abundance, and function, all of which can be used as proxies for overall reef health. Microbiomes are also undoubtedly *relevant* as bioindicators, due to their intimate connection to reefs and, in turn, their importance for overall reef and ecosystem health. While microbial samples from the water column and sediments can serve as adequate indicators of environmental changes, the microbiome of corals themselves can serve as an even more accurate proxy for environmental health. Their intimate connection with the reef system, the specificity of their responses to change, and their potential to provide predictive snapshots of a reef ecosystem's health all point to their potential for successful implementation as bioindicators.

4 Tools Available for Microbiome Bioindicator Monitoring

As the field of microbiology, particularly microbial ecology, continues to grow, so does the list of tools available for the analysis of microbial communities, which currently include cultivation, 16S and 18S tag sequencing, metagenomics, and metatranscriptomics (Fig. 2). Historically, studies using bacteria for monitoring of environmental health relied heavily on cultivation techniques (Cardenas and Tiedje 2008). For example, culture-based detection of fecal coliforms is a well-established and widely employed bioindicator of human fecal contamination in water bodies (Tan et al. 2015). Studies focusing on microbial communities in reef ecosystems have used cultivation techniques for the purposes of taxonomic identification of microbes present in a sample and genetic sequence retrieval (Haygood et al. 1999). Culture-based methods are relatively cheap and straightforward, and they allow for the targeting of specific bacteria for growth and analysis via specialized media. This is beneficial when targeting a particular bioindicator species for analyses of presence and abundance. However, in order for this method to be successful, the microorganisms chosen for analysis must be good indicators, and the specificity of this method does not allow for the discovery of other potential bioindicators. Furthermore, given that only around 1% bacteria are cultivable, the pool of potential culture-based bioindicators is quite limited. Culture-based methods also cannot provide information on environmental processes in which the microorganisms being cultured may be involved or on their function and contribution to an ecosystem.

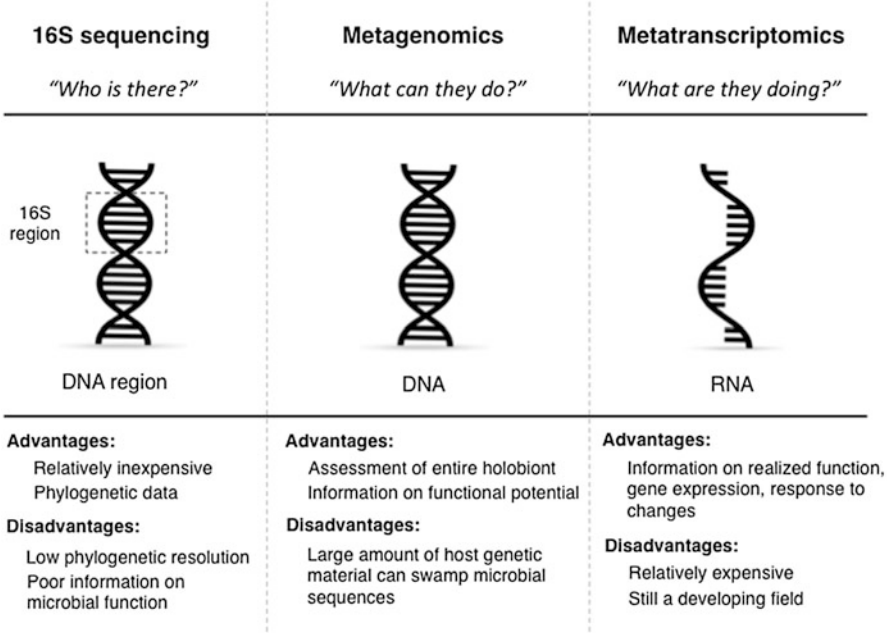

Fig. 2 Summary of the advantages and disadvantages of different sequencing tools available for analysis of microbial samples. Each tool can serve to answer different questions regarding the microbial community present in a sample and provide different levels of information

Amplicon-based sequencing of phylogenetically conserved genetic regions is often preferred over culture-based techniques due to the large amount of information it can provide on microbial life in an ecosystem. The processes of ribosomal DNA tag sequencing (e.g., 16S, 18S, and ITS) from coral tissue samples allow for the targeting of a particular genetic region for amplification and subsequent sequencing in order to identify the organisms present in a sample. The small subunit rRNA gene, also known as 16S in prokaryotes, is about 1,500 base pairs in length and is highly conserved, enabling amplification across most bacterial taxa (Janda and Abbott 2007). The eukaryotic counterpart is the 18S rRNA gene, which has similar features and can be used for the identification of microbial eukaryotes (Tan et al. 2015). In contrast, the ITS2 (internal transcribed spacer 2) region can be used for more targeted identification of *Symbiodinium*, a genus of symbiotic algae that form integral symbioses with corals (LaJeunesse 2001). Due to their low cost and high data yield, tag sequencing techniques have emerged as a cornerstone of microbiome analysis. These cultivation-independent methods are particularly popular in microbiome analyses of coral samples, and have been used to identify bacteria associated with specific species of coral, determine how bacterial communities change when corals are diseased, and analyze the link between bacterial communities and environmental stress (Babu et al. 2004; Ben-Haim et al. 2003; Bourne and Munn 2005; Cooney et al. 2002; Sunagawa et al. 2009; Ziegler et al. 2017).

High-throughput tag sequencing techniques allow for the discovery of new potential indicators and analysis of indicative bacterial responses to changes in the environment and stress, such as shifts in overall diversity and community structure. While this method is an improvement from culturing, in that it provides information on all bacteria present in a sample rather than a small subset, it still has its drawbacks; this method does not allow direct assessment of bacterial function, nor does it provide a high level of phylogenetic resolution. It also does not account for the possibility that bacteria grouped into a single operational taxonomic unit (OTU) (i.e., microbes with similar sequences) may actually be acting as separate functional entities in the coral holobiont.

To answer questions about microbial functional potential, researchers have turned to metagenomics. While tag sequencing targets a relatively short stretch of the microbial genome, metagenomic approaches provide data on the entire genetic repertoire present in a given sample (Cardenas and Tiedje 2008). Metagenomic approaches, therefore, provide insights into the functional potential of all organisms within a sample (Cardenas and Tiedje 2008; Streit and Schmitz 2004). Unlike 16S or 18S sequencing, which only characterizes either prokaryotes or eukaryotes, respectively, metagenome analysis characterizes all genes in a sample regardless of their phylogenetic origins (Ainsworth et al. 2010). Metagenomic analyses have been used to detect taxonomic, functional, and metabolic shifts in stressed and diseased corals (Kimes et al. 2010; Littman et al. 2011; Vega Thurber et al. 2009). For example, metagenomic approaches have uncovered that increases in temperature, excess nutrients, carbon loading, and reduced pH can lead to the increase of microbial genes involved in virulence, stress resistance, and sulfur metabolism (Vega Thurber et al. 2009). By providing a more holistic perspective on the genetic potential of the coral microbiome, metagenomic analyses can increase the accuracy of microbial indicators as well as the scope of detectable responses. Advances in metagenome analysis software have expanded the accessibility of metagenomics tools, allowing for rapid identification of draft genomes and intuitive inference of microbial population dynamics (Eren et al. 2015). Metagenomic approaches also come with their own technical challenges, as metagenomes extracted from coral samples tend to be comprised of mostly host DNA. To be able to apply metagenomics to coral microbiome analyses effectively, since full genomes are available for only a handful of coral species, techniques must be developed to remove coral host signal. Metagenome analyses only reveal the genetic potential of microorganisms; genetic data gleaned from metagenomes can provide predictions of function, but cannot confirm that a microorganism is, in fact, performing that function, due to lack of protein and transcriptomic evidence.

More recent advances have led the field of microbiome monitoring toward metatranscriptomics tools and methods for the purposes of confirming microbial function and metabolism. While metagenomics tools only describe the functional potential of a microorganism, metatranscriptomics (i.e., sequencing of RNA to infer gene expression) provides information on realized function (Martinez et al. 2016). Gene expression elucidates function as well as interactions between microorganisms and their environment or host (Martinez et al. 2016). Using metatranscriptomes,

researchers across disciplines have been able to answer important questions about host-symbiont-environment interactions by identifying and quantifying stress-related function by revealing cross talk among hosts and their symbionts during environmental changes (Luo et al. 2015; Moitinho-Silva et al. 2014). This information is particularly relevant in the context of microbes as bioindicators, given their intimate connection with environmental changes and stressors and the multitude of changes in function and gene expression that could serve as indicators for environmental perturbation. Metatranscriptomic sequencing of white plague-diseased corals revealed "stress signatures" in the bacterial community, in this case characterized by an increased abundance of proteins associated with DNA repair as well as an enrichment of genes associated with virulence and antibiotic resistance (Daniels et al. 2015). Researchers also observed metabolic shifts, specifically toward glucogenesis, ammonia assimilation, and sulfur assimilation (Daniels et al. 2015). Such results provide insight to differences in microbial function during periods of stress and disease and suggest that these changes may also have downstream effects on coral and/or ecosystem health. While this remains an emerging field, it holds the promise of elucidating the complex interactions that occur in the microbiome and strengthening the potential of coral microbiomes as bioindicators of reef health (Cardenas and Tiedje 2008).

5 Challenges and Future Directions

Despite the promise they show as bioindicators, the study of microorganisms still presents significant challenges. While the abundance of microbes in reef ecosystems provides many advantages, it can also complicate interpretation of the cause-and-effect relationship between shifts and environmental changes. While a shift in diversity, function, structure, or abundance might be directly related to a specific stressor or change, it could also result from a number of other factors. Coral species often differ significantly in their microbial assemblages and responses to ecosystem changes, providing different "normal" microbial baselines and bioindicative responses. Corals also contain PCR inhibitors, which can make amplicon-based analyses challenging. Host genetic sequences can also be substantially more abundant than microbial sequences, particularly in metagenomic studies. This can make it difficult to target and separate out microbial sequences for analysis. These hurdles hinder our ability to create microbial baselines, determine microbial specificity to environmental changes, and properly analyze microbial data, all of which are necessary when dealing with bioindicators. As a result, they represent significant roadblocks in the process of developing microbial bioindicator-based monitoring systems.

In addition to these technical challenges, an incomplete yet growing knowledge of the ecological and biological underpinnings of microbiomes limits our ability to effectively use microbiomes as bioindicators. Though microbiomes have been studied extensively in diverse systems, research on the microorganisms associated

with corals is still in its infancy. Limited understanding of baseline diversity, community structure, and function across different coral clades and species prevents the development of robust bioassays. Many innovative projects are attempting to tackle this problem by establishing "core" microbial members of the coral holobiont, information that is necessary when attempting to study and connect environmental changes to shifts in the coral microbiome (Ainsworth et al. 2015). Other studies are targeting specific microbial responses to stressors, using tools such as tag sequencing, metagenomics, and metatranscriptomics in order to draw clearer and more specific connections between stresses and microbial stress responses (Lee et al. 2016; Mouchka et al. 2010; Vega Thurber et al. 2009). Others still are attempting to identify the specific location of different microbial members within coral microhabitats (e.g., physiologically distinct compartments such as the skeleton, mucus, and tissue), in order to gain a better understanding of the functions of specific microorganisms within the coral host (Ainsworth et al. 2015). Glasl et al. (2017) outline the importance of such research for management efforts that seek to incorporate microorganisms as bioindicators and provide a detailed list of steps and measures that must be taken to successfully implement microbes as bioindicators.

The staggering rate of advancement in the field of genomics, particularly single-cell genomics (considered a new frontier in the "omics" fields), has great potential to expand our understanding of the roles of specific microorganisms in the coral holobiont (Wang and Bodovitz 2010). Current approaches to study the function of entire microbial communities (such as metagenomics or metatranscriptomics) are unable to provide functional information for potentially key microorganisms which may be less abundant and thus may receive lower or no coverage within the metagenome. Single-cell genomics can fill this gap, as it can be used to elucidate a fuller picture of a single uncultivated microorganism by providing its complete genome and thus full functional potential, and a small number of studies have already begun to implement this tool in both sponge and coral systems (Hentschel et al. 2012; Kamke et al. 2013; Pernice et al. 2012; Siegl et al. 2011). Similarly, proteomic approaches are beginning to gain traction, delving deeper into the roles of proteins in host-microbe interactions (de O Santos et al. 2011). Machine learning, another technological advancement referring to the development of algorithms to identify patterns and make predictions based on existing data, has also recently been applied to biological sciences and has great potential as a way to recognize previously invisible patterns in many different types of microbiome datasets (Tarca et al. 2007).

While a substantial gap remains between our current understanding of coral microbiomes and the knowledge base required to systematically and effectively employ microorganisms as bioindicators, continued technological advances and innovative studies are rapidly narrowing this gap.

6 Conclusion

As coral reefs continue to decline worldwide, it is imperative that we develop robust monitoring methods for the rapid identification of environmental changes driving coral loss. Such monitoring methods must be highly sensitive and provide information on whole ecosystem health in the face of changes in water quality and stressors. Given their intimate connection to corals and their ability to respond to a diverse array of stressors, coral microbiomes hold great potential as bioindicators of reef health. A system of microbial bioindicators would beautifully complement current reef monitoring methods that focus more on coral colony and macroorganismal health. Microbial bioindicators' sensitivity to alterations in the environment also shows that they have the potential to provide specific information on ecosystem changes that could serve as an early-warning system for effective adaptive management. Such a breakthrough could revolutionize reef conservation, as well as conservation efforts in other systems that aim to employ microorganisms for the development of more sensitive and accurate monitoring systems. Ultimately, the use of microbial bioindicators for the purposes of monitoring reef ecosystem health would aid conservation efforts significantly by providing more accurate information on the fluxes and changes occurring in the reef environment, allowing for more informed management decisions in the future.

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References

AIMS. Australian Institute of Marine Science Annual Report 2012–13. Townsville: Australian Institute of Marine Science; 2013.

Ainsworth TD, Thurber RV, Gates RD. The future of coral reefs: a microbial perspective. Trends Ecol Evol. 2010;25:233–40.

Ainsworth TD, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, Gates RD, Padilla-Gamiño JL, Spalding HL, Smith C, et al. The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. ISME J. 2015;9:2261–74.

Apprill A, Weber LG, Santoro AE. Distinguishing between microbial habitats unravels ecological complexity in coral microbiomes. mSystems. 2016;1:e00143–16.

Babu TG, Nithyanand P, Kannapiran E, Ravi AV, Pandian KS. Molecular identification of bacteria associated with the coral reef ecosystem of Gulf of Mannar Marine Biosphere Reserve using 16S rRNA sequences. In: Proceedings national seminar on new frontiers in marine bioscience research. Karaikudi: Alagappa University; 2004. p. 47–53.

Ben-Haim Y, Thompson FL, Thompson CC, Cnockaert MC, Hoste B, Swings J, Rosenberg E. Vibrio coralliilyticus sp. nov., a temperature-dependent pathogen of the coral Pocillopora damicornis. Int J Syst Evol Microbiol. 2003;53:309–15.

Bispo A, Cluzeau D, Creamer R, Dombos M, Graefe U, Krogh PH, Sousa JP, Peres G, Rutgers M, Winding A, et al. Indicators for monitoring soil biodiversity. Integr Environ Assess Manag. 2009;5:717–9.

Bloem J, Breure AM. Microbial indicators. Trace Met Other Contam Environ. 2003;6:259–82.

Bouchet P. The exploration of marine biodiversity: scientific and technological challenges. In: Duarte CM, editor. The exploration of marine biodiversity: scientific and technological challenges. Bilbao: Fundación BBVA; 2006. p. 31–6.

Bourne DG, Munn CB. Diversity of bacteria associated with the coral Pocillopora damicornis from the Great Barrier Reef. Environ Microbiol. 2005;7:1162–74.

Bourne DG, Webster NS. Coral reef bacterial communities. In: The prokaryotes. Berlin: Springer; 2013. p. 163–87.

Brookes PC. The use of microbial parameters in monitoring soil pollution by heavy metals. Biol Fertil Soils. 1995;19:269–79.

Bruno JF, Selig ER, Casey KS, Page CA, Willis BL, Harvell CD, Sweatman H, Melendy AM. Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol. 2007;5: e124.

Burns A, Ryder DS. Potential for biofilms as biological indicators in Australian riverine systems. Ecol Manage Restor. 2001;2:53–64.

Cardenas E, Tiedje JM. New tools for discovering and characterizing microbial diversity. Curr Opin Biotechnol. 2008;19:544–9.

Cárdenas A, Rodriguez-r LM, Pizarro V, Cadavid LF, Arévalo-Ferro C. Shifts in bacterial communities of two Caribbean reef-building coral species affected by white plague disease. ISME J. 2012;6:502.

Cooney RP, Pantos O, Le Tissier MD, Barer MR, Bythell JC, et al. Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. Environ Microbiol. 2002;4:401–13.

Cooper TF, Gilmour JP, Fabricius KE. Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programmes. Coral Reefs. 2009;28:589–606.

Daniels C, Baumgarten S, Yum LK, Michell CT, Bayer T, Arif C, Roder C, Weil E, Voolstra CR. Metatranscriptome analysis of the reef-building coral Orbicella faveolata indicates holobiont response to coral disease. Front Mar Sci. 2015;2:62.

de O Santos E, Alves N Jr, Dias GM, Mazotto AM, Vermelho A, Vora GJ, Wilson B, Beltran VH, Bourne DG, Le Roux F. Genomic and proteomic analyses of the coral pathogen Vibrio coralliilyticus reveal a diverse virulence repertoire. ISME J. 2011;5:1471.

De'ath G, Fabricius K. Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecol Appl. 2010;20:840–50.

Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002;8:881–90.

Ehrlich P, Ehrlich A. Extinction: the causes and consequences of the disappearance of species. New York: Random House; 1981.

Eren AM, Esen ÖC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO. Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ. 2015;3:e1319.

Fabricius KE, Cooper TF, Humphrey C, Uthicke S, De'ath G, Davidson J, LeGrand H, Thompson A, Schaffelke B. A bioindicator system for water quality on inshore coral reefs of the Great Barrier Reef. Mar Pollut Bull. 2012;65:320–32.

Foissner W, Berger H. A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshw Biol. 1996;35:375–482.

Galloway-Peña JR, Smith DP, Sahasrabhojane P, Ajami NJ, Wadsworth WD, Daver NG, Chemaly RF, Marsh L, Ghantoji SS, Pemmaraju N, et al. The role of the gastrointestinal microbiome in infectious complications during induction chemotherapy for acute myeloid leukemia. Cancer. 2016;122:2186–96.

Gil-Agudelo DL, Smith GW, Garzón-Ferreira J, Weil E, Petersen D. Dark spots disease and yellow band disease, two poorly known coral diseases with high incidence in Caribbean reefs. In: Coral health disease. Berlin: Springer; 2004. p. 337–49.

Gladfelter WB. White-band disease in Acropora palmata: implications for the structure and growth of shallow reefs. Bull Mar Sci. 1982;32:639–43.

Glasl B, Webster NS, Bourne DG. Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. Mar Biol. 2017;164:91.

Hallock P, Lidz BH, Cockey-Burkhard EM, Donnelly KB. Foraminifera as bioindicators in coral reef assessment and monitoring: the FORAM index. In: Coastal monitoring through partnerships. Dordrecht: Springer; 2003. p. 221–38.

Harvell D, Jordán-Dahlgren E, Merkel S, Rosenberg E, Raymundo L, Smith G, Weil E, Willis B. Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanography. 2007;20:172–95.

Haygood MG, Schmidt EW, Davidson SK, Faulkner DJ. Microbial symbionts of marine invertebrates: opportunities for microbial biotechnology. J Mol Microbiol Biotechnol. 1999;1:33–43.

Hentschel U, Piel J, Degnan SM, Taylor MW. Genomic insights into the marine sponge microbiome. Nat Rev Microbiol. 2012;10:641.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, et al. Coral reefs under rapid climate change and ocean acidification. Science. 2007;318:1737–42.

Holling CS. Adaptive environmental assessment and management. New York: Wiley; 1978.

Holt EA, Miller SW. Bioindicators: using organisms to measure environmental impacts. Nat Educ Knowl. 2011;3:8.

Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol. 2007;45:2761–4.

Jessen C, Lizcano JFV, Bayer T, Roder C, Aranda M, Wild C, Voolstra CR. In-situ effects of eutrophication and overfishing on physiology and bacterial diversity of the Red Sea coral Acropora hemprichii. PLoS One. 2013;8:e62091.

Jones RJ, Muller J, Haynes D, Schreiber U. Effects of herbicides diuron and atrazine on corals of the Great Barrier Reef, Australia. Mar Ecol Prog Ser. 2003;251:153–67.

Kamke J, Sczyrba A, Ivanova N, Schwientek P, Rinke C, Mavromatis K, Woyke T, Hentschel U. Single-cell genomics reveals complex carbohydrate degradation patterns in poribacterial symbionts of marine sponges. ISME J. 2013;7:2287.

Kimes NE, Van Nostrand JD, Weil E, Zhou J, Morris PJ. Microbial functional structure of Montastraea faveolata, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. Environ Microbiol. 2010;12:541–56.

Klaus JS, Janse I, Heikoop JM, Sanford RA, Fouke BW. Coral microbial communities, zooxanthellae and mucus along gradients of seawater depth and coastal pollution. Environ Microbiol. 2007;9:1291–305.

Knowlton N, Rohwer F. Multispecies microbial mutualisms on coral reefs: the host as a habitat. Am Nat. 2003;162:S51–62.

Knowlton N, Brainard RE, Fisher R, Moews M, Plaisance L, Caley MJ. Coral reef biodiversity. In: Life in the world's oceans: diversity distribution and abundance. Chichester: Wiley; 2010. p. 65–74.

Kriwy P, Uthicke S. Microbial diversity in marine biofilms along a water quality gradient on the Great Barrier Reef. Syst Appl Microbiol. 2011;34:116–26.

LaJeunesse TC. Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus Symbiodinium using the ITS region: in search of a "species" level marker. J Phycol. 2001;37:866–80.

Lee ST, Davy SK, Tang S-L, Fan T-Y, Kench PS. Successive shifts in the microbial community of the surface mucus layer and tissues of the coral Acropora muricata under thermal stress. FEMS Microbiol Ecol. 2015;91:fiv142.

Lee ST, Davy SK, Tang S-L, Kench PS. Mucus sugar content shapes the bacterial community structure in thermally stressed Acropora muricata. Front Microbiol. 2016;7:371.

Leite D, Falcão Salles J, Calderon E, Castro CBE, Bianchini A, Marques J, Van Elsas JD, Peixoto R. Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. Front Microbiol. 2018;9:833.

Littman R, Willis BL, Bourne DG. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. Environ Microbiol Rep. 2011;3:651–60.
Liu J, Weinbauer MG, Maier C, Dai M, Gattuso J-P. Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning. Aquat Microb Ecol. 2010;61:291–305.
Luo B, Gu W, Zhong J, Wang Y, Zhang G. Revealing crosstalk of plant and fungi in the symbiotic roots of sewage-cleaning Eichhornia crassipes using direct de novo metatranscriptomic analysis. Sci Rep. 2015;5:15407.
Mages M, Óvári M, Tümpling WV, Kröpfl K. Biofilms as bio-indicator for polluted waters? Anal Bioanal Chem. 2004;378:1095–101.
Martinez X, Pozuelo M, Pascal V, Campos D, Gut I, Gut M, Azpiroz F, Guarner F, Manichanh C. MetaTrans: an open-source pipeline for metatranscriptomics. Sci Rep. 2016;6:26447.
McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL. Responses of coral-associated bacterial communities to local and global stressors. Front Mar Sci. 2017;4:262.
Mendes S, Azul AM, Castro P, Römbke J, Sousa JP. Protecting soil biodiversity and soil functions: current status and future challenges. In: Biodiversity and education for sustainable development. Berlin: Springer; 2016. p. 249–63.
Moitinho-Silva L, Seridi L, Ryu T, Voolstra CR, Ravasi T, Hentschel U. Revealing microbial functional activities in the Red Sea sponge Stylissa carteri by metatranscriptomics. Environ Microbiol. 2014;16:3683–98.
Morrow KM, Moss AG, Chadwick NE, Liles MR. Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. Appl Environ Microbiol. 2012;78:6438–49.
Mouchka ME, Hewson I, Harvell CD. Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts. Integr Comp Biol. 2010;50:662–74.
Mulhall M. Saving the rainforest of the sea: an analysis of international efforts to conserve coral reefs. In: Duke environmental law and policy forum, vol. 19; 2008. p. 321.
Nielsen MN, Winding A, Binnerup S, Hansen BM, Hendriksen NB, Kroer N. Microorganisms as indicators of soil health. Copenhagen: National Environmental Research Institute; 2002.
Nugues MM, Smith GW, Hooidonk RJ, Seabra MI, Bak RP. Algal contact as a trigger for coral disease. Ecol Lett. 2004;7:919–23.
Nwuche CO, Ugoji EO. Effects of heavy metal pollution on the soil microbial activity. Int J Environ Sci Technol. 2008;5:409–14.
Parmar TK, Rawtani D, Agrawal YK. Bioindicators: the natural indicator of environmental pollution. Front Life Sci. 2016;9:110–8.
Parry M, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE, et al. Climate change 2007: impacts, adaptation and vulnerability. Cambridge: Cambridge University Press; 2007.
Peixoto RS, Rosado PM, Leite DC, Rosado AS, Bourne DG. Beneficial microorganisms for corals (BMC): proposed mechanisms for coral health and resilience. Front Microbiol. 2017;8:341.
Pernice M, Meibom A, Van Den Heuvel A, Kopp C, Domart-Coulon I, Hoegh-Guldberg O, Dove S. A single-cell view of ammonium assimilation in coral–dinoflagellate symbiosis. ISME J. 2012;6:1314.
Phillips DJ, Rainbow PS. Biomonitoring of trace aquatic contaminants. Berlin: Springer; 2013.
Plaisance L, Caley MJ, Brainard RE, Knowlton N. The diversity of coral reefs: what are we missing? PLoS One. 2011;6:e25026.
Pollock FJ, Lamb JB, Field SN, Heron SF, Schaffelke B, Shedrawi G, Bourne DG, Willis BL. Sediment and turbidity associated with offshore dredging increase coral disease prevalence on nearby reefs. PLoS One. 2014;9:e102498.
Rogers CS, Garrison G, Grober R, Hillis Z-M, Franke MA. Coral reef monitoring manual for the Caribbean and Western Atlantic. Virgin Islands National Park: St. John; 1994.
Rohwer F, Seguritan V, Azam F, Knowlton N. Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser. 2002;243:1–10.

Röthig T, Ochsenkühn MA, Roik A, Van Der Merwe R, Voolstra CR. Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. Mol Ecol. 2016;25:1308–23.
Siegl A, Kamke J, Hochmuth T, Piel J, Richter M, Liang C, Dandekar T, Hentschel U. Single-cell genomics reveals the lifestyle of Poribacteria, a candidate phylum symbiotically associated with marine sponges. ISME J. 2011;5:61.
Sims A, Zhang Y, Gajaraj S, Brown PB, Hu Z. Toward the development of microbial indicators for wetland assessment. Water Res. 2013;47:1711–25.
Sobolev D, Begonia M. Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. Int J Environ Res Public Health. 2008;5:450–6.
Stone D, Ritz K, Griffiths BG, Orgiazzi A, Creamer RE. Selection of biological indicators appropriate for European soil monitoring. Appl Soil Ecol. 2016;97:12–22.
Streit WR, Schmitz RA. Metagenomics – the key to the uncultured microbes. Curr Opin Microbiol. 2004;7:492–8.
Sunagawa S, DeSantis TZ, Piceno YM, Brodie EL, DeSalvo MK, Voolstra CR, Weil E, Andersen GL, Medina M. Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral Montastraea faveolata. ISME J. 2009;3:512–21.
Tan B, Ng CM, Nshimyimana JP, Loh L-L, Gin KY-H, Thompson JR. Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges, and future opportunities. Front Microbiol. 2015;6:1027.
Tarca AL, Carey VJ, Chen X, Romero R, Drăghici S. Machine learning and its applications to biology. PLoS Comput Biol. 2007;3:e116.
Thakur RK, Jindal R, Singh UB, Ahluwalia AS. Plankton diversity and water quality assessment of three freshwater lakes of Mandi (Himachal Pradesh, India) with special reference to planktonic indicators. Environ Monit Assess. 2013;185:8355.
Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale E, Kelly L, Rohwer F. Metagenomic analysis of stressed coral holobionts. Environ Microbiol. 2009;11:2148–63.
Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob Chang Biol. 2014;20:544–54.
Wang D, Bodovitz S. Single cell analysis: the new frontier in “omics”. Trends Biotechnol. 2010;28:281–90.
Wei H, Dong L, Wang T, Zhang M, Hua W, Zhang C, Pang X, Chen M, Su M, Qiu Y, et al. Structural shifts of gut microbiota as surrogate endpoints for monitoring host health changes induced by carcinogen exposure. FEMS Microbiol Ecol. 2010;73:577–86.
Witt V, Wild C, Uthicke S. Terrestrial runoff controls the bacterial community composition of biofilms along a water quality gradient in the Great Barrier Reef. Appl Environ Microbiol. 2012;78:7786–91.
Yergeau E, Lawrence JR, Sanschagrin S, Waiser MJ, Korber DR, Greer CW. Next-generation sequencing of microbial communities in the Athabasca River and its tributaries in relation to oil sands mining activities. Appl Environ Microbiol. 2012;78:7626–37.
Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE, McMinds R, Payet JP, Welsh R, Correa AM, Lemoine NP, Rosales S, et al. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat Commun. 2016;7:11833.
Zaneveld JR, McMinds R, Vega TR. Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nat Microbiol. 2017;2:17121.
Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR. Bacterial community dynamics are linked to patterns of coral heat tolerance. Nat Commun. 2017;8:14213.

Part III
Genetic Diversity, Population Structure, and Biogeography

Population Genomics of Marine Zooplankton

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Abstract The exceptionally large population size and cosmopolitan biogeographic distribution that distinguish many – but not all – marine zooplankton species generate similarly exceptional patterns of population genetic and genomic diversity and structure. The phylogenetic diversity of zooplankton has slowed the application of population genomic approaches, due to lack of genomic resources for closely related species and diversity of genomic architecture, including highly replicated genomes of many crustaceans. Use of numerous genomic markers, especially single nucleotide polymorphisms (SNPs), is transforming our ability to analyze population genetics and connectivity of marine zooplankton, and providing new understanding and different answers than earlier analyses, which typically used mitochondrial DNA and microsatellite markers. Population genomic approaches have confirmed that, despite high dispersal potential, many zooplankton species exhibit genetic structuring among geographic populations, especially at large ocean-basin scales, and have revealed patterns and pathways of population connectivity that do not always track ocean circulation. Genomic and transcriptomic resources are critically needed to allow further examination of micro-evolution and local adaptation, including identification of genes that show evidence of selection. These new tools will also enable further examination of the significance of small-scale genetic heterogeneity of marine zooplankton, to discriminate genetic "noise" in large and patchy populations from local adaptation to environmental conditions and change.

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1 Introduction

1.1 Introduction to Population Genomics

Population genomic approaches entail simultaneous sampling of numerous variable loci within a genome and allow inference of locus-specific effects (Baird et al. 2008). These powerful new techniques are transforming our understanding of the population genetics, connectivity, demographic history, and local adaptation of marine organisms (Crawford and Oleksiak 2016; Pogson 2016). Genotyping hundreds to thousands of genetic markers for multiple individuals across populations or species has enabled the identification of selectively neutral markers that can be used for a wide variety of analyses (Luikart et al. 2003; Baird et al. 2008). Discrimination of statistical "outlier" loci allows examination of the impacts of selection and evidence of local adaptation (Stapley et al. 2010). Whole-genome analysis of non-model organisms has enabled new insights into underlying evolutionary forces. However, significant challenges remain for whole-genome analysis of non-model organisms, thus necessitating and encouraging broad use of approaches that require little or no prior genomic data. These include reduced-representation genomic DNA libraries (Reitzel et al. 2013), genotyping-by-sequencing (GBS) (Elshire et al. 2011), and exon-capture (Hodges et al. 2007; De Wit et al. 2015; Jones and Good 2016), although the latter requires prior knowledge of gene architecture. In broad view, population genomic approaches have enormous potential to yield significant new understanding of the ecological and evolutionary dynamics of zooplankton and other marine organisms.

1.2 Introduction to Marine Zooplankton

1.2.1 Biodiversity

The marine zooplankton assemblage includes ~6,000 described species of holoplanktonic metazoan organisms that complete their entire cycle in the water column (Wiebe et al. 2010). The phylogenetic diversity of this assemblage is impressive, with 11 phyla and 27 orders represented (Bucklin et al. 2010b). However, these numbers most likely markedly underestimate the actual biodiversity – perhaps by several orders of magnitude – due to the presence of cryptic variation within geographically widespread species or sibling species swarms, as well as undiscovered species in under-sampled or explored habitats (Bucklin et al. 2010a; Beaugrand 2017).

Molecular approaches, including DNA barcoding and metabarcoding, are providing important new insights into this "hidden diversity" of marine zooplankton (Lindeque et al. 2013; Bucklin et al. 2016).

1.2.2 Biogeography

Global patterns of zooplankton biogeographic distributions have been well-characterized for the epipelagic (0–200 m) zone (Longhurst 2007). The many classical studies form a basis for ongoing examination of climate-driven range changes and regime shifts (de Young et al. 2008). In contrast, the deep ocean, including the mesopelagic (200–1,000 m) and bathypelagic (1,000–4,000 m), remains under-sampled and poorly known (but see Wiebe et al. 2010; Laakmann et al. 2012). Many species exhibit cosmopolitan distributions, with ranges spanning multiple ocean basins and broad latitudinal ranges (Peijnenburg and Goetze 2013). However, there are many exceptions to this oversimplified description, likely resulting from specific habitat requirements, restricted gene flow, or relict populations (Chust et al. 2016). Further complicating analysis of species distributional patterns are rather characteristic high ratios of local-to-global species diversity; a net sample from oceanic waters may contain hundreds of species of copepods or ~10% of the global total (Kuriyama and Nishida 2006).

1.2.3 Life History

Many zooplankton species have life histories entailing multiple stages with different microhabitat preferences and requirements. Some exhibit alternation of sexual and asexual generations. Most are relatively short-lived organisms, with generation spans from several months to a couple of years. As a group, marine zooplankton are useful indicators of impacts of environmental variability or climate change, since they are rapid responders in terms of species distribution and abundance. The exceptional diversity of marine zooplankton – in terms of phylogenetic biodiversity, pelagic biogeography, and life history variation – provide a unique opportunity to examine ecological and evolutionary genomic responses. This review will summarize new knowledge resulting from population genomic examination of the genetic diversity and structure, phylogeography and connectivity, demographic history, and local adaptation of marine metazoan holozooplankton.

1.3 Genomic Resources for Marine Zooplankton

1.3.1 Published Genomic Resources

It can be argued that there are no universally accepted model species among the marine zooplankton; in many cases, there are no closely related model organisms to which extrapolations or comparisons can be made (Ellegren 2014). However, the number of marine zooplankton species targeted for genome-scale studies is growing, including species ranging phylogenetically from the Cnidaria to the Urochordata and including ecologically important or keystone species for some pelagic ecosystems, such as the Southern Ocean salp, *Salpa thompsoni* (Jue et al. 2016) (Table 1).

For the most part, marine zooplankton species targeted for reference sequencing and assembly have been identified by their impact on comparative genomic studies or as part of larger genome consortia. An example of this latter group is the genome sequence for the copepod *Eurytemora affinis*, a species targeted for sequencing as part of the i5K Pilot Project aimed at sequencing 28 arthropod genomes (i5K Consortium 2013; Eyun et al. 2017). Currently, assembled genomes are available for species representing only a snapshot of some of the major lineages of eukaryotes and a small sampling of the species diversity of the pelagic realm (Table 1). A significant factor in the identification of a target species for a genome assembly effort is the estimated genome size. Notably, all the reference genomes available are from organisms whose genome size estimates are significantly smaller than 1 GB, presumably since the depth of coverage required is low enough to represent a feasible investment of resources in terms of fiscal and computational effort. While reference quality assemblies are ideal (e.g., *Oikopleura dioica*; Denoeud et al. 2010), lower coverage assemblies can still provide a high enough N50 value (i.e., the weighted median statistic such that 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value) to afford extensive gene predictions (e.g., Jue et al. 2016).

Recently, mining genome databases such as NCBI and the SRA (Short Read Archive) for partial genome sequences has afforded broader comparisons among species lacking a fully assembled genome. For example, a newly derived reference for the common estuarine copepod *E. affinis* was compared to short-read genomic sequence data from two other copepods, the freshwater cyclopoid copepod, *Mesocyclops edax* (SRX246444 and SRX246445; Sun et al. 2014) and the North Atlantic copepod, *Calanus finmarchicus* (SRX456026; Smolina et al. 2014), revealing species-specific adaptations of the chemosensory related gene families to environments (Eyun et al. 2017).

1.3.2 Genome Size in the Zooplankton

The average estimated genome sizes (haploid nuclear DNA contents) of holoplankton species are in general far above 1 GB (Fig. 1) and vary more than

Table 1 Holozooplankton species for which genome assemblies and accompanying statistics are publicly available (as of June 2017)

	Tunicates			Ctenophores		Copepods			
	Oikopleura dioica								
	Main assembly	Allelic assembly	*Salpa thompsoni*	*Pleurobrachia bachei*	*Mnemiopsis leidyi*	*Eurytemora affinis*	*Oithona nana*	*Tigriopus kingsejongensi*	*Tigriopus californicus*
Assembly name	ASM20953v1	ASM20955v1	Salp genome 1.0	P.bachei_draft_genome_v.1.1	GCA_000226015.1	Eaff_1.0	O. Nana v.1.0	NA	TCALIF_v1.0
Estimated genome size (MB)	68.46	Allelic assembly	602[a]	170	150	616.14[b]	85	298	245[c]
Assembly size	70,471,451	45,141,193	318,747,957	156,121,975	155,865,547	494,890,867	85,010,107	305,712,242	184,634,130
Predicted protein-coding genes	18,020	18,020	13,186	19,523	16,548	29,783	15,359	12,772	14,536
Coverage	14×	N/A[d]	20×	200×	160×	75×	N/A[d]	65×	N/A[d]
Number of scaffolds	1,260	4,196	478,281	21,979	5,100	6,899	4,626	27,823	2,365
Length of N50 scaffold (bp)	395,387	21,890	934	20,628	187,314	862,645	400,614	159,218	298,012
Number of N50 scaffold (L50)	35	478	79,492	1,646	242	163	60	N/A[d]	180
Number of contigs	5,917	6,678	590,021	38,864	24,884	122,625	7,437	48,368	26,175
Length of N50 contig (bp)	24,932	10,847	636	6,132	11,936	5,738	38,620	17,566	14,799
Number of N50 contig	718	985	136,534	6,078	3,653	19,338	463	N/A[d]	3,352
Length of gaps (bp)	3,938,358	2,655,217	14,945,692	19,276,734	5,525,119	107,316,113	2,943,785	10,474,460	N/A[d]
References	Denoeud et al. (2010)	Denoeud et al. (2010)	Jue et al. (2016)	Moroz et al. (2014)	Ryan et al. (2013)	Eyun et al. (2017)	Madoui et al. (2017)	Kang et al. (2017)	https://i5k.nal.usda.gov/Tigriopus_californicus

Estimated genome sizes are based on assembly statistics unless otherwise noted
[a]Genome size estimate independent of assembly (Jue et al. 2016)
[b]Genome size estimate independent of assembly (Rasch et al. 2004)
[c]Genome size estimate independent of assembly (Wyngaard and Rasch 2000)
[d]Not available/not provided

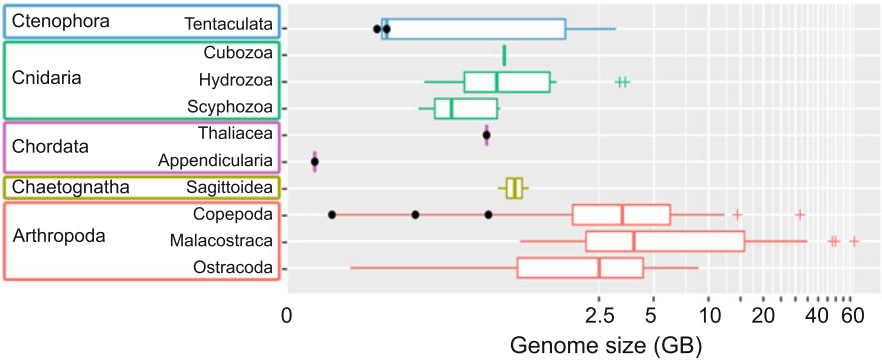

Fig. 1 Distribution of estimated genome sizes in representative holozooplankton phyla. Black dots indicate sequenced genomes. Genome size estimations are from Gregory (2017), Jeffery et al. (2017), Leinaas et al. (2016), Ryan et al. (2013), Moroz et al. (2014), and Madoui et al. (2017)

900-fold, from 0.07 GB in *O. dioica* (Appendicularia) to 63.2 GB in *Ampelisca macrocephala* (Amphipoda). Variation of genome sizes in marine zooplankton is especially large within the Copepoda with >370-fold variation among species (Leinaas et al. 2016; Madoui et al. 2017) followed by Ostracoda and Malacostraca with around 80-fold and 70-fold variation of genome size among species, respectively (Gregory 2017; Jeffery et al. 2017). To date, genome size has been investigated for 115 species of zooplankton, with poor representation of important phyla, including Chaetognatha, Cnidaria, Ctenophora, Mollusca, and Chordata.

Several trends or patterns are emerging from genomic analyses of crustaceans, although only a few species have been studied to date. First, a positive relationship between genome size (C-value) and body size has been observed in copepods (McLaren et al. 1988; Wyngaard and Rasch 2000), amphipods (Hessen and Persson 2009), and ostracods (Jeffery et al. 2017). However, there is considerable variability in genome size both among species of similar body size (Gregory et al. 2000; Leinaas et al. 2016) and within species due to environmental conditions (McLaren et al. 1988; Escribano et al. 1992; Leinaas et al. 2016). Second, genome size has been associated with specific habitats and environmental conditions. Marine crustaceans are likely to have larger genomes than freshwater and terrestrial ones (Jeffrey 2015; Alfsnes et al. 2017); within the marine realm, polar species tend to have larger genomes compared to temperate species (Hessen and Persson 2009; Jeffrey 2015; Leinaas et al. 2016). Jeffrey (2015) hypothesizes that such large genomes may result from the expansion of transposable elements and other repetitive elements, due to relaxed selection for rapid development or reduced constraints on body size in predictable and stable marine polar environments, compared to more fluctuating environments.

Causes and mechanisms of genome size variability and particularly expansion of genome sizes are still not known. Among eukaryotes, genome size is positively correlated with gene number, average intron size, and the number of introns per genome (Elliott and Gregory 2015). The main drivers of genome size expansion are

suggested to be whole-genome duplication (polyploidization) or partial duplication events and proliferation of noncoding elements (Dufresne and Jeffery 2011).

Information on genome size, genome sequence, and karyotype is sparse in marine zooplankton, limiting our understanding of genome evolution. Nevertheless, evidence from insects and crustaceans suggest that accumulation of transposable and repetitive elements may be the primary contributor to their large genome sizes (Alfsnes et al. 2017), while polyploidization is probably not the most common driver of genome evolution in zooplankton (Gregory and Hebert 1999). For example, species of the copepod genera *Calanus* and *Pseudocalanus* exhibit quantum shifts in genome size (C-values) within each genus but share similar chromosome complements (McLaren et al. 1989).

Partial duplication or amplification of genomic regions may be common in large genomes of zooplankton, particularly for ribosomal rDNA and protein-coding genes. Among eukaryotes, rDNA copy number correlates positively with genome size (Prokopowich et al. 2003). For species of *Calanus*, 18S rDNA gene copy number has been found to approximately double between *C. finmarchicus* (15,300 copies; 2C = 12.95 pg) and *Calanus glacialis* (33,500 copies; 2C = 24.20 pg; Wyngaard et al. 1995). Transcriptomic analysis has indicated the presence of multi-copy gene families originating from multiple duplications of an ancestral gene in copepods (Lenz et al. 2014; Yang et al. 2014), euphausiids (Toullec et al. 2013; Sales et al. 2017), and pteropods (Maas et al. 2015; Thabet et al. 2017).

1.3.3 Mitochondrial Genomes

Fragments of mitochondrial DNA were among the first molecular tools used to tackle questions related to zooplankton species identification, phylogenetics, phylogeography, and population genetics. For example, the cytochrome oxidase subunit I is preferentially used as a barcode for metazoans (Schindel and Miller 2005) and has been used frequently for marine zooplankton (Bucklin et al. 2007, 2010a, 2011; Blanco-Bercial et al. 2014).

Recent technological advances are allowing routine sequencing of whole mitochondrial genomes (mitogenomes), with marked increase in the power of phylogenetic and phylogeographic analyses compared to use of short mtDNA sequences. Applications such as shotgun sequencing of genomic DNA using high throughput sequencing technologies afford opportunities to capture other genomes that may be resident within a sample, such as mitochondrial DNA. Given the smaller target genome size (12–20 KB), mitogenomes are easier to subsample from larger datasets or to assemble using a PCR-build approach (Maricic et al. 2010; Hahn et al. 2013; Kollias et al. 2015).

Mitogenomics is a promising field of research that will contribute new insights into the phylogenetic history and evolution of planktonic species. For example, sequencing the mitogenome of the chaetognath, *Spadella cephaloptera*, allowed resolution of the phylogenetic position of the chaetognaths within Protostome lineages (Papillon et al. 2004). Only a few mitogenomes have been published thus

far – especially when the species diversity of zooplankton is considered – and within those, unexpected features appear to be more common than previously thought. Mitogenomes are publicly available for a number of ecologically important species representing diverse phylogenetic lineages of marine zooplankton (Table 2), and additional complete mitochondrial assemblies may be found within incompletely explored genomic data. Nonetheless, the sequencing and assembly of complete mitogenomes of marine zooplankton species has progressed at a much slower pace than for other vertebrate groups (Genome 10K Community of Scientists 2009; GIGA Community of Scientists 2014).

Table 2 Mitochondrial genomes available for marine zooplankton species, with corresponding lengths

Taxon and species	Citation	Length (bp)
Copepoda		
Calanus finmarchicus	Weydmann et al. (2017)	>29,462
Calanus glacialis	Weydmann et al. (2017)	>27,342
Calanus hyperboreus	Kim et al. (2013)	17,910
Calanus sinicus	Minxiao et al. (2011)	>20,460
Paracyclopina nana	Ki et al. (2009)	15,981
Tigriopus californicus	Burton et al. (2007)	14,600
Tigriopus japonicus	Machida et al. (2002)	14,628
Tigriopus sp.	Jung et al. (2006)	14,301
Euphausiacea		
Euphausia pacifica	Shen et al. (2011)	16,898
Euphausia superba	Shen et al. (2010)	>15,498
Ostracoda		
Vargula hilgendorfii	Ogoh and Ohmiya (2004)	15,923
Amphipoda		
Onisimus nanseni	Ki et al. (2010)	14,734
Decapoda		
Acetes chinensis	Kim et al. (2012)	15,740
Cnidaria		
Aurelia aurita	Shao et al. (2006)	16,937
Cassiopea frondosa	Kayal et al. (2011)	15,949
Chrysaora quinquecirrha	Hwang et al. (2014)	16,775
Ctenophora		
Mnemiopsis leidyi	Pett et al. (2011)	10,000
Pleurobrachia bachei	Kohn et al. (2012)	11,016
Chaetognatha		
Sagitta decipiens	Miyamoto et al. (2010)	11,121
Sagitta enflata	Miyamoto et al. (2010)	12,631
Sagitta ferox	Li et al. (2016)	12,153
Sagitta nagae	Miyamoto et al. (2010)	11,459
Paraspadella gotoi	Helfenbein et al. (2004)	11,423
Pterosagitta draco	Wei et al. (2016)	10,426
Spadella cephaloptera	Papillon et al. (2004)	11,905

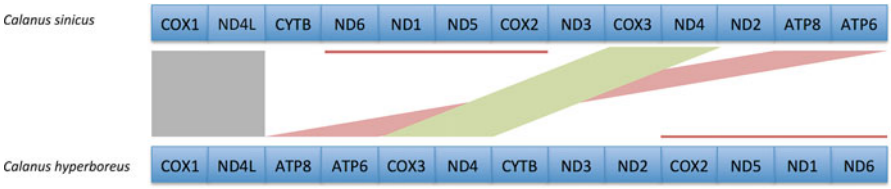

Fig. 2 Comparison of the mitochondrial gene order between *Calanus sinicus* and *Calanus hyperboreus*. Only the 13 protein-coding genes are represented. Rectilinear shapes show genes for which the order is conserved between the two species; red lines indicate genes with the same sequence but in reverse order between the species

In animals, the mitogenome is relatively well conserved, with 36 or 37 genes, including two for rRNAs, 22 for tRNAs, and 12 or 13 for protein-coding genes. The mitogenomes available for marine zooplankton species indicate a general trend of high intra- and interspecific variability. Rearrangement of gene order is exceptionally common and has been documented in amphipods (Ki et al. 2010) and ctenophores (Kohn et al. 2012), with some of the genes relocated to the nuclear genome (Pett et al. 2011). Copepods also show marked variability among congeneric species and among genera (Fig. 2; Jung et al. 2006; Minxiao et al. 2011). The most exceptional cases of mitochondrial variability documented to date are in the chaetognaths, *S. cephaloptera* and *Sagitta elegans*, for which natural populations exhibit unprecedented levels of intraspecific divergence (Marlétaz et al. 2017).

The variability observed in the mitogenomes of different species/lineages is also apparent in the gene content and size of these mitogenomes (Table 2). The smallest mitogenome reported is the ctenophore, *Mnemiopsis leidyi*, with only 10 kb, which is missing 25 genes (Pett et al. 2011). Within the chaetognaths, mitogenomes are also very reduced compared to other metazoans, missing several common genes (Helfenbein et al. 2004; Papillon et al. 2004). On the other hand, the longest mitogenomes documented belong to the copepods and are, up to 20 kb (Minxiao et al. 2011). Several mitogenomes were found to contain multiple copies of some sequences (Ogoh and Ohmiya 2004; Burton et al. 2007), or short tandem repeats, similar to microsatellites (Shen et al. 2011).

1.3.4 Transcriptomic Resources

For some species, especially those with large, duplicated, and/or evolutionarily divergent genomes, analysis of transcriptomes has proven more feasible, accurate, and cost-effective (De Wit et al. 2016). Transcriptomic data have the further advantage of allowing identification and annotation of target genes used in the examination of genomic micro-evolution and local adaptation (Havird and Santos 2016). Transcriptomic data including partial reference transcriptomes are available for a number of marine zooplankton species (Table 3).

Table 3 Summary of transcriptomic resources for marine zooplankton species

Phylum and species	BioProject	Contig total	Contig max. length	Contigs total length	Contigs annotated	Transcripts	N50	Genes	Citation
Cnidaria									
Alatina alata	PRJNA312373	31,737	32,591	48,508,802	No	31,776	2,545	20,173	Ames et al. (2016)
Rhopilema esculentum	PRJNA318143	148,857	30,742	121,470,903	No	NA	NA	NA	Li et al. (2017b)
Aurelia aurita	PRJNA252562	252,170	46,960	180,188,094	No	24,264	1,761	10,285	Brekhman et al. (2015)
Ctenophora									
Mnemiopsis leidyi	PRJNA344880	140,842	29,348	137,638,938	No	NA	NA	NA	Sanchez Alvarado (2016)
Arthropoda: Copepoda									
Acartia fossae		100,383	8,174		No		769		Eyun et al. (2017)
Calanus finmarchicus	PRJNA236983	28,954	2,945	10,223,122	No	251,042	354	13,057	Smolina et al. (2014)
Calanus finmarchicus	PRJNA236528	206,012	23,068	205,455,659	Yes		1,418		Lenz et al. (2014)
Calanus finmarchicus	PRJNA231164	241,140	25,048	160,760,719	No				Tarrant et al. (2014)
Calanus glacialis	PRJNA237014	36,880	4,021	15,748,490	No	242,602	471	18,387	Smolina et al. (2014)
Calanus glacialis	PRJNA274584	54,344	7,507	33,214,362	No	16,998	620	16,998	Ramos et al. (2015)
Calanus sinicus		69,751				69,751	1,127	43,417	Yang et al. (2014)
Calanus sinicus			3,923		No	29,458	513		Eyun et al. (2017)
Eucyclops serrulatus	PRJNA231234	51,528	16,342	36,645,141	No				Baratti et al. (2015)
Eurytemora affinis	PRJNA278152	107,445	26,685	142,143,154	No	29,783			Munro et al. (2015)
Eurytemora affinis	PRJNA242763	138,088	23,627	143,733,589	Yes				Almeida and Tarrant (2016)

Eurytemora affinis		88,104	26,685						Eyun et al. (2017)
Paracyclopina nana	PRJNA268783	60,687	27,858	95,849,484	Yes	67,179	4,178	12,474	Lee et al. (2015)
Pseudocalanus acuspes	PRJNA296544	207,302	12,713	59,236,626	Yes	69,555	1,348	28,879	De Wit et al. (2016)
Tigriopus kingsejongensis	PRJNA283925	38,250	7,809	36,497,199	Yes				Lee (2016b)
Tigriopus kingsejongensis			23,942	28,850,726		40,172	1,093	12,772	Kang et al. (2017)
Tigriopus californicus	PRJNA263967	12,067	13,452	14,966,851	No				Barreto et al. (2011)
Tigriopus californicus	PRJNA263967	12,075	13,452	14,902,878	No				Barreto et al. (2011)
Tigriopus californicus		106,317	27,644	NA	Yes	106,317	2,837	12,573	Pereira et al. (2016)
Tigriopus californicus		60,840	8,614				1,510		Eyun et al. (2017)
Tigriopus japonicus	PRJNA274317	54,758	23,769	82,981,758	Yes		3,565		Kim et al. (2015)
Arthropoda: Euphausiacea									
Euphausia superba			11,127		Yes	15,347	520	7,942	Meyer et al. (2015)
Euphausia superba		22,177	8,515		Yes			5,563	Clark et al. (2011)
Euphausia superba		133,962		129,183,922	Yes		1,294	27,928	Sales et al. (2017)
Euphausia crystallorophias		42,632					8,341		Toullec et al. (2013)
Meganyctiphanes norvegica	PRJNA324094	405,497	26,644	222,530,071	No	NA	NA	NA	Blanco-Bercial and Maas (2017)

(continued)

Table 3 (continued)

Phylum and species	BioProject	Contig total	Contig max. length	Contigs total length	Contigs annotated	Transcripts	N50	Genes	Citation
Arthropoda: Amphipoda									
Talitrus saltator	PRJNA297565	156,706	22,032	151,674,147	Yes		968		O'Grady et al. (2016)
Arthropoda: Mysidacea									
Neomysis awatschensis	PRJNA287057	22,141	10,398	14,999,154	Yes	22,141	801		Kim et al. (2016)
Mollusca: Pteropoda									
Clio pyramidata	PRJNA231010	45,735				45,735	852	30,800	Maas et al. (2015)
Clione limacina	PRJNA314884	477,401	30,190	258,267,445	Yes	300,994	816	181,879	Thabet et al. (2017)
Limacina antarctica	PRJNA295792	81,226	7,935	59,791,880	No	402,273	500	81,229	Johnson and Hofmann (2016)
Limacina helicina	PRJNA386290	53,121	12,358	31,790,000	Yes		796		Koh et al. (2015)
Urochordata: Tunicata									
Oikopleura dioica	PRJNA269316	54,949	23,096	66,526,340	No				Wang et al. (2015)
Oikopleura dioica	PRJNA269317	86,898		70,800,000		57,962	1,806	16,423	Wang et al. (2015)
Salpa thompsoni	PRJNA279245	217,849	30,785	151,741,986	No	216,931	1,163	26,413	Jue et al. (2016) and Batta-Lona et al. (2017)

Transcript and gene numbers are indicated as presented in the original study. Note that different methodologies were employed across these datasets (e.g., Trinity, MIRA_Newbler, Evigene, FPKM filtered, etc.) that render cross-comparisons of gene and transcript numbers among species equivocal

2 Applications of Population Genomics for Marine Zooplankton

2.1 Population Genetic Diversity and Structure

Although many zooplankton species exhibit broad geographic distributions and appear to have high dispersal potential, both biological and physical environmental processes may limit gene flow. Previous studies have revealed significant genetic differentiation of geographic populations of marine organisms over a range of spatial scales (Hellberg 2009; Weersing and Toonen 2009). Two general principles may be gleaned from many studies of zooplankton population genetics: first, zooplankton are quite variable in many different molecular characters; second, this variability is resolved into genetically divergent, geographically distinct populations for only some species and at some temporal and spatial scales (Peijnenburg and Goetze 2013).

Ocean processes that are thought to be significant for population genetic structuring of zooplankton are currents, persistent eddies, ocean gyres, and other physical ocean structures from the mesoscale (10 s–100 s km) to large scale (100 s–1,000 s km). The physical structure of the ocean can alter the timing of reproduction and mortality events, providing biological barriers to gene flow. Geological features – continents, islands, and other landforms, continental shelves, seamounts, and ocean ridges – may form natural barriers to dispersal. In contrast, cosmopolitan species, which range from 40°N to 40°S and are found in every ocean basin, may have few barriers to dispersal throughout their range. These species may exhibit large-scale spatial population genetic structure due to isolation by distance (i.e., reproductive isolation resulting when the geographic range of the species far exceeds the dispersal potential of an individual).

The temporal stability of population genetic diversity and structure is an important consideration and useful metric. Since zooplankton are subject to transport in ocean currents, temporal stability of population genetic characters may indicate retention of local populations or local recruitment. An unfortunate aspect of many studies of zooplankton populations is the collection of samples from different regions during different years, thus confounding spatial and temporal variation. In relatively few studies, spatial and temporal contributions to population genetic structure have been analyzed separately using appropriately collected samples (Goetze et al. 2015; Iacchei et al. 2017).

Patterns of genetic diversity and structure have been examined over a wide range of spatial scales for species representing many lineages of the zooplankton assemblage. Some species have been shown to be panmictic, such as the jellyfish *Pelagia noctiluca* (Stopar et al. 2010) and Antarctic krill *Euphausia superba* (Deagle et al. 2015). Many species exhibit geographic variation reflecting geographic barriers and/or circulation patterns: for example, the intertidal copepod *Tigriopus californicus* (Renaut and Dion-Côté 2016) and arrow worms *Eukrohnia hamata* (Kulagin et al. 2014) and *Caecosagitta macrocephala* (Miyamoto et al. 2010), to name a few. A number of

species show large-scale patterns of genetic diversity associated with latitudinal gradients (e.g., Francisco et al. 2014) and among ocean basins, including *E. hamata* (Miyamoto et al. 2012) and copepods *Pleuromamma abdominalis* (Hirai and Tsuda 2015) and *Oithona similis* (Cornils et al. 2017).

The occurrence and significance of small-scale genetic patchiness in marine zooplankton populations remain a subject of study and disagreement. Such variability has been considered to reflect the genetic "noise" of large and under-sampled populations of copepods (e.g., Goetze et al. 2015). Small-scale heterogeneity was considered to reflect advective transport from diverse recruitment sources in the Antarctic krill, *E. superba* (Batta-Lona et al. 2011).

Due to nearly universal application in population genetic studies, hierarchical analysis of variance using Wright's *F*-statistics (Excoffier et al. 1992) provides useful benchmarks for comparisons among species, regions, and environments. However, *F*-statistics have assumptions that are surely not met for zooplankton (Hellberg 2009), including genetic equilibrium conditions, symmetrical migration, and stable populations. The usefulness of *F*-statistics is further limited by the very large population sizes of many zooplankton, which result in relatively larger confidence intervals for very small *F* values (Waples 1998), and thus a lack of statistical significance for high gene flow species (see Waples et al. 2008). At least partly for this reason, population genetic studies of marine species have also employed various measures of oceanographic distance (Hansen and Hemmer-Hansen 2007; McGovern et al. 2010; Alberto et al. 2011; Schunter et al. 2011) and approaches such as seascape genetics (Galindo et al. 2010).

Until recently, population genetic studies have most frequently been conducted with markers representing a very small fraction of the genome, such as individual mitochondrial or nuclear genes and microsatellites (see reviews by Avise 2009; Hellberg 2009; Peijnenburg and Goetze 2013). Rates of divergence and amounts of variation differ among these markers, but many studies have documented significant genetic differentiation of zooplankton populations at large, ocean basin scales using mitochondrial DNA (e.g., Goetze 2005; Goetze and Ohman 2010; Miyamoto et al. 2010; Blanco-Bercial et al. 2011; Miller et al. 2012; Norton and Goetze 2013; Dawson et al. 2015) and microsatellite markers (Bolte et al. 2013; Andrews et al. 2014). A number of studies have used mitochondrial DNA markers to resolve population structure of zooplankton populations associated with physical barriers to gene flow, including ocean circulation, for copepods (Aarbakke et al. 2011; Blanco-Bercial et al. 2011, 2014) and euphausiids (Bucklin et al. 1997; Zane et al. 1998, 2000; Zane and Patarnello 2000; Papetti et al. 2005; Patarnello et al. 2010).

Both mitochondrial and microsatellite markers continue to be widely used for population genetic analysis of zooplankton, allowing useful comparisons among diverse species and ocean environments. Studies using single markers have limitations, not least that results may differ among studies using different markers (Avise et al. 2016). In addition to their limited analytical power, studies using multiple markers can yield discordant conclusions. In particular, the haploid nature and uniparental inheritance of mitochondrial markers, and consequent smaller effective

population size, may generate differences from results using nuclear markers (Toews and Brelsford 2012).

Population genomic approaches can also be used for phylogeographic analysis (i.e., the description of the geographical distributions of the genetic lineages within a population or species; Avise 2009; Avise et al. 2016). Such analysis allows for the characterization of dispersal and quantitative estimation of the rate and direction of exchange among populations. Recent reviews of larval dispersal and population connectivity (Cowen and Sponaugle 2009) and gene flow (Hellberg 2009) in the ocean have provided comprehensive assessment and analyses for marine organisms. Quantitative estimates of population persistence and directional (asymmetric) migration can also entail approaches that are less sensitive to lack of population stability and nonequilibrium conditions, typical of marine organisms (Knowles 2009). Analysis of patterns of gene flow has revealed that pathways of population connectivity of marine organisms do not always mimic major ocean currents (Kool et al. 2013; Riginos et al. 2016), even for zooplankton (Blanco-Bercial and Bucklin 2016; Questel et al. 2016).

Phylogeographic analysis can also provide a window into the evolutionary history of a population or species. Results can be interpreted to estimate and understand the age of the lineage in terms of time to coalescence (i.e., the common ancestral gene from which all current copies of the gene are descended), as well as imprints of demographic history on populations and species (Knowles 2009). Among marine zooplankton, mitochondrial markers have been used most regularly to infer demographic history (e.g., Peijnenburg et al. 2005; Aarbakke et al. 2014; Cornils et al. 2017), including marine invasions (Cristescu 2015; Lee 2016a; Sherman et al. 2016), population expansions and contractions (Edmands 2001), geographic isolation giving rise to speciation events (Lee 2000; Peijnenburg et al. 2004; Miyamoto et al. 2010), and divergence of genetic lineages following major global climate events (Papadopoulos et al. 2005; Blanco-Bercial et al. 2011; Milligan et al. 2011).

2.2 From Population Genetics to Population Genomics

Recent advances in High-Throughput Sequencing (HTS) have created exceptional new opportunities for analysis of population genetic diversity and structure of natural populations. Tens of thousands of genomic Single Nucleotide Polymorphisms (SNPs) can be detected and screened for use as genetic makers of population genetic diversity and structure (Helyar et al. 2011; Reitzel et al. 2013). Such population genomic approaches are being widely used among marine organisms (Bierne et al. 2016), including fishes (Hemmer-Hansen et al. 2014). In addition, HTS is yielding both deep coverage and nucleotide-level resolution in simultaneous or multiplexed analysis of numerous genes (e.g., Bybee et al. 2011). Such population genomic approaches are yielding a new view of population structure and

connectivity of marine species, based on statistical discrimination of neutral, selected, and hitchhiker loci (Gagnaire and Gaggiotti 2016).

Over the last three decades, genetic research has showed continuous development and a high turnover of molecular markers, from partial DNA sequencing, restriction fragment length polymorphism (RFLP), random amplified polymorphism detection (RAPD), and amplified fragment length polymorphism (AFLP) to microsatellites, insertion-deletion polymorphism (InDel), and SNP (Schlötterer et al. 2014). Historically, the development of markers was difficult and expensive for non-model organisms. However, the advent of HTS has revolutionized this by allowing the use of genome-wide markers in any organism and for low cost (Ekblom and Galindo 2011). Although simultaneous discovery and genotyping of genome-wide variation has become feasible for tens of individuals with small genome sizes (<1 GB), the individual sequencing of hundreds of individuals with large genomes remains prohibitively expensive (Narum et al. 2013). In addition, sequencing of the complete genome for all individuals is often unnecessary and inflates the bioinformatics demands (Narum et al. 2013). Therefore, for many studies including population genomics, it is more efficient to sequence a limited number of targeted loci, thus increasing their coverage and chance to detect true polymorphism (Ekblom and Galindo 2011).

A revolutionizing solution to address this situation was the development of GBS approaches that allow sequencing with high throughput technology of a targeted fraction of the genome via various reduced-representation protocols (see review by Crawford and Oleksiak 2016). These approaches result in discovery and simultaneous genotyping of thousands of SNPs even in species with large genomes and little or no previous genomic information. GBS relies on various reduced-representation protocols to target a genome fraction, but four protocols are currently the most popular: RNA-seq, Ampli-seq, Cap-seq (i.e., capture enrichment), and RAD-seq (Davey and Blaxter 2010; Reitzel et al. 2013). Published reduced-representation genomic resources are currently available for several species of marine zooplankton, such as the copepods, *T. californicus* (Foley et al. 2011), *C. finmarchicus* (Smolina 2015), and *Centropages typicus* (Blanco-Bercial and Bucklin 2016); and the euphausiid, *E. superba* (Deagle et al. 2015). The number of studies using reduced representation for population genomics in marine zooplankton may be expected to expand in the near future.

The power of genomic SNPs for resolution of regional- to large-scale population structure of zooplankton has been demonstrated for several key species (see Case Studies, below). A large-scale population genetic analysis using genomic SNPs demonstrated that RAD-seq methods performed poorly in the copepod, *C. finmarchicus*, which has a large and complex genome (Smolina 2015). Subsequent studies of this species using targeted resequencing (e.g., Cap-seq) showed promise for accurate SNP identification and detection of genetic structuring for this species (Choquet et al. unpublished data). Similarly, a study of the copepod, *C. typicus*, by Blanco-Bercial and Bucklin (2016) using 1,000 s of genomic SNPs

obtained by RAD-seq revealed evidence of population structure, in contrast to an earlier study based on mitochondrial gene sequences (Castellani et al. 2012).

Genomic SNPs that show evidence of selection can provide markers of micro-evolution and local adaptation, including identification of the key genes involved in these phenomena. The use of many thousands of genomic markers will also enable further examination of the significance of small-scale genetic heterogeneity of marine zooplankton, including distinguishing genetic "noise" in large and patchy populations from local adaptation to environmental conditions. Large-scale SNP genotyping studies remain very scarce in zooplankton species, but as more studies based on these approaches are published, it will be important to resolve differing conclusions based on the various technical approaches and genetic markers employed.

2.3 Genomic Basis of Adaptation

Population genomic approaches have provided powerful new tools for detection of impacts of selection and evidence of local adaptation (Stapley et al. 2010). Patterns of variation of genomic markers can be statistically evaluated for non-neutrality and correlation with population dynamic, environmental, and evolutionary conditions and drivers (Gagnaire et al. 2015). Non-neutral markers showing evidence of selection can be used to reveal adaptation of populations to local conditions across a species range (Whitehead 2012), although other evolutionary drivers, including introgression and hitchhiking, can also cause such departures from neutrality for genomic traits (Bierne et al. 2013). Nielsen et al. (2009) concluded that few published studies have convincingly documented that non-neutral traits reflect local adaptation, citing reviews by Hedrick (2006) and Levasseur et al. (2007). Recent advances in statistical analysis of genomic markers are enabling more sensitive and accurate detection of local adaptation (Gayral et al. 2013; Savolainen et al. 2013; De Wit et al. 2015), although these are much more powerful for species with well-characterized genomes, which allows exome capture and sequencing (Jones and Good 2016).

Patterns of differential gene expression can also provide useful insights into local adaptive responses of marine organisms to environmental conditions. There are a number of such studies of marine zooplankton, including target-gene and whole-transcriptome analyses of differential gene expression patterns associated with stress responses and environmental variability (Lauritano et al. 2012; Schoville et al. 2012; De Pittà et al. 2013; Smolina et al. 2015, 2016; Roncalli et al. 2016; Batta-Lona et al. 2017). The genetic and genomic bases of such gene expression differences have received considerable attention (see review by Romero et al. 2014).

2.4 Metagenetics and Metabarcoding

The exceptional challenge of species identification in zooplankton assemblages, resulting from both phylogenetic diversity and sibling species swarms, has encouraged the development of genetic approaches for both stand-alone and integrative use with morphological taxonomic methods (Bucklin et al. 2016). Metagenetic and metabarcoding approaches analyze DNA recovered from environmental samples and can reflect the biodiversity of entire pelagic communities (de Vargas et al. 2015), with the advantage of detecting "hidden diversity" of marine zooplankton (Lindeque et al. 2013). These studies use "universal" PCR primers to amplify one or more gene regions for high throughput sequencing yielding tens of millions of sequences, which are subsequently resolved into operational taxonomic units (OTUs) that can either be matched to reference databases for identification of taxa or used for various statistical measures of biodiversity (Leray and Knowlton 2016). Metabarcoding studies of marine zooplankton have ranged from analysis of the global ocean (Bik et al. 2012; de Vargas et al. 2015) to studies focused on particular habitats and ecosystems, such as estuaries (Abad et al. 2016), the Red Sea (Pearman and Irigoien 2015), among others. Challenges remain for quantitative analysis of taxa using metabarcoding, although recent studies have shown some correlation between OTU frequency and taxon biomass (Hirai et al. 2015; Sun et al. 2015).

The continuing development of sequencing technologies may soon allow a full metagenomics approach, where DNA extracted from environmental samples is sequenced and whole genomes are reconstructed from the data. These data will be invaluable resources for diverse population genomic approaches, including analysis of population genetic diversity and structure, detection of loci under selection, and genomic bases of adaptations of zooplankton species to environmental variation. Currently, both technical and bioinformatics challenges limit the use of metagenomics to species with small genomes, such as the copepod, *Oithona nana* (Madoui et al. 2017).

3 Case Studies of Marine Zooplankton

Population genomic approaches, entailing simultaneous sampling of numerous variable loci within a genome and the inference of locus-specific effects (Black et al. 2001; Luikart et al. 2003), are only very recently being used for analysis of marine zooplankton. Comparison between results from population genetic studies using single markers (usually mitochondrial or microsatellite DNA) and HTS genomic markers are particularly useful to evaluate the power and precision of population genomic approaches for analysis of genetic structure, connectivity, demographic history, and local adaptation.

Several of the marine zooplankton species analyzed using population genomic approaches belong to the crustacean Subclass Copepoda, which comprises more

species than any other zooplankton group, including many that are ecologically important, numerically predominant, and geographically widespread. Genomic analysis of copepods has been a focus of research, although progress has been hampered by the exceptionally large genome sizes of many species (Bron et al. 2011; Wyngaard et al. 2011; Jeffrey 2015).

3.1 Calanus finmarchicus *(Copepoda)*

The planktonic copepod *C. finmarchicus* (Fig. 3) is thought to be the most abundant metazoan in the ocean; the species is ubiquitous in coastal and open ocean cold-temperate regions of the North Atlantic Ocean (Planque et al. 1997); within this area, the species may contribute >70% of total copepod biomass (Head et al. 2003) and occupies a pivotal position in ocean food webs (Falk-Petersen et al. 2007). Population genetic studies using mitochondrial DNA (e.g., Bucklin et al. 1996) and microsatellites (Provan et al. 2009) have shown high levels of gene flow and little or no significant population genetic structure at any spatial scale. Studies using SNPs in targeted gene regions suggested genetic differentiation among samples from different water masses and ocean basins (Bucklin and Kaartvedt 2000; Unal and Bucklin 2010; Fig. 4). Population genomic analyses of *C. finmarchicus* have been impeded by the large size of its genome (C-value = 6.48 pg; McLaren et al. 1988), typical of crustaceans. Smolina (2015) used a GBS approach (ddRADseq; Peterson et al. 2012) to characterize genomic SNPs in pooled samples of *C. finmarchicus* collected across the North Atlantic Ocean. Significant population differentiation was observed among locations, although the allelic nature of the SNP variants in the pooled samples could not be confirmed due to the highly replicated genome

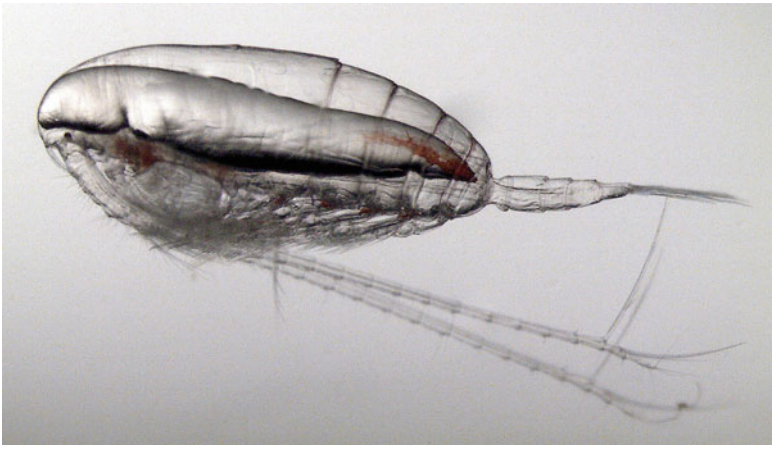

Fig. 3 *Calanus finmarchicus* (Copepoda) Photo courtesy of Cameron Thompson (University of Maine, USA)

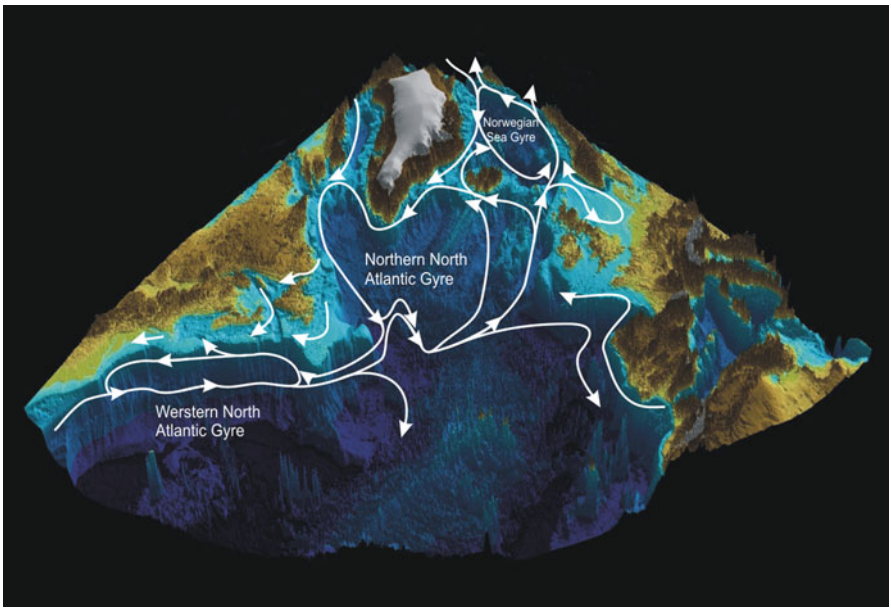

Fig. 4 Circulation patterns and bathymetry of the North Atlantic Ocean basin, providing the foundation of the three-gyre hypothesis for basin-scale dispersal of the copepod *C. finmarchicus*. Figure from Wiebe et al. (2009)

(Smolina 2015). An ongoing study by this group is analyzing genomic SNPs in targeted gene regions to allow confirmation of allelic variation despite genome size (Choquet et al. 2017a). A partial reference transcriptome for the species (Lenz et al. 2014) is allowing the evaluation of evidence of local adaptation based on transcriptomic and target gene analysis (e.g., Roncalli et al. 2016).

3.2 Centropages typicus *(Copepoda)*

Blanco-Bercial and Bucklin (2016) used genomic SNPs detected by 2b-RADseq analysis (Wang et al. 2012) to examine population genetic structure of the copepod *C. typicus* (Fig. 5) in the North Atlantic Ocean. Thousands of genomic SNP markers were identified; loci showing evidence of positive selection were removed from analysis (Foll and Gaggiotti 2008). Statistical analysis of molecular variance (Excoffier and Lischer 2010) revealed significant differences between continental shelf populations of the NE and NW Atlantic populations, in contrast with an earlier study by Castellani et al. (2012), which showed no structuring using a mitochondrial COI gene region, but some differentiation of NE and NW Atlantic populations based on a nuclear rRNA internal transcribed spacer (ITS) region. GBS (RADtag sequences) of *C. typicus* yielded 675 loci used by Blanco-Bercial and Bucklin (2016)

Fig. 5 *Centropages typicus* (Copepoda) Photo courtesy of Julie Ambler (Millersville University, USA and NatureAtlas.org)

to test hypotheses of dispersal and directional migration (Beerli 2012). Among five different gene flow models (Fig. 6), the full migration model showed the highest support. These results demonstrate the power of population genomic approaches to resolve patterns and pathways of dispersal of a high gene flow species in a dynamic and complex current system. Such analyses can also be used to examine the genomic basis of observed local adaptation of this species to environmental variability among regions or along a latitudinal gradient (Carlotti et al. 2007).

3.3 Tigriopus californicus *(Copepoda)*

The tidepool copepod, *T. californicus*, shows exceptional levels of small-scale population genetic heterogeneity associated with the habitat structure of the rocky shoreline, based on studies using mitochondrial markers (Rawson et al. 2000; Burton et al. 2007). The species may be considered to be a model species for studies of evolutionary divergence and local adaptation (Raisuddin et al. 2007). The rapid rate of evolutionary divergence of mitochondrial genes is thought to contribute to the potential for local adaptation but may also cause low hybrid fitness by disrupting

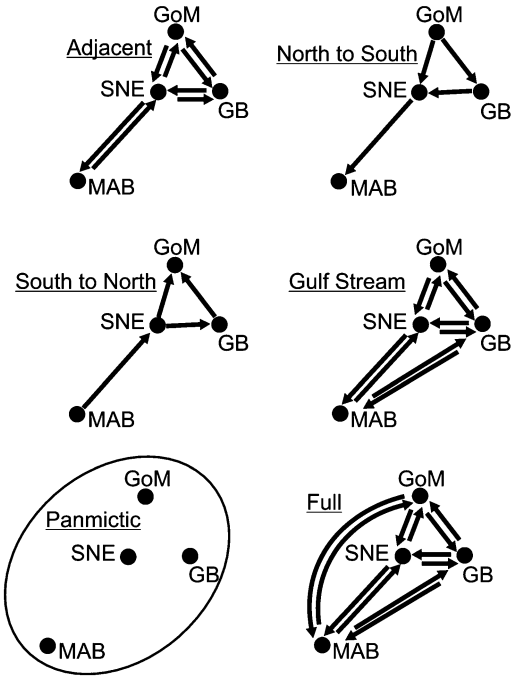

Fig. 6 Hypothesized models of gene flow and population connectivity of the copepod *C. typicus*. The full migration model (lower right in diagram) showed the highest likelihood among the considered models based on Bayesian analysis. Abbreviations refer to regions of the Northwest Atlantic continental shelf: Georges Bank (GB), Gulf of Maine (GoM), Mid-Atlantic Bight (MAB), Southern New England (SNE). Figure from Blanco-Bercial and Bucklin (2016)

gene complexes (Burton et al. 2013). The mitochondrial genome has been sequenced (Barreto et al. 2011; Pereira et al. 2016). A genomic SNP linkage map (Foley et al. 2011) and a partial draft genome (https://i5k.nal.usda.gov/Tigriopus_californicus) serve as useful resources for characterizing population genetic diversity and structure. More recently, the capacity of this species to adapt to local condition and stressors has been explored using population genomic and transcriptomic approaches (Lima and Willett 2017; Pereira et al. 2017).

3.4 Acartia tonsa *(Copepoda)*

The rapid cladogenesis – and perhaps cryptic speciation – of the estuarine copepod, *Acartia tonsa*, has been extensively studied along the Atlantic coastline of the USA using mtDNA marker genes (Caudill and Bucklin 2004; Chen and Hare 2008, 2011). The species has been intensively studied in laboratory culture, partly as food for aquacultured fish (Jepsen et al. 2017) and partly as a model organism for studies of the genetic basis of local adaptation and micro-evolution (Drillet et al. 2008). Responses to environmental stressors have been examined using genomic and transcriptomic approaches (Nilsson et al. 2014; Petkeviciute et al. 2015; Rahlff et al. 2017).

3.5 Euphausia superba *(Euphausiacea)*

The Antarctic krill, *E. superba* (Fig. 7), is a keystone species of the Southern Ocean pelagic ecosystem, whose high abundance, markedly patchy distribution, and swarming behavior have long been a subject of research (Siegel and Watkins 2016). The population genetic consequences of this exceptional life history have been studied over many decades using varied markers, including allozymes, mitochondrial DNA, and microsatellites. Many studies have revealed similar patterns of genetic diversity, whereby variation within locations far outweighs that between locations, with consistent evidence of lack of large-scale population differentiation (see review by Jarman and Deagle 2016). Two studies using mitochondrial markers found evidence of significant small-scale patchiness: Batta-Lona et al. (2011) hypothesized that genetic differences among samples resulted from advective transport from distinct recruitment centers in the Western Antarctic Peninsula region. Zane et al. (1998) found genetic differentiation between samples collected in the Weddell Sea and South Georgia. Although the statistical significance of these findings has been questioned (see Bortolotto et al. 2011), small-scale patchiness – or genetic "noise" – may be a consequence of the life history of this unique species and/or evidence of local adaptation. Evidence of micro-evolution and local adaptation by Antarctic krill has been shown in genetic and functional analysis of target genes, including thioredoxin (Li et al. 2017a), clock genes (Jones and Good 2016), heat shock proteins (Papot et al. 2016), and opsins (Biscontin et al. 2016), among others. Population genomic analysis of Antarctic krill was introduced by Deagle et al. (2015), who examined circum-Antarctic genetic diversity and structure using both RADseq and mitochondrial (ND1 and COI) markers. The large and highly replicated genome of *E. superba* (47.7 GB, Jeffery 2012) prevented the

Fig. 7 *Euphausia superba* (Euphausiacea) Photo courtesy of Russell R. Hopcroft (University of Alaska, Fairbanks, USA and Census of Marine Life)

Fig. 8 *Meganyctiphanes norvegica* (Euphausiacea) Photo courtesy of Uwe Kils (Rutgers University, USA)

discrimination of allelic variation versus that between copies at separate loci (see above), which was addressed by analysis of sequence counts at variable nucleotide sites, rather than the derived genotypes. This study confirmed earlier findings of the large-scale panmixia of Antarctic krill populations (Deagle et al. 2015).

3.6 Meganyctiphanes norvegica *(Euphausiacea)*

The northern krill *Meganyctiphanes norvegica* (Fig. 8) is abundant throughout the North Atlantic and western Mediterranean Sea. The species exhibits clear genetic differentiation among geographic populations based on various mtDNA markers (see review by Patarnello et al. 2010). Consistent evidence of local adaptation of the species, including enzyme activities (Saborowski and Buchholz 2002), is now being analyzed using differential gene expression made possible by a reference transcriptome (Blanco-Bercial and Maas (2017).

3.7 Pleurobrachia bachei *(Ctenophora)*

A draft genome of the ctenophore *Pleurobrachia bachei* (Fig. 9) revealed the possible preservation of "ancient molecular toolkits" (Moroz et al. 2014), which are lost in other lineages. The exceptional nature of the genomic architecture of this species can provide new understanding of the genomic basis of their evolutionary success and potential for adaptation. Integrative and comparative analysis of genomic and transcriptomic data of this and another ctenophore species *M. leidyi* demonstrated the phylogenetic position of the phylum as the first metazoan lineage (Ryan et al. 2013; Moroz et al. 2014).

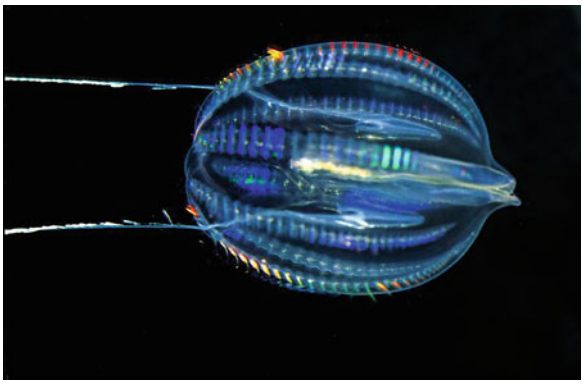

Fig. 9 *Pleurobrachia bachei* (Ctenophora) Photo courtesy of Dave Wrobel (www.wrobelphoto.com)

3.8 Spadella cephaloptera *(Chaetognatha)*

Arrow worms are predatory zooplankton that occupy key positions in pelagic food webs. The phylum comprises many species with cosmopolitan-but-disjunct biogeographical distributions, which has allowed interesting comparisons among species. Population genetic diversity and structure of several chaetognath species have been explored using both mtDNA and microsatellites (Peijnenburg et al. 2004, 2006; Faure and Casanova 2006; Miyamoto et al. 2010; Kulagin et al. 2014). Large-scale studies have also allowed examination of the demographic histories of the species (Peijnenburg et al. 2005). Analysis of the mitochondrial genome of *S. cephaloptera* (Fig. 10) yielded evidence of exceptional intraspecific variation (Marlétaz et al. 2017), and resolved the phylogenetic position of the Chaetognatha within Protostome lineages (Papillon et al. 2004).

3.9 Salpa thompsoni *(Tunicata, Thaliacea)*

The Southern Ocean salp *S. thompsoni* (Fig. 11) is a pivotal species in the pelagic ecosystem of Antarctic regions, including the Western Antarctic Peninsula, one of the fastest warming regions of the world's oceans. A reference transcriptome for *S. thompsoni* is available, although only 18% of the 216,931 sequences were associated with predicted, hypothetical, or known proteins (Batta-Lona et al. 2017). Another recent study (Jue et al. 2016) produced a preliminary reference genome for the species, identified more than 50% of sequences, and generated both SNP variant and INDEL predictions as a resource for future phylogenetic and population studies. The genome of this species shows evidence of a rapid

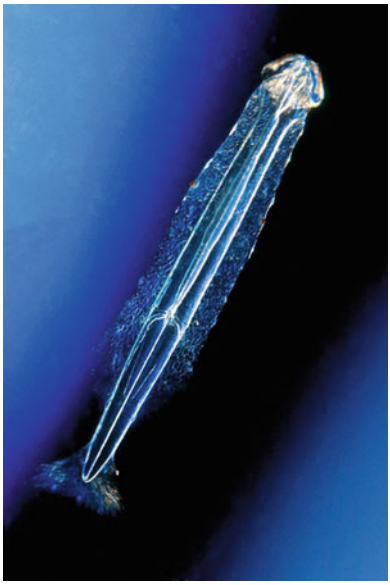

Fig. 10 *Spadella cephaloptera* (Chaetognatha) Photo courtesy of Peter Parks (Image Quest 3-D)

Fig. 11 *Salpa thompsoni* (Tunicata, Thaliacea) Photo courtesy of Lawrence P. Madin (Woods Hole Oceanographic Institution, USA)

evolutionary rate – consistent with other Urochordata (Denoeud et al. 2010; Tsagkogeorga et al. 2012). An initial survey of small RNAs revealed the presence of known, conserved miRNAs, novel miRNA genes, and unique piRNAs for various developmental stages (Jue et al. 2016), suggesting possible genomic bases of the successful adaptation of the species to the changing climate of the Southern Ocean.

4 Present-Day Challenges and Future Opportunities

4.1 Additional Genomic Resources for Marine Zooplankton Species

Pelagic zones represent one of the largest (by volume) habitats on Earth, with highly diverse and ecologically important assemblages of zooplankton, which can serve as early warning indicators of climate change. Genomic resources are needed to facilitate both intra- and interspecies comparative studies of genetic diversity and structure, phylogeography, demographic history, and adaptive evolution. Importantly, marine zooplankton provide a diverse and useful assemblage to move forward novel studies of the genomic basis of adaptation and evolutionary divergence. Yet the exceptional phylogenetic diversity of marine zooplankton exacerbates the challenges of ensuring that reference genomes are available for abundant and ecologically important species or their close relatives.

Whole-genome sequencing initiatives should cover a wide range of genome sizes to uncover trends in genome evolution and new elements of genome organization. For instance, sequencing of the salp genome revealed novel miRNA genes and unique piRNAs (Jue et al. 2016), while the genome of Pacific sea gooseberry, *P. bachei*, is apparently lacking the canonical miRNA machinery and HOX genes (Moroz et al. 2014).

Stimulating discoveries are anticipated from sequencing the exceptionally large genomes of many crustaceans, including euphausiids, copepods, and amphipods, which may reveal novel regulation of repetitive elements, functional divergence of gene duplication and concomitant novel functions of various gene copies, and correlation between genome size and DNA methylation levels in metazoans (e.g., Lechner et al. 2013). From a practical perspective, even low-coverage genomes will increase the robustness of population genomic approaches by facilitating a diverse range of methods, including in silico digestion of genome sequences for RAD-seq techniques, higher mapping rates for DNA and RNA-derived sequences, and the development of baits for sequence capture experiments.

Despite their ecological importance in pelagic food webs and their phylogenetic diversity, marine zooplankton have been – and continue to be – largely ignored in the prioritization of species for genomic and transcriptomic analysis. For example, a list of top priority species for reference genome determination from Voolstra et al. (2017) includes only one marine zooplankton species, the mid-water shrimp, *Acanthephyra purpurea*.

4.2 Sampling Zooplankton in the Global Ocean

Sampling zooplankton accurately and effectively is a challenge due both to the nature of the pelagic habitat and the frequently immense population sizes of the

organisms compared to sampling capacity. It is essential to keep in mind that planktonic organisms most usually occur in patchy distributions, and that some of them are able to avoid the sampling equipment. The origin of these planktonic assemblages or patches has been discussed over many years (e.g., Levin and Segel 1976) and some experimental studies have shown species-specific patterns (Omori and Hamner 1982). Avoidance behaviors also vary among species, and a number of studies have shown that net size and design can markedly impact avoidance and improve the accuracy of sampling of dense and diverse assemblages (Wiebe 1968; Skjoldal et al. 2013; Wiebe et al. 2013). Novel instrumentation designs are now allowing pairing of net sampling with optical and acoustical technologies to allow adaptive sampling of target species of particular interest and importance.

4.3 Species Identification

Accurate and precise identification of species is critical for any study, yet for most zooplankton groups this goal is challenging – at best. Morphological identification has been shown to be unreliable for numerous species, including sibling species of the copepods *Pseudocalanus* (Bailey et al. 2015) and *Calanus* (Choquet et al. 2017b). Both transcriptomic and genomic resources are invaluable in allowing the design of rapid and inexpensive protocols for accurate discrimination and identification of sibling and cryptic species of marine zooplankton (e.g., Smolina et al. 2015).

4.4 Genomic Analysis of Small-Sized Organisms

Zooplankton species are often very small and thus the yield of DNA extractions is limited. This is not an issue for current HTS methods, which usually require a very small amount of DNA (10 s ng). The ongoing development of new sequencing platforms and technologies will likely allow longer sequencing reads and thus better genome and transcriptome assemblies. There is a continuing need to ensure that even the tiniest organisms will be amenable to any new developments in sequencing technologies and instrumentation.

4.5 Genomic Basis of Adaptation

Marine environments are experiencing rapid changes in critically important processes and parameters, including temperature, light penetration, nutrient availability, and ocean acidification, among many others. The resultant changes in species physiological condition, ecological functioning, and biogeographical distribution

and abundance will inexorably alter pelagic ecosystems in trajectories that are difficult to predict. How species may acclimate and/or adapt to environmental change, and how their interactions within the pelagic food web may be altered, can be examined at many levels. A powerful and important approach lies in examining the underlying genomic mechanisms that facilitate successful adaptation to changing environmental conditions. Although any given species may be uniquely impacted by the physical and biological parameters accompanying shifts in global climate profiles, processes involved in responses to climate change at the molecular level may share common features across species, such as the evolution of gene networks associated with environmental stress responses. Genomic resources are proving instrumental in garnering new insights into organism–environment interactions, including responses to environmental variability associated with climate change. However, we still lack a fundamental understanding of genomic features that afford plasticity and facilitate adaptive responses. These challenges can only be met with comprehensive genomic and transcriptomic resources that will allow comparative analysis to investigate the mechanisms underlying the responses of marine zooplankton to the changing environmental conditions throughout the global ocean.

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References

Aarbakke ONS, Bucklin A, Halsband C, Norrbin F. Discovery of *Pseudocalanus moultoni* (Frost 1989) in Northeast Atlantic waters based on mitochondrial COI sequence variation. J Plankton Res. 2011;33:1487–95. https://doi.org/10.1093/plankt/fbr057.

Aarbakke ONS, Bucklin A, Halsband C, Norrbin F. Comparative phylogeography and demographic history of five sibling species of *Pseudocalanus* (Copepoda: Calanoida) in the North Atlantic Ocean. J Exp Mar Biol Ecol. 2014;461:479–88. https://doi.org/10.1016/j.jembe.2014.10.006.

Abad D, Albaina A, Aguirre M, et al. Is metabarcoding suitable for estuarine plankton monitoring? A comparative study with microscopy. Mar Biol. 2016;163(7):1–13. https://doi.org/10.1007/s00227-016-2920-0.

Alberto F, Raimondi PT, Reed DC, et al. Isolation by oceanographic distance explains genetic structure for *Macrocystis pyrifera* in the Santa Barbara channel. Mol Ecol. 2011;20:2543–54. https://doi.org/10.1111/j.1365-294X.2011.05117.x.

Alfsnes K, Leinaas HP, Hessen DO. Genome size in arthropods; different roles of phylogeny, habitat and life history in insects and crustaceans. Ecol Evol. 2017;7(15):5939–47. https://doi.org/10.1002/ece3.3163.

Almeida AA, Tarrant AM. Vibrio elicits targeted transcriptional responses from copepod hosts. FEMS Microbiol Ecol. 2016;92. https://doi.org/10.1093/femsec/fiw072.

Ames CL, Ryan JF, Bely AE, et al. A new transcriptome and transcriptome profiling of adult and larval tissue in the box jellyfish *Alatina alata*: an emerging model for studying venom, vision and sex. BMC Genomics. 2016;17:650. https://doi.org/10.1186/s12864-016-2944-3.

Andrews KR, Norton EL, Fernandez-Silva I, et al. Multilocus evidence for globally distributed cryptic species and distinct populations across ocean gyres in a mesopelagic copepod. Mol Ecol. 2014;23:5462–79. https://doi.org/10.1111/mec.12950.

Avise JC. Phylogeography: retrospect and prospect. J Biogeogr. 2009;36:3–15. https://doi.org/10.1111/j.1365-2699.2008.02032.x.

Avise JC, Bowen BW, Ayala FJ. In the light of evolution X: comparative phylogeography. Proc Natl Acad Sci U S A. 2016;113:7957–61. https://doi.org/10.1073/pnas.1604338113.

Bailey J, Rynearson T, Durbin EG. Species composition and abundance of copepods in the morphologically cryptic genus *Pseudocalanus* in the Bering Sea. Deep Sea Res Part II Top Stud Oceanogr. 2015;134:173–80. https://doi.org/10.1016/j.dsr2.2015.04.017.

Baird NA, Etter PD, Atwood TS, et al. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One. 2008;3:1–7. https://doi.org/10.1371/journal.pone.0003376.

Baratti M, Cattonaro F, Di Lorenzo T, et al. Genomic resources notes accepted 1 October 2014–30 November 2014. Mol Ecol Resour. 2015;15:458–9. https://doi.org/10.1111/1755-0998.12368.

Barreto FS, Moy GW, Burton RS. Interpopulation patterns of divergence and selection across the transcriptome of the copepod *Tigriopus californicus*. Mol Ecol. 2011;20:560–72. https://doi.org/10.1111/j.1365-294X.2010.04963.x.

Batta-Lona PG, Bucklin A, Wiebe PH, et al. Population genetic variation of the Southern Ocean krill, *Euphausia superba*, in the western Antarctic Peninsula region based on mitochondrial single nucleotide polymorphisms (SNPs). Deep Sea Res Part II Top Stud Oceanogr. 2011;58:1652–61. https://doi.org/10.1016/j.dsr2.2010.11.017.

Batta-Lona PG, Maas AE, O'Neill RJ, et al. Transcriptomic profiles of spring and summer populations of the Southern Ocean salp, *Salpa thompsoni*, in the western Antarctic Peninsula region. Polar Biol. 2017;40:1261–76. https://doi.org/10.1007/s00300-016-2051-6.

Beaugrand G. Plankton biodiversity and biogeography. In: Castellani C, Edwards M, editors. Marine plankton: a practical guide to ecology, methodology, and taxonomy. Oxford: Oxford University Press; 2017. p. 12–23.

Beerli P. Migrate documentation version 3.2.1. Tallahasee: Florida State University; 2012.

Bierne N, Roze D, Welch JJ. Pervasive selection or is it . . .? Why are FST outliers sometimes so frequent? Mol Ecol. 2013;33:2061–4. https://doi.org/10.1111/mec.12241.

Bierne N, Bonhomme F, Arnaud-Haond AS. Dedicated population genomics for the silent world: the specific questions of marine population genetics. Curr Zool. 2016;62:545–50. https://doi.org/10.1093/cz/zow107.

Bik HM, Porazinska DL, Creer S, et al. Sequencing our way towards understanding global eukaryotic biodiversity. Trends Ecol Evol. 2012;27:233–43. https://doi.org/10.1016/j.tree.2011.11.010.

Biscontin A, Frigato E, Sales G, et al. The opsin repertoire of the Antarctic krill *Euphausia superba*. Mar Genomics. 2016;29:61–8. https://doi.org/10.1016/j.margen.2016.04.010.

Black WC, Baer CF, Antolin MF, DuTeau NM. Population genomics: genome-wide sampling of insect populations. Annu Rev Entomol. 2001;46:441–69. https://doi.org/10.1146/annurev.ento.46.1.441.

Blanco-Bercial L, Álvarez-Marqués F, Bucklin A. Comparative phylogeography and connectivity of sibling species of the marine copepod *Clausocalanus* (Calanoida). J Exp Mar Biol Ecol. 2011;404:108–15. https://doi.org/10.1016/j.jembe.2011.05.011.

Blanco-Bercial L, Cornils A, Copley N, Bucklin A. DNA barcoding of marine copepods: assessment of analytical approaches to species identification. PLoS Curr. 2014;6. https://doi.org/10.1371/currents.tol.cdf8b74881f87e3b01d56b43791626d2.

Blanco-Bercial L, Bucklin A. New view of population genetics of zooplankton: RAD-seq analysis reveals population structure of the North Atlantic planktonic copepod *Centropages typicus*. Mol Ecol. 2016;25:1566–80. https://doi.org/10.1111/mec.13581.

Blanco-Bercial L, Maas AE. A transcriptomic resource for the northern krill Meganyctiphanes norvegica based on a short-term temperature exposure experiment. Mar Genomics. 2017. https://doi.org/10.1016/j.margen.2017.05.013.

Bolte S, Fuentes V, Haslob H, et al. Population genetics of the invasive ctenophore *Mnemiopsis leidyi* in Europe reveal source-sink dynamics and secondary dispersal to the Mediterranean Sea. Mar Ecol Prog Ser. 2013;485:25–36. https://doi.org/10.3354/meps10321.

Bortolotto E, Bucklin A, Mezzavilla M, et al. Gone with the currents: lack of genetic differentiation at the circum-continental scale in the Antarctic krill *Euphausia superba*. BMC Genet. 2011;12:32. https://doi.org/10.1186/1471-2156-12-32.

Brekhman V, Malik A, Haas B, et al. Transcriptome profiling of the dynamic life cycle of the scypohozoan jellyfish *Aurelia aurita*. BMC Genomics. 2015;16:74. https://doi.org/10.1186/s12864-015-1320-z.

Bron JE, Frisch D, Goetze E, et al. Observing copepods through a genomic lens. Front Zool. 2011;8:22. https://doi.org/10.1186/1742-9994-8-22.

Bucklin A, Kaartvedt S. Population genetics of drifting (*Calanus* spp.) and resident (*Acartia clausi*) plankton in Norwegian fjords. J Plankton Res. 2000;22:1237–51. https://doi.org/10.1093/plankt/22.7.1237.

Bucklin A, Sundt RC, Dahle G. The population genetics of *Calanus finmarchicus* in the North Atlantic. Ophelia. 1996;44:29–45.

Bucklin A, Smolenack SB, Bentley AM, Wiebe PH. Gene flow patterns of the euphausiid, *Meganyctiphanes norvegica*, in the NW Atlantic based on mtDNA sequences for cytochrome b and cytochrome oxidase I. J Plankton Res. 1997;19:1763–81. https://doi.org/10.1093/plankt/19.11.1763.

Bucklin A, Wiebe PH, Smolenack SB, et al. DNA barcodes for species identification of euphausiids (Euphausiacea, Crustacea). J Plankton Res. 2007;29:483–93. https://doi.org/10.1093/plankt/fbm031.

Bucklin A, Hopcroft RR, Kosobokova KN, et al. DNA barcoding of Arctic ocean holozooplankton for species identification and recognition. Deep Sea Res Part II Top Stud Oceanogr. 2010a;57:40–8. https://doi.org/10.1016/j.dsr2.2009.08.005.

Bucklin A, Ortman BD, Jennings RM, et al. A "Rosetta stone" for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean). Deep Sea Res Part II Top Stud Oceanogr. 2010b;57:2234–47. https://doi.org/10.1016/j.dsr2.2010.09.025.

Bucklin A, Steinke D, Blanco-Bercial L. DNA barcoding of marine metazoa. Annu Rev Mar Sci. 2011;3:471–508. https://doi.org/10.1146/annurev-marine-120308-080950.

Bucklin A, Lindeque PK, Rodriguez-Ezpeleta N, et al. Metabarcoding of marine zooplankton: prospects, progress and pitfalls. J Plankton Res. 2016;38:393–400. https://doi.org/10.1093/plankt/fbw023.

Burton RS, Byrne RJ, Rawson PD. Three divergent mitochondrial genomes from California populations of the copepod *Tigriopus californicus*. Gene. 2007;403:53–9. https://doi.org/10.1016/j.gene.2007.07.026.

Burton RS, Pereira RJ, Barreto FS. Cytonuclear genomic interactions and hybrid breakdown. Annu Rev Ecol Evol Syst. 2013;44:281–302. https://doi.org/10.1146/annurev-ecolsys-110512-135758.

Bybee SM, Bracken-Grissom HD, Hermansen RA, et al. Directed next generation sequencing for phylogenetics: an example using Decapoda (Crustacea). Zool Anz. 2011;250:497–506. https://doi.org/10.1016/j.jcz.2011.05.010.

Carlotti F, Bonnet D, Halsband-Lenk C. Development and growth rates of *Centropages typicus*. Prog Oceanogr. 2007;72:164–94. https://doi.org/10.1016/j.pocean.2007.01.011.

Castellani C, Lindley AJ, Wootton M, et al. Morphological and genetic variation in the North Atlantic copepod, *Centropages typicus*. J Mar Biol Assoc U K. 2012;92:99–106. https://doi.org/10.1017/S0025315411000932.

Caudill CC, Bucklin A. Molecular phylogeography and evolutionary history of the estuarine copepod, *Acartia tonsa*, on the Northwest Atlantic coast. Hydrobiologia. 2004;511:91–102. https://doi.org/10.1023/B:HYDR.0000014032.05680.9d.

Chen G, Hare MP. Cryptic ecological diversification of a planktonic estuarine copepod, *Acartia tonsa*. Mol Ecol. 2008;17:1451–68. https://doi.org/10.1111/j.1365-294X.2007.03657.x.

Chen G, Hare MP. Cryptic diversity and comparative phylogeography of the estuarine copepod *Acartia tonsa* on the US Atlantic coast. Mol Ecol. 2011;20:2425–41. https://doi.org/10.1111/j.1365-294X.2011.05079.x.

Choquet M, Alves Monteiro HJ, Bengtsson-Palme J, Hoarau G. The complete mitochondrial genome of the copepod *Calanus glacialis*. Mitochondrial DNA Part B. 2017a;2:506–7. https://doi.org/10.1080/23802359.2017.1361357.

Choquet M, Smolina I, Soreide JE, Hoarau G. New insight on the population structure of *Calanus finmarchicus* in the North Atlantic using next-generation sequencing technologies. In: Proceedings of the 13th international conference on Copepoda, Los Angeles, 2017b.

Chust G, Villarino E, Chenuil A, et al. Dispersal similarly shapes both population genetics and community patterns in the marine realm. Sci Rep. 2016;6:28730. https://doi.org/10.1038/srep28730.

Clark MS, Thorne MAS, Toullec JY, et al. Antarctic krill 454 pyrosequencing reveals chaperone and stress transcriptome. PLoS One. 2011;6:1–17. https://doi.org/10.1371/journal.pone.0015919.

Cornils A, Wend-Heckmann B, Held C. Global phylogeography of *Oithona similis* s.l. (Crustacea, Copepoda, Oithonidae) – a cosmopolitan plankton species or a complex of cryptic lineages? Mol Phylogenet Evol. 2017;107:473–85. https://doi.org/10.1016/j.ympev.2016.12.019.

Cowen RK, Sponaugle S. Larval dispersal and marine population connectivity. Annu Rev Mar Sci. 2009;1:443–66. https://doi.org/10.1146/annurev.marine.010908.163757.

Crawford DL, Oleksiak MF. Ecological population genomics in the marine environment. Brief Funct Genomics. 2016;15:342–51. https://doi.org/10.1093/bfgp/elw008.

Cristescu ME. Genetic reconstructions of invasion history. Mol Ecol. 2015;24:2212–25. https://doi.org/10.1111/mec.13117.

Davey JL, Blaxter MW. RADseq: next-generation population genetics. Brief Funct Genomics. 2010;9:416–23. https://doi.org/10.1093/bfgp/elq031.

Dawson MN, Cieciel K, Decker MB, et al. Population-level perspectives on global change: genetic and demographic analyses indicate various scales, timing, and causes of scyphozoan jellyfish blooms. Biol Invasions. 2015;17:851–67. https://doi.org/10.1007/s10530-014-0732-z.

De Pittà C, Biscontin A, Albiero A, et al. The Antarctic krill *Euphausia superba* shows diurnal cycles of transcription under natural conditions. PLoS One. 2013;8(7):e68652. https://doi.org/10.1371/journal.pone.0068652.

de Vargas C, Audic S, Henry N, et al. Eukaryotic plankton diversity in the sunlit ocean. Science. 2015;348:1261605. https://doi.org/10.1126/science.1261605.

De Wit P, Pespeni MH, Palumbi SR. SNP genotyping and population genomics from expressed sequences – current advances and future possibilities. Mol Ecol. 2015;24:2310–23. https://doi.org/10.1111/mec.13165.

De Wit P, Dupont S, Thor P. Selection on oxidative phosphorylation and ribosomal structure as a multigenerational response to ocean acidification in the common copepod *Pseudocalanus acuspes*. Evol Appl. 2016;9:1112–23. https://doi.org/10.1111/eva.12335.

de Young B, Barange M, Beaugrand G, et al. Regime shifts in marine ecosystems: detection, prediction and management. Trends Ecol Evol. 2008;23:402–9. https://doi.org/10.1016/j.tree.2008.03.008.

Deagle BE, Faux C, Kawaguchi S, et al. Antarctic krill population genomics: apparent panmixia, but genome complexity and large population size muddy the water. Mol Ecol. 2015;24:4943–59. https://doi.org/10.1111/mec.13370.

Denoeud F, Henriet S, Mungpakdee S. Plasticity of animal genome architecture unmasked by rapid evolution of a pelagic tunicate. Science. 2010;80:1381–6.

Drillet G, Goetze E, Jepsen PM, et al. Strain-specific vital rates in four *Acartia tonsa* cultures, I: strain origin, genetic differentiation and egg survivorship. Aquaculture. 2008;280:109–16. https://doi.org/10.1016/j.aquaculture.2008.04.005.

Dufresne F, Jeffery N. A guided tour of large genome size in animals: what we know and where we are heading. Chromosom Res. 2011;19:925–38. https://doi.org/10.1007/s10577-011-9248-x.

Edmands S. Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. Mol Ecol. 2001;10:1743–50. https://doi.org/10.1046/j.0962-1083.2001.01306.x.

Ekblom R, Galindo J. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity. 2011;107:1–15. https://doi.org/10.1038/hdy.2010.152.

Ellegren H. Genome sequencing and population genomics in non-model organisms. Trends Ecol Evol. 2014;29:51–63. https://doi.org/10.1016/j.tree.2013.09.008.

Elliott TA, Gregory TR. What's in a genome? The C-value enigma and the evolution of eukaryotic genome content. Philos Trans R Soc Lond Ser B Biol Sci. 2015;370:20140331. https://doi.org/10.1098/rstb.2014.0331.

Elshire RJ, Glaubitz JC, Sun Q, et al. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One. 2011;6:1–10. https://doi.org/10.1371/journal.pone.0019379.

Escribano R, McLaren IA, Breteler WCMK. Innate and acquired variation of nuclear DNA contents of marine copepods. Genome. 1992;35:602–10. https://doi.org/10.1139/g92-090.

Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010;10:564–7. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992;131:479–91. https://doi.org/10.1007/s00424-009-0730-7.

Eyun S, Young Soh H, Posavi M, et al. Evolutionary history of chemosensory-related gene families across the arthropoda. Mol Biol Evol. 2017;34:1838–62. https://doi.org/10.1093/molbev/msx147.

Falk-Petersen S, Pavlov V, Timofeev S, Sargent J. Climate variability and possible effects on Arctic food chains: the role of *Calanus*. In: Arctic apline ecosystems and people in a changing environment. Berlin, Heidelberg: Springer; 2007. p. 147–66.

Faure E, Casanova JP. Comparison of chaetognath mitochondrial genomes and phylogenetical implications. Mitochondrion. 2006;6:258–62. https://doi.org/10.1016/j.mito.2006.07.004.

Foley BR, Rose CG, Rundle DE, et al. A gene-based SNP resource and linkage map for the copepod *Tigriopus californicus*. BMC Genomics. 2011;12:568. https://doi.org/10.1186/1471-2164-12-568.

Foll M, Gaggiotti O. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics. 2008;180:977–93. https://doi.org/10.1534/genetics.108.092221.

Francisco SM, Robalo JI, Levy A, Almada VC. In search of phylogeographic patterns in the northeastern Atlantic and adjacent seas. In: Evolutionary biology: genome evolution, speciation, coevolutions and origin of life. Cham: Springer; 2014. p. 323–38.

Gagnaire PA, Gaggiotti OE. Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. Curr Zool. 2016;62:603–16. https://doi.org/10.1093/cz/zow088.

Gagnaire PA, Broquet T, Aurelle D, et al. Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. Evol Appl. 2015;8:769–86. https://doi.org/10.1111/eva.12288.

Galindo HM, Pfeiffer-Herbert AS, McManus MA, et al. Seascape genetics along a steep cline: using genetic patterns to test predictions of marine larval dispersal. Mol Ecol. 2010;19:3692–707. https://doi.org/10.1111/j.1365-294X.2010.04694.x.

Gayral P, Melo-Ferreira J, Glémin S, et al. Reference-free population genomics from next-generation transcriptome data and the vertebrate-invertebrate gap. PLoS Genet. 2013;9(4): e1003457. https://doi.org/10.1371/journal.pgen.1003457.

Genome 10K Community of Scientists. Genome 10K: a proposal to obtain whole-genome sequence for 10000 vertebrate species. J Hered. 2009;100:659–74. https://doi.org/10.1093/jhered/esp086.

GIGA Community of Scientists. The Global Invertebrate Genomics Alliance (GIGA): developing community resources to study diverse invertebrate genomes. J Hered. 2014;105:1–18. https://doi.org/10.1093/jhered/est084.

Goetze E. Global population genetic structure and biogeography of the oceanic copepods *Eucalanus hyalinus* and *E. spinifer*. Evolution. 2005;59:2378–98. https://doi.org/10.1554/05-077.1.

Goetze E, Ohman MD. Integrated molecular and morphological biogeography of the calanoid copepod family Eucalanidae. Deep Sea Res Part II Top Stud Oceanogr. 2010;57:2110–29. https://doi.org/10.1016/j.dsr2.2010.09.014.

Goetze E, Andrews KR, Peijnenburg KTCA, et al. Temporal stability of genetic structure in a mesopelagic copepod. PLoS One. 2015;10:1–16. https://doi.org/10.1371/journal.pone.0136087.

Gregory TR. Animal genome size databse. 2017. http://www.genomesize.com. Accessed 20 Jun 2017.

Gregory TR, Hebert PDN. The modulation of DNA content: proximate causes and ultimate consequences. Genome Res. 1999;9:317–24. https://doi.org/10.1101/gr.9.4.317.

Gregory TR, Hebert PDN, Kolasa J. Evolutionary implications of the relationship between genome size and body size in flatworms and copepods. Heredity. 2000;84:201–8. https://doi.org/10.1046/j.1365-2540.2000.00661.x.

Hahn C, Bachmann L, Chevreux B. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads – a baiting and iterative mapping approach. Nucleic Acids Res. 2013;41(13):e129. https://doi.org/10.1093/nar/gkt371.

Hansen MM, Hemmer-Hansen J. Landscape genetics goes to sea. J Biol. 2007;6:6. https://doi.org/10.1186/jbiol59.

Havird JC, Santos SR. Here we are, but where do we go? A systematic review of crustacean transcriptomic studies from 2014–2015. Integr Comp Biol. 2016;56:1055–66. https://doi.org/10.1093/icb/icw061.

Head EJH, Harris LR, Yashayaev I. Distributions of *Calanus* spp. and other mesozooplankton in the Labrador Sea in relation to hydrography in spring and summer (1995–2000). Prog Oceanogr. 2003;59:1–30. https://doi.org/10.1016/S0079-6611(03)00111-3.

Hedrick PW. Genetic polymorphism in heterogeneous environments: the age of genomics. Annu Rev Ecol Evol Syst. 2006;37:67–93. https://doi.org/10.1146/annurev.ecolsys.37.091305.110132.

Helfenbein KG, Fourcade HM, Vanjani RG, Boore JL. The mitochondrial genome of *Paraspadella gotoi* is highly reduced and reveals that chaetognaths are a sister group to protostomes. Proc Natl Acad Sci U S A. 2004;101:10639–43. https://doi.org/10.1073/pnas.0400941101.

Hellberg ME. Gene flow and isolation among populations of marine animals. Annu Rev Ecol Evol Syst. 2009;40:291–310. https://doi.org/10.1146/annurev.ecolsys.110308.120223.

Helyar SJ, Hemmer-Hansen J, Bekkevold D, et al. Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. Mol Ecol Resour. 2011;11:123–36. https://doi.org/10.1111/j.1755-0998.2010.02943.x.

Hemmer-Hansen J, Overgaard N, Hemmer-Hansen J, et al. Population genomics of marine fishes: next generation prospects and challenges. Biol Bull. 2014;227:117–32. https://doi.org/10.1086/BBLv227n2p117.

Hessen DO, Persson J. Genome size as a determinant of growth and life-history traits in crustaceans. Biol J Linn Soc. 2009;98:393–9. https://doi.org/10.1111/j.1095-8312.2009.01285.x.

Hirai J, Tsuda A. Metagenetic community analysis of epipelagic planktonic copepods in the tropical and subtropical pacific. Mar Ecol Prog Ser. 2015;534:65–78. https://doi.org/10.3354/meps11404.

Hirai J, Tsuda A, Goetze E. Extensive genetic diversity and endemism across the global range of the oceanic copepod *Pleuromamma abdominalis*. Prog Oceanogr. 2015;138:77–90. https://doi.org/10.1016/j.pocean.2015.09.002.

Hodges E, Xuan Z, Balija V, et al. Genome-wide in situ exon capture for selective resequencing. Nat Genet. 2007;39:1522–7. https://doi.org/10.1038/ng.2007.42.

Hwang D-S, Park E, Won Y-J, et al. Complete mitochondrial genome of the jellyfish, *Chrysaora quinquecirrha* (Cnidaria, Scyphozoa). Mitochondrial DNA. 2014;25:25–6. https://doi.org/10.3109/19401736.2013.775272.

i5K Consortium. The i5K initiative: advancing arthropod genomics for knowledge, human health, agriculture, and the environment. J Hered. 2013;104:595–600. https://doi.org/10.1093/jhered/est050.

Iacchei M, Butcher E, Portner E, Goetze E. It's about time: insights into temporal genetic patterns in oceanic zooplankton from biodiversity indices. Limnol Oceanogr. 2017;62(5):1836–52. https://doi.org/10.1002/lno.10538.

Jarman SN, Deagle BE. Genetics of Antarctic krill. In: Siegel V, editor. Biology and ecology of Antarctic krill, Advances in polar ecology. Cham: Springer; 2016. p. 247–77.

Jeffery NW. The first genome size estimates for six species of krill (Malacostraca, Euphausiidae): large genomes at the north and south poles. Polar Biol. 2012;35:959–62. https://doi.org/10.1007/s00300-011-1137-4.

Jeffery NW. Genome size diversity and evolution in the crustacea. Guelph: University of Guelph; 2015.

Jeffery NW, Ellis EA, Oakley TH, Gregory TR. The genome sizes of ostracod crustaceans correlate with body size and evolutionary history, but not environment. J Hered. 2017;108:701–6. https://doi.org/10.1093/jhered/esx055.

Jepsen PM, Bjørbæk NS, Rayner TA, et al. Recommended feeding regime and light climate in live feed cultures of the calanoid copepod *Acartia tonsa* Dana. Aquac Int. 2017;25:635–54. https://doi.org/10.1007/s10499-016-0063-4.

Johnson KM, Hofmann GE. A transcriptome resource for the Antarctic pteropod *Limacina helicina antarctica*. Mar Genomics. 2016;28:25–8. https://doi.org/10.1016/j.margen.2016.04.002.

Jones MR, Good JM. Targeted capture in evolutionary and ecological genomics. Mol Ecol. 2016;25:185–202. https://doi.org/10.1111/mec.13304.

Jue NK, Batta-Lona PG, Trusiak S, et al. Rapid evolutionary rates and unique genomic signatures discovered in the first reference genome for the southern ocean salp, *Salpa thompsoni* (Urochordata, Thaliacea). Genome Biol Evol. 2016;8:3171–86. https://doi.org/10.1093/gbe/evw215.

Jung SO, Lee YM, Park TJ, et al. The complete mitochondrial genome of the intertidal copepod *Tigriopus* sp. (Copepoda, Harpactidae) from Korea and phylogenetic considerations. J Exp Mar Bio Ecol. 2006;333:251–62. https://doi.org/10.1016/j.jembe.2005.12.047.

Kang S, Ahn D, Lee JH, et al. The genome of the Antarctic-endemic copepod, *Tigriopus kingsejongensis*. Gigascience. 2017;6(1):1–9. https://doi.org/10.1093/gigascience/giw010.

Kayal E, Bentlage B, Collins AG, et al. Evolution of linear mitochondrial genomes in medusozoan cnidarians. Genome Biol Evol. 2011;4:1–12. https://doi.org/10.1093/gbe/evr123.

Ki JS, Lee KW, Park HG, et al. Phylogeography of the copepod *Tigriopus japonicus* along the Northwest Pacific rim. J Plankton Res. 2009;31:209–21. https://doi.org/10.1093/plankt/fbn100.

Ki JS, Hop H, Kim SJ, et al. Complete mitochondrial genome sequence of the Arctic gammarid, *Onisimus nanseni* (Crustacea; Amphipoda): novel gene structures and unusual control region features. Comp Biochem Physiol Part D Genomics Proteomics. 2010;5:105–15. https://doi.org/10.1016/j.cbd.2010.02.002.

Kim S, Kim J, Choi H-G, et al. Complete mitochondrial genome of the northern mauxia shrimp *Acetes chinensis* (Decapoda, Dendrobranchiata, Sergestoidae). Mitochondrial DNA. 2012;23:28–30. https://doi.org/10.3109/19401736.2011.643878.

Kim S, Lim BJ, Min GS, Choi HG. The complete mitochondrial genome of Arctic *Calanus hyperboreus* (Copepoda, Calanoida) reveals characteristic patterns in calanoid mitochondrial genome. Gene. 2013;520:64–72. https://doi.org/10.1016/j.gene.2012.09.059.

Kim HS, Lee BY, Won EJ, et al. Identification of xenobiotic biodegradation and metabolism-related genes in the copepod *Tigriopus japonicus* whole transcriptome analysis. Mar Genomics. 2015;24:207–8. https://doi.org/10.1016/j.margen.2015.05.011.

Kim HS, Hwang DS, Lee BY, et al. De novo assembly and annotation of the marine mysid (Neomysis awatschensis) transcriptome. Mar Genomics. 2016;28:41–3. https://doi.org/10.1016/j.margen.2016.05.001.

Knowles LL. Statistical phylogeography. Annu Rev Ecol Evol Syst. 2009;40:593–612. https://doi.org/10.1146/annurev.ecolsys.38.091206.095702.

Koh HY, Lee JH, Han SJ, et al. A transcriptomic analysis of the response of the arctic pteropod *Limacina helicina* to carbon dioxide-driven seawater acidification. Polar Biol. 2015;38:1727–40. https://doi.org/10.1007/s00300-015-1738-4.

Kohn AB, Citarella MR, Kocot KM, et al. Rapid evolution of the compact and unusual mitochondrial genome in the ctenophore, *Pleurobrachia bachei*. Mol Phylogenet Evol. 2012;63:203–7. https://doi.org/10.1016/j.ympev.2011.12.009.

Kollias S, Poortvliet M, Smolina I, Hoarau G. Low cost sequencing of mitogenomes from museum samples using baits capture and ion torrent. Conserv Genet Resour. 2015;7:345–8. https://doi.org/10.1007/s12686-015-0433-7.

Kool JT, Moilanen A, Treml EA. Population connectivity: recent advances and new perspectives. Landsc Ecol. 2013;28:165–85. https://doi.org/10.1007/s10980-012-9819-z.

Kulagin DN, Stupnikova AN, Neretina TV, Mugue NS. Spatial genetic heterogeneity of the cosmopolitan chaetognath *Eukrohnia hamata* (Möbius, 1875) revealed by mitochondrial DNA. Hydrobiologia. 2014;721:197–207. https://doi.org/10.1007/s10750-013-1661-z.

Kuriyama M, Nishida S. Species diversity and niche-partitioning in the pelagic copepods of the family Scolecitrichidae (Calanoida). Crustaceanna. 2006;79:293–317.

Laakmann S, Auel H, Kochzius M. Evolution in the deep sea: biological traits, ecology and phylogenetics of pelagic copepods. Mol Phylogenet Evol. 2012;65:535–46. https://doi.org/10.1016/j.ympev.2012.07.007.

Lauritano C, Procaccini G, Ianora A. Gene expression patterns and stress response in marine copepods. Mar Environ Res. 2012;76:22–31. https://doi.org/10.1016/j.marenvres.2011.09.015.

Lechner M, Marz M, Ihling C, et al. The correlation of genome size and DNA methylation rate in metazoans. Theory Biosci. 2013;132:47–60. https://doi.org/10.1007/s12064-012-0167-y.

Lee BY, Kim HS, Choi BS, et al. RNA-seq based whole transcriptome analysis of the cyclopoid copepod *Paracyclopina nana* focusing on xenobiotics metabolism. Comp Biochem Physiol Part D Genomics Proteomics. 2015;15:12–9. https://doi.org/10.1016/j.cbd.2015.04.002.

Lee CE. Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations". Evolution. 2000;54:2014–27. https://doi.org/10.1111/j.0014-3820.2000.tb01245.x.

Lee CE. Evolutionary mechanisms of habitat invasions, using the copepod *Eurytemora affinis* as a model system. Evol Appl. 2016a;9:248–70. https://doi.org/10.1111/eva.12334.

Lee JS. Transcriptome profiling of the Antarctic copepod Tigriopus kingsejongensis (Crustacea, Harpacticoida) by Illumina RNA-seq. BioProject Acc. No. PRJNA283925. Direct Submission 2016b. https://www.ncbi.nlm.nih.gov/nuccore/859378166

Leinaas HP, Jalal M, Gabrielsen TM, Hessen DO. Inter- and intraspecific variation in body- and genome size in calanoid copepods from temperate and arctic waters. Ecol Evol. 2016;6:5585–95. https://doi.org/10.1002/ece3.2302.

Lenz PH, Roncalli V, Hassett RP, et al. De novo assembly of a transcriptome for *Calanus finmarchicus* (crustacea, copepoda) – the dominant zooplankter of the North Atlantic Ocean. PLoS One. 2014;9(2):e88589. https://doi.org/10.1371/journal.pone.0088589.

Leray M, Knowlton N. Censusing marine eukaryotic diversity in the twenty-first century. Philos Trans R Soc London Ser B. 2016;371:1–9. https://doi.org/10.1098/rstb.2015.0331.

Levasseur A, Orlando L, Bailly X, et al. Conceptual bases for quantifying the role of the environment on gene evolution: the participation of positive selection and neutral evolution. Biol Rev. 2007;82:551–72. https://doi.org/10.1111/j.1469-185X.2007.00024.x.

Levin SA, Segel LA. Hypothesis for origin of planktonic patchiness. Nature. 1976;259:659. https://doi.org/10.1038/259659a0.

Li F, Ma L, Zhang H, et al. A thioredoxin from Antarctic microcrustacean (*Euphausia superba*): cloning and functional characterization. Fish Shellfish Immunol. 2017a;63:376–83. https://doi.org/10.1016/j.fsi.2017.02.035.

Li P, Yang M, Ni S, et al. Complete mitochondrial genome sequence of the pelagic chaetognath, *Sagitta ferox*. Mitochondrial DNA. 2016;1736:1–2. https://doi.org/10.3109/19401736.2015.1106508.

Li Y, Zhou Z, Tian M, et al. Exploring single nucleotide polymorphism (SNP), microsatellite (SSR) and differentially expressed genes in the jellyfish (Rhopilema esculentum) by transcriptome sequencing. Mar Genomics. 2017b;34:31–7. https://doi.org/10.1016/j.margen.2017.01.007.

Lima TG, Willett CS. Locally adapted populations of a copepod can evolve different gene expression patterns under the same environmental pressures. Ecol Evol. 2017;7:4312–25. https://doi.org/10.1002/ece3.3016.

Lindeque PK, Parry HE, Harmer RA, et al. Next generation sequencing reveals the hidden diversity of zooplankton assemblages. PLoS One. 2013;8:1–14. https://doi.org/10.1371/journal.pone.0081327.

Longhurst AR. Ecological geography of the sea. Amsterdam: Elsevier; 2007.

Luikart G, England PR, Tallmon D, et al. The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet. 2003;4:981–94. https://doi.org/10.1038/nrg1226.

Maas AE, Lawson GL, Tarrant AM. Transcriptome-wide analysis of the response of the thecosome pteropod *Clio pyramidata* to short-term CO_2 exposure. Comp Biochem Physiol Part D Genomics Proteomics. 2015;16:1–9. https://doi.org/10.1016/j.cbd.2015.06.002.

Machida RJ, Miya MU, Nishida M, Nishida S. Complete mitochondrial DNA sequence of *Tigriopus japonicus* (Crustacea: Copepoda). Mar Biotechnol. 2002;4:406–17. https://doi.org/10.1007/s10126-002-0033-x.

Madoui M-A, Poulain J, Sugier K, et al. New insights into global biogeography, population structure and natural selection from the genome of the epipelagic copepod *Oithona*. Mol Ecol. 2017;38:42–9. https://doi.org/10.1111/mec.14214.

Maricic T, Whitten M, Pääbo S. Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. PLoS One. 2010;5:9–13. https://doi.org/10.1371/journal.pone.0014004.

Marlétaz F, Parco Y, Shenglin L, Peijnenburg KTCA. Extreme mitogenomic variation in natural populations of chaetognaths. Genome Biol Evol. 2017;9:1–21. https://doi.org/10.1093/gbe/evx090.

McGovern TM, Keever CC, Saski CA, et al. Divergence genetics analysis reveals historical population genetic processes leading to contrasting phylogeographic patterns in co-distributed species. Mol Ecol. 2010;19:5043–60. https://doi.org/10.1111/j.1365-294X.2010.04854.x.

McLaren IA, Sevigny J-M, Corkett CJ. Body sizes, development rates, and genome sizes among *Calanus* species. Hydrobiologia. 1988;167/168:275–84.

McLaren IA, Laberge E, Corkett CJ, Sevigny J-M. Life cycles of four species of *Pseudocalanus* in Nova Scotia. Can J Zool. 1989;67:552–8. https://doi.org/10.1139/z89-078.

Meyer B, Martini P, Biscontin A, et al. Pyrosequencing and de novo assembly of Antarctic krill (*Euphausia superba*) transcriptome to study the adaptability of krill to climate-induced environmental changes. Mol Ecol Resour. 2015;15:1460–71. https://doi.org/10.1111/1755-0998.12408.

Miller B, von der Heyden S, Gibbons M. Significant population genetic structuring of the holoplanktic scyphozoan *Pelagia noctiluca* in the Atlantic Ocean. Afr J Mar Sci. 2012;34:425–30. https://doi.org/10.2989/1814232X.2012.726646.

Milligan PJ, Stahl EA, Schizas NV, Turner JT. Phylogeography of the copepod *Acartia hudsonica* in estuaries of the northeastern United States. Hydrobiologia. 2011;666:155–65. https://doi.org/10.1007/s10750-010-0097-y.

Minxiao W, Song S, Chaolun L, Xin S. Distinctive mitochondrial genome of Calanoid copepod *Calanus sinicus* with multiple large non-coding regions and reshuffled gene order: useful molecular markers for phylogenetic and population studies. BMC Genomics. 2011;12:73. https://doi.org/10.1186/1471-2164-12-73.

Miyamoto H, Machida RJ, Nishida S. Genetic diversity and cryptic speciation of the deep sea chaetognath *Caecosagitta macrocephala* (Fowler, 1904). Deep Res Part II Top Stud Oceanogr. 2010;57:2211–9. https://doi.org/10.1016/j.dsr2.2010.09.023.

Miyamoto H, Machida RJ, Nishida S. Global phylogeography of the deep-sea pelagic chaetognath *Eukrohnia hamata*. Prog Oceanogr. 2012;104:99–109. https://doi.org/10.1016/j.pocean.2012.06.003.

Moroz LL, Kocot KM, Citarella MR, et al. The ctenophore genome and the evolutionary origins of neutral systems. Nature. 2014;510:109–14. https://doi.org/10.1038/nature13400.

Munro JB, Posavi M, Brady A, et al. Sex-biased gene expression in the common copepod Eurytemora affinis. BioProject Acc. No. PRJNA278152. Direct Submission 2015. https://www.ncbi.nlm.nih.gov/nuccore/1102726653

Narum SR, Buerkle CA, Davey JW, et al. Genotyping-by-sequencing in ecological and conservation genomics. Mol Ecol. 2013;22:2841–7. https://doi.org/10.1111/mec.12350.

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D. Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol. 2009;18:3128–50. https://doi.org/10.1111/j.1365-294X.2009.04272.x.

Nilsson B, Jepsen PM, Rewitz K, Hansen BW. Expression of hsp70 and ferritin in embryos of the copepod *Acartia tonsa* (Dana) during transition between subitaneous and quiescent state. J Plankton Res. 2014;36:513–22. https://doi.org/10.1093/plankt/fbt099.

Norton EL, Goetze E. Equatorial dispersal barriers and limited population connectivity among oceans in a planktonic copepod. Limnol Oceanogr. 2013;58:1581–96. https://doi.org/10.4319/lo.2013.58.5.1581.

O'Grady JF, Hoelters LS, Swain MT, Wilcockson DC. Identification and temporal expression of putative circadian clock transcripts in the amphipod crustacean *Talitrus saltator*. Peer J. 2016;4:e2555. https://doi.org/10.7717/peerj.2555.

Ogoh K, Ohmiya Y. Complete mitochondrial DNA sequence of the sea-firefly, *Vargula hilgendorfii* (Crustacea, Ostracoda) with duplicate control regions. Gene. 2004;327:131–9. https://doi.org/10.1016/j.gene.2003.11.011.

Omori M, Hamner WM. Patchy distribution of zooplankton: behavior, population assessment and sampling problems. Mar Biol. 1982;72:193–200. https://doi.org/10.1007/BF00396920.

Papadopoulos LN, Peijnenburg KTCA, Luttikhuizen PC. Phylogeography of the calanoid copepods *Calanus helgolandicus* and *C. euxinus* suggests Pleistocene divergences between Atlantic, Mediterranean, and Black Sea populations. Mar Biol. 2005;147:1353–65. https://doi.org/10.1007/s00227-005-0038-x.

Papetti C, Zane L, Bortolotto E, et al. Genetic differentiation and local temporal stability of population structure in the euphausiid *Meganyctiphanes norvegica*. Mar Ecol Prog Ser. 2005;289:225–35. https://doi.org/10.3354/meps289225.

Papillon D, Perez Y, Caubit X, Le Parco Y. Identification of chaetognaths as protostomes is supported by the analysis of their mitochondrial genome. Mol Biol Evol. 2004;21:2122–9. https://doi.org/10.1093/molbev/msh229.

Papot C, Cascella K, Toullec JY, Jollivet D. Divergent ecological histories of two sister Antarctic krill species led to contrasted patterns of genetic diversity in their heat-shock protein (hsp70) arsenal. Ecol Evol. 2016;6:1555–75. https://doi.org/10.1002/ece3.1989.

Patarnello T, Papetti C, Zane L. Genetics of Northern krill (*Megantyctiphanes norvegica* Sars). In: Tarling G (ed) Biology of Northern krill, vol 57. Cambridge: Academic Press; 2010.

Pearman JK, Irigoien X. Assessment of zooplankton community composition along a depth profile in the central Red Sea. PLoS One. 2015;10:1–14. https://doi.org/10.1371/journal.pone.0133487.

Peijnenburg KTCA, Goetze E. High evolutionary potential of marine zooplankton. Ecol Evol. 2013;3:2765–83. https://doi.org/10.1002/ece3.644.

Peijnenburg KTCA, Breeuwer JAJ, Pierrot-Bults AC, Menken SBJ. Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. Evolution. 2004;58:1472–87. https://doi.org/10.1554/03-638.

Peijnenburg KTCA, Van Haastrecht EK, Fauvelot C. Present-day genetic composition suggests contrasting demographic histories of two dominant chaetognaths of the North-East Atlantic, *Sagitta elegans* and *S. setosa*. Mar Biol. 2005;147:1279–89. https://doi.org/10.1007/s00227-005-0041-2.

Peijnenburg KTCA, Fauvelot C, Breeuwer JAJ, Menken SBJ. Spatial and temporal genetic structure of the planktonic *Sagitta setosa* (Chaetognatha) in European seas as revealed by mitochondrial and nuclear DNA markers. Mol Ecol. 2006;15:3319–38. https://doi.org/10.1111/j.1365-294X.2006.03002.x.

Pereira RJ, Barreto FS, Pierce NT, et al. Transcriptome-wide patterns of divergence during allopatric evolution. Mol Ecol. 2016;25:1478–93. https://doi.org/10.1111/mec.13579.

Pereira RJ, Sasaki MC, Burton RS. Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity. Proc R Soc B Biol Sci. 2017;284:20170236. https://doi.org/10.1098/rspb.2017.0236.

Peterson BK, Weber JN, Kay EH, et al. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One. 2012;7(5):e37135. https://doi.org/10.1371/journal.pone.0037135.

Petkeviciute E, Kania PW, Skovgaard A. Genetic responses of the marine copepod *Acartia tonsa* (Dana) to heat shock and epibiont infestation. Aquacult Rep. 2015;2:10–6. https://doi.org/10.1016/j.aqrep.2015.04.001.

Pett W, Ryan JF, Pang K, et al. Extreme mitochondrial evolution in the ctenophore *Mnemiopsis leidyi*: insight from mtDNA and the nuclear genome. Mitochondrial DNA. 2011;22:130–42. https://doi.org/10.3109/19401736.2011.624611.

Planque B, Graeme Hay IC, Ibanez F, Gamble JC. Large scale spatial variations in the seasonal abundance of *Calanus finmarchicus*. Deep Res Part I Top Stud Oceanogr. 1997;44:315–26.

Pogson GH. Studying the genetic basis of speciation in high gene flow marine invertebrates. Curr Zool. 2016;62:643–53. https://doi.org/10.1093/cz/zow093.

Prokopowich CD, Gregory TR, Crease TJ. The correlation between rDNA copy number and genome size in eukaryotes. Genome. 2003;46:48–50. https://doi.org/10.1139/g02-103.

Provan J, Beatty GE, Keating SL, et al. High dispersal potential has maintained long-term population stability in the North Atlantic copepod *Calanus finmarchicus*. Proc Biol Sci. 2009;276:301–7. https://doi.org/10.1098/rspb.2008.1062.

Questel JM, Blanco-Bercial L, Hopcroft RR, Bucklin A. Phylogeography and connectivity of the *Pseudocalanus* (Copepoda: Calanoida) species complex in the eastern North Pacific and the Pacific Arctic region. J Plankton Res 2016; 1–14. https://doi.org/10.1093/plankt/fbw025.

Rahlff J, Peters J, Moyano M, et al. Short-term molecular and physiological responses to heat stress in neritic copepods *Acartia tonsa* and *Eurytemora affinis*. Comp Biochem Physiol Part A Mol Integr Physiol. 2017;203:348–58. https://doi.org/10.1016/j.cbpa.2016.11.001.

Raisuddin S, Kwok KWH, Leung KMY, et al. The copepod Tigriopus: a promising marine model organism for ecotoxicology and environmental genomics. Aquat Toxicol. 2007;83:161–73. https://doi.org/10.1016/j.aquatox.2007.04.005.

Ramos AA, Weydmann A, Cox CJ, et al. A transcriptome resource for the copepod *Calanus glacialis* across a range of culture temperatures. Mar Genomics. 2015;23:27–9. https://doi.org/10.1016/j.margen.2015.03.014.

Rasch EM, Lee CE, Wyngaard GA. DNA-Feulgen cytophotometric determination of genome size for the freshwater-invading copepod *Eurytemora affinis*. Genome. 2004;47:559–64. https://doi.org/10.1139/G04-014.

Rawson PD, Brazeau DA, Burton RS. Isolation and characterization of cytochrome c from the marine copepod *Tigriopus californicus*. Gene. 2000;248:15–22.

Reitzel AM, Herrera S, Layden MJ, et al. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Mol Ecol. 2013;22:2953–70. https://doi.org/10.1111/mec.12228.

Renaut S, Dion-Côté AM. History repeats itself: genomic divergence in copepods. Mol Ecol. 2016;25:1417–9. https://doi.org/10.1111/mec.13577.

Riginos C, Crandall ED, Liggins L, et al. Navigating the currents of seascape genomics: how spatial analyses can augment population genomic studies. Curr Zool. 2016;62:581–601. https://doi.org/10.1093/cz/zow067.

Romero IG, Ruvinsky I, Gilad Y. Comparative studies of gene expression and the evolution of gene regulation. Nat Rev Genet. 2014;13:505–16. https://doi.org/10.1038/nrg3229.Comparative.

Roncalli V, Cieslak MC, Lenz PH. Transcriptomic responses of the calanoid copepod *Calanus finmarchicus* to the saxitoxin producing dinoflagellate *Alexandrium fundyense*. Nat Publ Group 2016; 1–13. https://doi.org/10.1038/srep25708.

Ryan JF, Pang K, Schnitzler CE, et al. The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. Science. 2013;342:1242592. https://doi.org/10.1126/science.1242592.

Saborowski R, Buchholz F. Metabolic properties of northern krill, *Meganyctiphanes norvegica*, from different climatic zones. I. Respiration and excretion. Mar Biol. 2002;140:547–56. https://doi.org/10.1007/s00227-001-0730-4.

Sales G, Deagle BE, Calura E, et al. KrillDB: a de novo transcriptome database for the Antarctic krill (*Euphausia superba*). PLoS One. 2017;12:1–12. https://doi.org/10.1371/journal.pone.0171908.

Sanchez Alvarado A. Transcriptome - adult Mnemiopsis leidyi. BioProject Acc. No. PRJNA344880. Direct Submission 2016. https://mnemiopsis.github.io/transcriptome.html

Savolainen O, Lascoux M, Merilä J. Ecological genomics of local adaptation. Nat Rev Genet. 2013;14:807–20. https://doi.org/10.1038/nrg3522.

Schindel DE, Miller SE. DNA barcoding a useful tool for taxonomists. Nature. 2005;435:17.

Schlötterer C, Tobler R, Kofler R, Nolte V. Sequencing pools of individuals – mining genome-wide polymorphism data without big funding. Nat Rev Genet. 2014;15:749–63. https://doi.org/10.1038/nrg3803.

Schoville SD, Barreto FS, Moy GW, et al. Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcriptome of the copepod *Tigriopus californicus*. BMC Evol Biol. 2012;12:170. https://doi.org/10.1186/1471-2148-12-170.

Schunter C, Carreras-Carbonell J, MacPherson E, et al. Matching genetics with oceanography: directional gene flow in a Mediterranean fish species. Mol Ecol. 2011;20:5167–81. https://doi.org/10.1111/j.1365-294X.2011.05355.x.

Shao Z, Graf S, Chaga OY, Lavrov DV. Mitochondrial genome of the moon jelly *Aurelia aurita* (Cnidaria, Scyphozoa): a linear DNA molecule encoding a putative DNA-dependent DNA polymerase. Gene. 2006;381:92–101. https://doi.org/10.1016/j.gene.2006.06.021.

Shen X, Wang H, Ren J, et al. The mitochondrial genome of *Euphausia superba* (Prydz Bay) (Crustacea: Malacostraca: Euphausiacea) reveals a novel gene arrangement and potential molecular markers. Mol Biol Rep. 2010;37:771–84. https://doi.org/10.1007/s11033-009-9602-7.

Shen X, Wang H, Wang M, Liu B. The complete mitochondrial genome sequence of *Euphausia pacifica* (Malacostraca: Euphausiacea) reveals a novel gene order and unusual tandem repeats. Genome. 2011;54:911–22. https://doi.org/10.1139/g11-053.

Sherman CDH, Lotterhos KE, Richardson MF, et al. What are we missing about marine invasions? Filling in the gaps with evolutionary genomics. Mar Biol. 2016;163:1–24. https://doi.org/10.1007/s00227-016-2961-4.

Siegel V, Watkins JL. Distribution, biomass and demography of Antarctic krill, *Euphausia superba*. In: Volker S, editor. Biology and ecology of Antarctic krill. Cham: Springer; 2016. p. 21–100.

Skjoldal HR, Wiebe PH, Postel L, et al. Intercomparison of zooplankton (net) sampling systems: results from the ICES/GLOBEC sea-going workshop. Prog Oceanogr. 2013;108:1–42. https://doi.org/10.1016/j.pocean.2012.10.006.

Smolina I. *Calanus* in the North Atlantic: species identification, stress response, and population genetic structure. PhD Aquatic Biosciences 2015; 15, p 64.

Smolina I, Kollias S, Poortvliet M, et al. Genome- and transcriptome-assisted development of nuclear insertion/deletion markers for *Calanus* species (Copepoda: Calanoida) identification. Mol Ecol Resour. 2014;14:1072–9. https://doi.org/10.1111/1755-0998.12241.

Smolina I, Kollias S, Møller E, et al. Contrasting transcriptome response to thermal stress in two key zooplankton species, *Calanus finmarchicus* and *C. glacialis*. Mar Ecol Prog Ser. 2015;534:79–93. https://doi.org/10.3354/meps11398.

Smolina I, Harmer R, Lindeque P, Hoarau G. Reduced up-regulation of gene expression in response to elevated temperatures in the mid-Atlantic population of *Calanus finmarchicus*. J Exp Mar Biol Ecol. 2016;485:88–93. https://doi.org/10.1016/j.jembe.2016.09.003.

Stapley J, Reger J, Feulner PGD, et al. Adaptation genomics: the next generation. Trends Ecol Evol. 2010;25:705–12. https://doi.org/10.1016/j.tree.2010.09.002.

Stopar K, Ramšak A, Trontelj P, Malej A. Lack of genetic structure in the jellyfish *Pelagia noctiluca* (Cnidaria: Scyphozoa: Semaeostomeae) across European seas. Mol Phylogenet Evol. 2010;57:417–28. https://doi.org/10.1016/j.ympev.2010.07.004.

Sun C, Wyngaard G, Walton DB, et al. Billions of basepairs of recently expanded, repetitive sequences are eliminated from the somatic genome during copepod development. BMC Genomics. 2014;15:186. https://doi.org/10.1186/1471-2164-15-186.

Sun C, Zhao Y, Li H, et al. Unreliable quantitation of species abundance based on high-throughput sequencing data of zooplankton communities. Aquat Biol. 2015;24:9–15. https://doi.org/10.3354/ab00629.

Tarrant AM, Baumgartner MF, Hansen BH, et al. Transcriptional profiling of reproductive development, lipid storage and molting throughout the last juvenile stage of the marine copepod *Calanus finmarchicus*. Front Zool. 2014;11:91. https://doi.org/10.1186/s12983-014-0091-8.

Thabet AA, Maas AE, Saber SA, Tarrant AM. Assembly of a reference transcriptome for the gymnosome pteropod *Clione limacina* and profiling responses to short-term CO_2 exposure. Mar Genomics. 2017;34:39–45. https://doi.org/10.1016/j.margen.2017.03.003.

Toews DPL, Brelsford A. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol. 2012;21:3907–30. https://doi.org/10.1111/j.1365-294X.2012.05664.x.

Toullec JY, Corre E, Bernay B, et al. Transcriptome and peptidome characterisation of the main neuropeptides and peptidic hormones of a euphausiid: the Ice krill, *Euphausia crystallorophias*. PLoS One. 2013;8(8):e71609. https://doi.org/10.1371/journal.pone.0071609.

Tsagkogeorga G, Cahais V, Galtier N. The population genomics of a fast evolver: high levels of diversity, functional constraint, and molecular adaptation in the tunicate *Ciona intestinalis*. Genome Biol Evol. 2012;4:740–9. https://doi.org/10.1093/gbe/evs054.

Unal E, Bucklin A. Basin-scale population genetic structure of the planktonic copepod *Calanus finmarchicus* in the North Atlantic Ocean. Prog Oceanogr. 2010;87:175–85. https://doi.org/10.1016/j.pocean.2010.09.017.

Voolstra CR, Wörheide G, Lopez JV. Advancing genomics through the Global Invertebrate Genomics Alliance (GIGA). Invertebr Syst. 2017;31:1–7. https://doi.org/10.1071/IS16059.

Wang S, Meyer E, McKay JK, Matz MV. 2b-RAD: a simple and flexible method for genome-wide genotyping. Nat Methods. 2012;9:808–10. https://doi.org/10.1038/nmeth.2023.

Wang K, Omotezako T, Kishi K, et al. Maternal and zygotic transcriptomes in the appendicularian, *Oikopleura dioica*: novel protein-encoding genes, intra-species sequence variations, and trans-spliced RNA leader. Dev Genes Evol. 2015;225:149–59. https://doi.org/10.1007/s00427-015-0502-7.

Waples RS. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J Hered. 1998;89:438–50. https://doi.org/10.1093/jhered/89.5.438.

Waples RS, Punt AE, Cope JM. Integrating genetic data into management of marine resources: how can we do it better? Fish Fish. 2008;9:423–49. https://doi.org/10.1111/j.1467-2979.2008.00303.x.

Weersing K, Toonen RJ. Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser. 2009;393:1–12. https://doi.org/10.3354/meps08287.

Wei S, Li P, Yang M, et al. The mitochondrial genome of the pelagic chaetognath, *Pterosagitta draco*. Mitochondrial DNA Part B. 2016;1:515–6. https://doi.org/10.1080/23802359.2016.1197055.

Weydmann A, Przyłucka A, Lubośny M, et al. Mitochondrial genomes of the key zooplankton copepods Arctic Calanus glacialis and North Atlantic Calanus finmarchicus with the longest crustacean non-coding regions. Sci Rep. 2017;7:13702. https://doi.org/10.1038/s41598-017-13807.

Whitehead A. Comparative genomics in ecological physiology: toward a more nuanced understanding of acclimation and adaptation. J Exp Biol. 2012;215:884–91. https://doi.org/10.1242/jeb.058735.

Wiebe PH. Plankton patchiness: effects on repeated net tows. Limnol Oceanogr. 1968;13:315–21. https://doi.org/10.4319/lo.1968.13.2.0315.

Wiebe PH, Harris RP, St. John MA, et al. BASIN: basin-scale analysis, synthesis, and integration. Science Plan and Implementation Strategy. GLOBEC Report 27: iii 2009; p 43.

Wiebe PH, Bucklin A, Madin L, et al. Deep-sea sampling on CMarZ cruises in the Atlantic Ocean – an introduction. Deep Sea Res Part II Top Stud Oceanogr. 2010;57:2157–66. https://doi.org/10.1016/j.dsr2.2010.09.018.

Wiebe PH, Lawson GL, Lavery AC, et al. Improved agreement of net and acoustical methods for surveying euphausiids by mitigating avoidance using a net-based LED strobe light system. ICES J Mar Sci. 2013;70:650–64. https://doi.org/10.1093/icesjms/fsr005.

Wyngaard GA, Rasch EM. Patterns of genome size in the copepoda. Hydrobiologia. 2000;417:43–56. https://doi.org/10.1023/A:1003855322358.

Wyngaard GA, McLaren IA, White MM, Sévigny JM. Unusually high numbers of ribosomal RNA genes in copepods (Arthropoda: Crustacea) and their relationship to genome size. Genome. 1995;38:97–104. https://doi.org/10.1139/g95-012.

Wyngaard GA, Rasch EM, Connelly BA. Unusual augmentation of germline genome size in *Cyclops kolensis* (Crustacea, Copepoda): further evidence in support of a revised model of chromatin diminution. Chromosom Res. 2011;19:911–23. https://doi.org/10.1007/s10577-011-9234-3.

Yang EJ, Ha HK, Kang S-H. Microzooplankton community structure and grazing impact on major phytoplankton in the Chukchi sea and the western Canada basin, Arctic ocean. Deep Sea Res Part II Top Stud Oceanogr. 2014;120:91–102. https://doi.org/10.1016/j.dsr2.2014.05.020.

Zane L, Patarnello T. Krill: a possible model for investigating the effects of ocean currents on the genetic structure of a pelagic invertebrate. Can J Fish Aquat Sci. 2000;57:16–23. https://doi.org/10.1139/f00-166.

Zane L, Ostellari L, Maccatrozzo L, et al. Molecular evidence for genetic subdivision of Antarctic krill (*Euphausia superba* Dana) populations. Proc Biol Sci. 1998;265:2387–91.

Zane L, Ostellari L, Maccatrozzo L, et al. Genetic differentiation in a pelagic crustacean (*Meganyctiphanes norvegica*: Euphausiacea) from the north East Atlantic and the Mediterranean Sea. Mar Biol. 2000;136:191–9. https://doi.org/10.1007/s002270050676.

Population Genomics of Early-Splitting Lineages of Metazoans

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Abstract Population genetics/genomics investigates gene flow, genetic diversity within and between populations, inbreeding, and effective population size, among other parameters. We here review the literature available on this topic in early-splitting lineages of metazoans (Porifera, Ctenophora, and Cnidaria). To date, great variation in population genetic patterns has been demonstrated in marine invertebrates, but because genetic structure results from the interplay of both biological and physical factors at different temporal and spatial scales, the complexity of these processes is not yet well known. This knowledge gap limits our ability to predict connectivity patterns according to species' biological traits and environmental factors. A general conclusion of our review is that in most sponges and brooding cnidarians, the short dispersal potential of the larvae, together with oceanographic circulation, is behind the strong genetic structure observed, whereas species with long pelagic larval duration (e.g., some broadcast spawners) or planktonic adults (jellyfish and ctenophores) display large variation in genetic patterns, from panmixia to local structuring, because a long pelagic life stage does not always ensure connectivity at large spatial scales. Additionally, the contribution of asexual propagation to local recruitment across phyla may also influence a populations' genetic structure. The future use of genome-wide scans of the species, together with oceanographic modeling and methodologies to measure larval dispersal, will provide us with meaningful data to understand the role of biotic and abiotic factors driving genetic patterns in marine metazoans and allow us to make predictions about genetic trends with implications for conservation and management.

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1 Introduction

Population genetics is the discipline that investigates the distribution of genetic diversity within and between populations in response to long-term processes of selection, genetic drift, mutation, and gene flow (Hartl et al. 1997; Grosberg and Cunningham 2001; Selkoe et al. 2014). Marine species, similar to their terrestrial counterparts, are structured into genetically divergent populations, which are then linked to each other to a greater or lesser degree by means of gene flow. Gene flow depends on biotic and abiotic factors, and the resulting population structuring has large demographic and evolutionary impact on the species (Avise et al. 1987; Avise 2000; Grosberg and Cunningham 2001; Palumbi 2003). Population structure and gene flow can be used as an approximation to measure connectivity, a crucial factor for the sustainability of marine resources, since the survival of marine populations is strongly intertwined with the survival of peripheral populations (Frankham et al. 2010; Beger et al. 2014; Holland et al. 2017). In most benthic invertebrates, the dispersal potential of the species depends on the larvae, but during the last years, empirical data have shown that the pelagic larval duration (PLD) is not a strong predictor of population structure (Weersing and Toonen 2009; Riginos et al. 2011; Selkoe and Toonen 2011; Coelho and Lasker 2016), pointing out the importance of multiple factors shaping marine species' connectivity patterns. The genetic structure of populations, and thus their connectivity, results from the interplay of both biological characteristics (e.g., free-swimming developmental stages, sessility/vagility, behavior, and reproduction systems, among others) and physical factors (e.g., geographical barriers, bathymetry, oceanographic circulation, and climatic events, among others) at different temporal and spatial scales (Avise et al. 1987; Coelho and Lasker 2016). Great variation in the spatial distribution of genetic diversity has been demonstrated in marine organisms (Grosberg and Cunningham 2001; Selkoe et al. 2008, 2014; Holland et al. 2017), and the genetic patterns can vary dramatically at similar spatial scales even between closely related species with similar life histories (see some examples in Ayre and Hughes 2000; Dong et al. 2015, 2016; Taboada and Pérez-Portela 2016; Holland et al. 2017, among many others).

Characterizing population genetic structure is used not only to infer gene flow but also to measure levels of diversity, inbreeding, effective population size, and other evolutionary and ecologically relevant parameters, such as the prevalence of sexual versus asexual propagation and introgression between species. It is considered a complex task, and the different methods available to characterize genetic structure require accounting for different assumptions about the given populations (Grosberg and Cunningham 2001; Holland et al. 2017), one of which, the Hardy-Weinberg equilibrium, is frequently violated in marine invertebrates due to several

methodological and biological reasons (e.g., Hellberg 2007; Uriz and Turon 2012; Adjeroud et al. 2014; Glynn et al. 2016; Pérez-Portela et al. 2016).

The field of population genetics radically improved with the use of DNA sequence data to infer molecular microevolutionary processes. In the past, a single marker from the mitochondrial genome or dozens of allozymes or AFLPs would be used to test hypotheses about population differentiation and gene flow (see examples in Benzie et al. 1994; Miller 1997; Holland et al. 2004). Recently, evolutionary biology is rapidly shifting from a genetic to a genomic perspective, with the use of thousands of DNA markers across the whole genomes of hundreds of individuals (Gagnaire et al. 2015; Benestan et al. 2016). Population genomics now offers an excellent opportunity to explore demographics and fine-scale genetic structure across space and over time in marine invertebrates for which no previous genomic knowledge is required (Reitzel et al. 2013); but a new, remarkable use of population genomics is to identify alleles and/or loci under selection and the potential link of genomic variation and adaptation to local conditions in non-model species (see Catchen et al. 2017), therefore providing us with the tools to understand the ultimate drivers of phenotypic and genotypic variation in the wild.

1.1 Population Genetics and Genomics as a Tool for Conservation and Management

Studies describing the genetic/genomic structure of populations are critical to understand the interplay of species' dispersal ability, life-history traits, demographic events, and environmental barriers and so are providing a relevant relationship between ecology and evolution (Palumbi 1994; Avise 2000). The complexities of both evolutionary and ecological processes, which finally determine the distribution of species' genetic diversity, are not yet well known in marine invertebrates. This knowledge gap limits our ability to predict connectivity patterns according to biological and environmental features and the molecular processes underpinning population dynamics and demographic events, such as planktonic *blooms* [dense and large aggregations of individuals that rapidly increase in biomass (Purcell et al. 2007; Fuentes et al. 2010)]. In the current changing marine habitats, not only is the survival of many marine species seriously threatened by direct and indirect human activities, but also jellyfish and ctenophore *blooms* (Purcell and Arai 2001; Purcell et al. 2007) and invasive species (a topic addressed in another chapter of this book) seem to be increasing in frequency and intensity, causing severe problems (Lee et al. 2013; Bayha et al. 2015) that should be addressed with the right tools.

Population genetic and genomic data have been increasingly integrated in management and conservation strategies during the last decades (Moritz 1994; Waples 2002; Allendorf et al. 2004; Garner et al. 2016). For example, molecular ecology analyses have proved useful to measure the extent of connectivity between marine protected areas (Bell 2008; Cros et al. 2016), determine relevant evolutionary units

and scales for population conservation (e.g., Santangelo and Abbiati 2001), identify sink and source populations, measure effective population sizes, detect bottleneck events in populations suffering disease-related decimation and overexploitation (e.g., Pérez-Portela et al. 2015; Riesgo et al. 2016), and investigate local adaptation in endangered species and introgression between species (Garner et al. 2016); therefore, genomic data holds tremendous potential for conservation and management if applied rigorously. In this sense, the use of genomic approaches and genome-wide scans has simplified the process of genotyping large number of samples in a cost-effective way and provided more statistical power than more "classic" markers such as microsatellite loci (Garner et al. 2016).

In this chapter we focus our attention on the population genetics/genomics of the following early-splitting lineages of metazoans: Porifera, Ctenophora, and Cnidaria. Even though the population genetic patterns of marine invertebrates are far less known than those of marine vertebrates (Coelho and Lasker 2016), the last decade has witnessed substantial improvement, thanks to the introduction of hypervariable markers, such as microsatellite loci and, more recently, single-nucleotide polymorphisms (SNPs) (Fig. 1). However, there is still a remarkable bias among phyla and groups: most of the population genetic studies have been conducted on cnidarians (Fig. 2) and within cnidarians on Anthozoa, mainly coral reef-building species (Fig. 3). Given that reviews on anthozoan population genetics and connectivity are more abundant than on the other early-splitting metazoan groups, we provide a more extensive overview of the research done on less-known groups within Cnidaria, the Medusozoa, and the other two phyla, Porifera and Ctenophora.

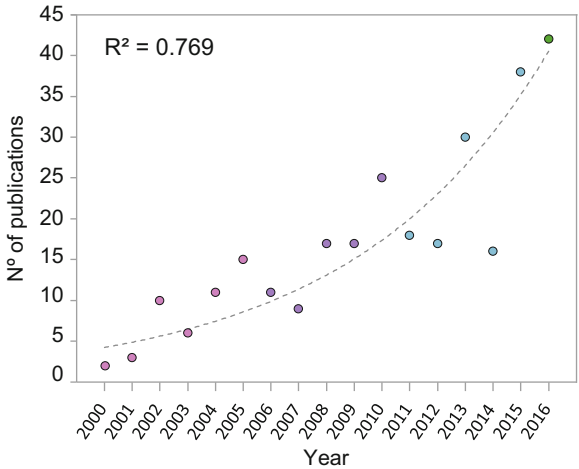

Fig. 1 Population genetic studies in early-splitting lineages of metazoans (Porifera, Ctenophora, and Cnidaria pooled). Graph of the exponential increase in the number of studies since 2000

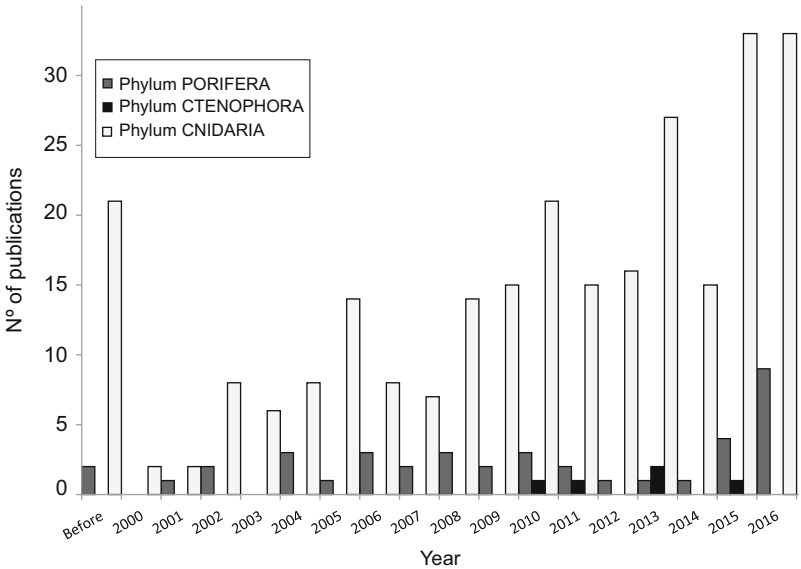

Fig. 2 Number of population genetic studies per year in three marine phyla: Porifera, Ctenophora, and Cnidaria

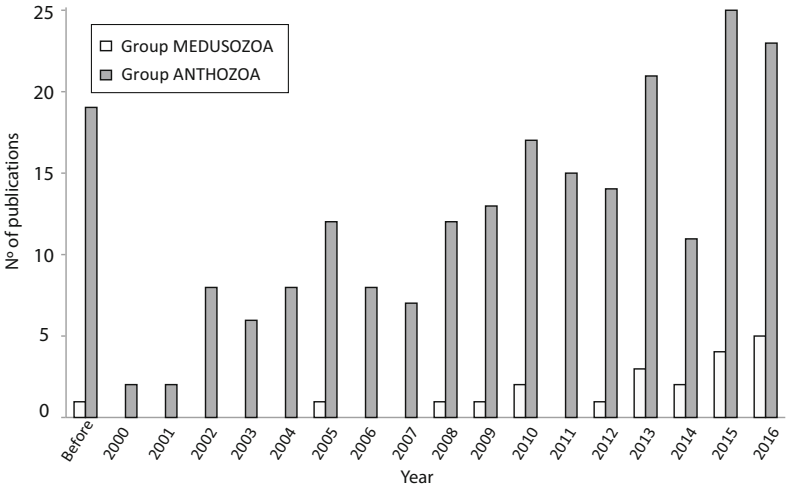

Fig. 3 Number of population genetic studies per year in Medusozoa and Anthozoa (Cnidaria)

2 Phylum Porifera

Sponges, the so-called species of the phylum Porifera, are crucial components of the benthic assemblages because of both their abundance and the ecosystem services they provide (e.g., De Goeij et al. 2013). They are used as refuge by myriad invertebrates, and sometimes vertebrates, and are basic food resources for multiple animals (e.g., Cerrano et al. 2000). They are distributed all over the world, in both freshwater and all kinds of seawater habitats, exhibiting an astonishing depth range, from the intertidal to the bathyal zones. But their importance is not only at the ecological level; they are also important in the biomedical field since they harbor a wide array of natural products with proven cytotoxic activity in tumor treatment and as antibacterial effectors (Mehbub et al. 2014).

The phylum Porifera is comprised of four classes: Demospongiae, Hexactinellida, Calcarea, and Homoscleromorpha; most sponges are hermaphroditic and viviparous species (Riesgo et al. 2014), although all reproductive strategies (hermaphroditism/gonochorism, viviparity/oviparity) are present in the phylum (Maldonado and Riesgo 2009a). Usually sponge larvae fall within the range of 50 to 500 μm, although some could reach 5–6 mm (Maldonado 2006). These small larvae are all lecithotrophic (Maldonado 2006), most of them being free-swimming and ciliated (Fig. 4a), although some are crawling larvae (Borojevic 1967; Bergquist et al. 1970; Ayling 1980; Maldonado and Riesgo 2009b). Free-swimming sponge larvae are capable of actively swimming in the first few centimeters after being released; however, they rapidly become entrapped by the viscous forces acting in the water molecules (Maldonado 2006), limiting their swimming capability. In addition, their shape and ciliary motions (Fig. 4a) provide them with poor abilities as long-distance dispersal propagules. Overall, sponge larvae are considered short-lived, lasting from minutes to less than 15 days of life in the seawater column (see Maldonado 2006). Only the hoplitomella larvae, which are unciliated and bear arm protrusions (Fig. 4b), are considered as adapted to long-distance dispersion (Topsent 1903; Trégouboff 1939, 1942; Vacelet 1999; Maldonado 2006).

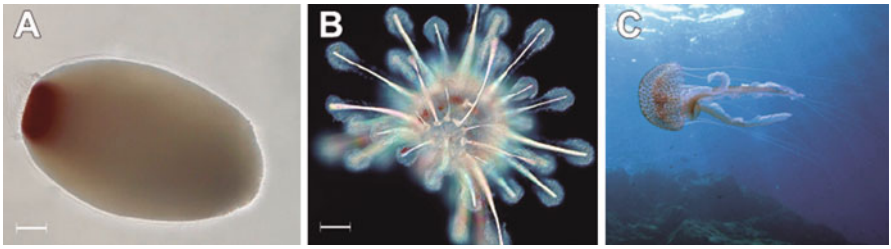

Fig. 4 Pictures of dispersal stages in early-splitting lineages of metazoans. (**a**) Ciliated parenchymella larva of a demosponge, (**b**) hoplitomella larva of a thoosid sponge (Porifera), and (**c**) adult of the jellyfish *Pelagia noctiluca* (Cnidaria, Medusozoa). Scale bar: 50 μm. Pictures featured in (**a**) and (**b**) were courtesy of Rafael Martín-Ledo

Sponges are currently under severe threats in many areas, with drastically reduced populations (Bell et al. 2015), but ironically, some sponge populations are actually growing and expanding their distribution ranges. This is particularly true for bioeroding sponges in coral reefs, which present a dramatic threat to the viability of healthy coral populations (Bell et al. 2015). In this context, understanding the genetic diversity and connectivity patterns of sponges is important in designing strategies to palliate the effects of habitat degradation and population survival. The molecular ecology of sponges has been thoroughly reviewed in the past decades (Wörheide et al. 2005; Uriz and Turon 2012), but the field is growing so rapidly, thanks to the introduction of microsatellite and SNP markers, that new and more detailed patterns are emerging and should be addressed comprehensively.

2.1 Genetic Markers for Population Genetics in Sponges

In sponges, several DNA markers have been used for population genetics studies, including allozymes (Benzie et al. 1994; Davis et al. 1996; Miller et al. 2001; Whalan et al. 2008), different mitochondrial genes (see examples in Bentlage and Wörheide 2007; Hoshino et al. 2008; López-Legentil and Pawlik 2009; Rua et al. 2011; Voigt et al. 2012), and nuclear genetic fragments such as the *ribosomal internal transcribed spacers—ITS1* and *ITS2* (Wörheide et al. 2002; Bentlage and Wörheide 2007; Hoshino et al. 2008; Wörheide et al. 2008; Andreakis et al. 2012; Becking et al. 2013; Ekins et al. 2016), among others (DeBiasse et al. 2010). More recently, the use of multilocus approaches has become widespread for sponges, ranging from 3 to 14 microsatellites (Duran et al. 2004a; Blanquer et al. 2009; Blanquer and Uriz 2011; Dailianis et al. 2011; Bell et al. 2014; Chaves-Fonnegra et al. 2015; Pérez-Portela et al. 2015; Riesgo et al. 2016; Guardiola et al. 2012, 2016; Padua et al. 2017) to tens of single-nucleotide polymorphisms, although limited to only one study (Brown et al. 2017). Mitochondrial DNA, extensively used in phylogeography and population genetics of metazoans (Avise et al. 1987; Avise 2000), presents significantly lower levels of variability in some early-splitting invertebrate lineages (sponges and cnidarians) than in other invertebrate groups (Shearer et al. 2002; Huang et al. 2008), making it unsuitable for many studies requiring fine intraspecific resolution and high intrapopulation genetic diversity. This low variability has been demonstrated in a number of studies in sponges (see examples in Xavier et al. 2010; Dailianis et al. 2011; León-Pech et al. 2015; Riesgo et al. 2016; Setiawan et al. 2016), although exceptions exist (e.g., Duran et al. 2004b; López-Legentil and Pawlik 2009; DeBiasse et al. 2014; de Bakker et al. 2016). On the other hand, the *ITS* markers seem to be extremely polymorphic even intraindividually (Wörheide et al. 2008; Ekins et al. 2016), although not for all sponges (Wörheide et al. 2002; Léon-Pech et al. 2015). In general, although microsatellite loci have to be *de novo* isolated and optimized for each species, they are reliable and accurate for detecting genetic structure and demographic events in sponges (Calderon et al. 2006; Blanquer and Uriz 2010, 2011; Dailianis et al. 2011; Bell et al. 2014, 2015; Giles et al. 2015).

2.2 Population Differentiation: Isolation by Distance and Oceanographic Fronts

In general, all sponge species exhibit strong genetic structure at local and regional scales along their population ranges, regardless of the markers used (Benzie et al. 1994; Duran et al. 2004a, b; Bentlage and Wörheide 2007; DeBiasse et al. 2010, 2014; Dailianis et al. 2011; Pérez-Portela et al. 2015; de Bakker et al. 2016; Riesgo et al. 2016; Brown et al. 2017; Padua et al. 2017). In the studies where microsatellites were used, the average of one of the most commonly applied statistics for measuring genetic differentiation between populations, the fixation index F_{ST}, ranged from 0.023 to 0.24, with higher values associated, in general, with greater distances between populations (Fig. 5a). Interestingly, when using microsatellites in sponges,

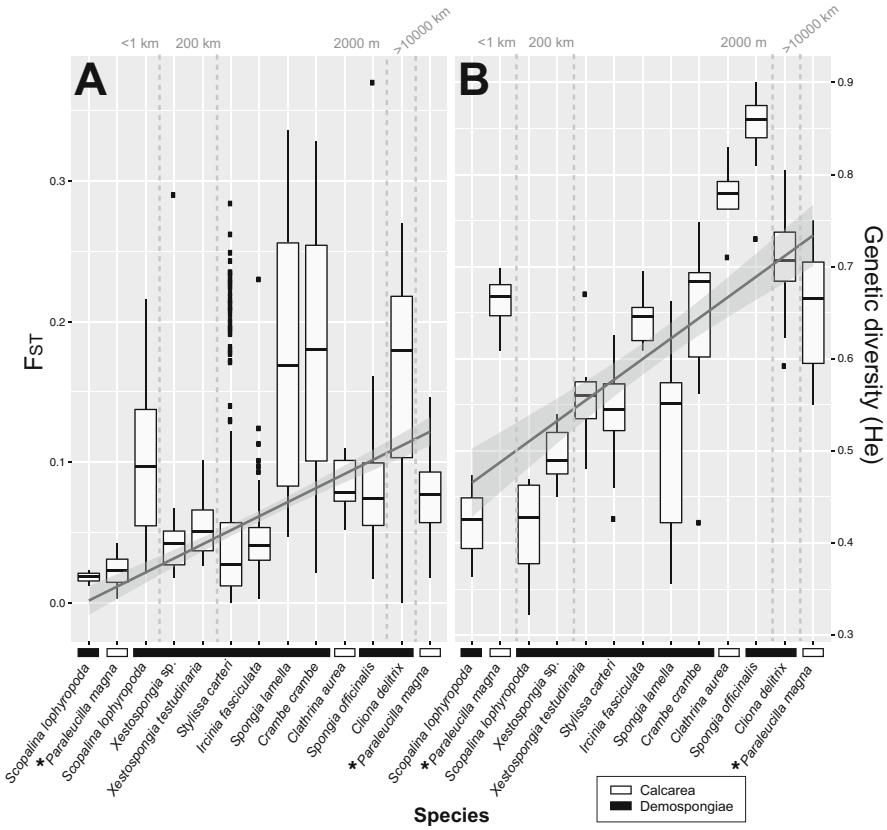

Fig. 5 Genetic divergence and diversity in sponges (Porifera). (**a**) Boxplots of genetic distances between populations based on the microsatellites (F_{ST}) and (**b**) expected heterozygosity (H_e) species. On both graphs we observe the line of correlation (and confidence interval) between both variables (F_{ST} or H_e) and geographical scales of those studies. The geographical scale of each study is represented by kilometers on the top the graphs. * Invasive species

most (if not all) pairwise comparisons of F_{ST} between sites were significant, regardless of the F_{ST} value (e.g., Dailianis et al. 2011; Riesgo et al. 2016; Padua et al. 2017). Significant F_{ST} values are indicative of strong population structure; this structure has been mostly attributed to the low dispersal abilities and philopatric behavior of their larvae, which promote populations' self-recruitment and local retention that finally increase populations' divergence over space. Most sponge larvae known have been reported to swim for less than 2 weeks (Maldonado 2006), but the geographical distance that can potentially be covered during such time can vary from dozens of meters to kilometers depending on the marine currents. However, the very few studies on spatial autocorrelation in sponges provide settlement distances of less than 200 m (Bell et al. 2014; Giles et al. 2015), although in some cases not more than 65 cm (Calderon et al. 2006; Blanquer and Uriz 2011), creating patterns of small-scale spatial genetic structure within sites. In addition to the poor swimming capabilities of the lecithotrophic sponge larvae, which are unable to disperse long distances or overstep small physical discontinuities (e.g., small submarine walls and local unidirectional currents) (Blanquer et al. 2009), it seems that major genetic breaks can also be explained by large oceanographic fronts acting as low permeable barriers to gene flow (see below).

When using *cytochrome c oxidase I (COI)*, the average Φ_{ST} (equivalent to F_{ST}) ranged from values close to zero in *Neoaulaxinia zingiberadix* (0.000), *Neoshcrammeniella fulvodesmus* (0.002), and *Isabella mirabilis* (0.005) (Ekins et al. 2016) indicating panmictic populations (or low genetic resolution) to moderate values of genetic divergence in *Xestospongia muta* (0.3) (de Bakker et al. 2016) and *Chondrosia reniformis* (0.2) (Villamor et al. 2014) at regional scales and up to very high values in *Cliona vermifera* (0.8) across the Pacific coast of Central America (León-Pech et al. 2015) likely indicating cryptic speciation in the last example. In *Rhopaloeides odorabile* (Whalan et al. 2008), virtually no structure was detected, coupled with low values of genetic diversity for *COI* and for different allozymes, although in this case the sampling area was no larger than 80 km^2. But even in the cases where the differentiation was extremely low, like in *I. mirabilis* or *R. odorabile*, the Φ_{ST} values were significant between some populations (Whalan et al. 2008; Ekins et al. 2016), suggesting local restricted gene flow that could be due to geographical distance or other factors, like interreef circulation patterns (Whalan et al. 2008).

In many cases, the strong population differentiation in sponges is correlated to geographical distances (Duran et al. 2004a, b; Xavier 2009; Xavier et al. 2010; Dailianis et al. 2011; Bell et al. 2014; Chaves-Fonnegra et al. 2015; León-Pech et al. 2015; Pérez-Portela et al. 2015), although sometimes these two parameters are not correlated (Wörheide et al. 2008; DeBiasse et al. 2010 Bell et al. 2014; Giles et al. 2015; Riesgo et al. 2016). In these cases, major population differentiation appears to be driven by the disruptive effect of oceanographic fronts (DeBiasse et al. 2010, 2016; Bell et al. 2014; Riesgo et al. 2016) and/or environmental patterns (Giles et al. 2015), but more often than not, the chaotic structure observed cannot be explained by the abovementioned factors (Bell et al. 2014), and nonrandom mating among individuals and the stochasticity in reproductive success are the most likely reasons

to explain the structure observed. In the Mediterranean, several oceanographic fronts have been found to be relatively impermeable to gene flow for benthic organisms (Patarnello et al. 2007; Villamor et al. 2014), including sponges: the Almeria-Oran front, described as the real biogeographical boundary between the Atlantic and Mediterranean basins, seems to be the most important one, dividing the Atlantic and Mediterranean genetic stocks of all sponges studied across this area (Dailianis et al. 2011; Pérez-Portela et al. 2015; Riesgo et al. 2016). Also, in the Wider Caribbean, oceanographic circulation seems to be behind the genetic discontinuities found for sponges across the Caribbean Sea, Gulf of Mexico, and Atlantic Coast of Florida (DeBiasse et al. 2016). In Northern Australia, the barrier created along the Torres Strait could be behind the highly structured populations of *Ianthella basta* (Andreakis et al. 2012). But there is no information about any other known oceanographic fronts causing genetic breaks in sponges in any other area of the world, and much more effort is needed to understand geographical patterns of the genetic diversity of Porifera species.

2.3 Genetic Diversity and Inbreeding

The genetic divergence of the *COI* gene used for some population genetics studies in the sponge field is usually very low, with the haplotypic diversity very close to 0 in many demosponges (Wörheide 2006; Xavier et al. 2010; León-Pech et al. 2015; Ekins et al. 2016). However, in some other demosponges, the average values of haplotypic diversity for the "Folmer or 5′ end" partition of *COI* can be higher, for instance, 0.48 in *Callyspongia vaginalis* in a study covering the Wider Caribbean (DeBiasse et al. 2010; DeBiasse and Hellberg 2015). Other authors found that the I3-M11 partition of *COI* was more variable at the species level for some sponges, and subsequently, several researchers have used it for population genetics (Erpenbeck et al. 2006; López-Legentil and Pawlik 2009; de Bakker et al. 2016). The genetic diversity of other mitochondrial markers like *nad5* and *ATP8* has also been explored; these markers also show extremely low diversity (Hoshino et al. 2008; Xavier et al. 2010). In contrast, it appears that in *Cliona vermifera*, *ATP6* was highly variable (León-Pech et al. 2015). Given this great variability in the divergence of the mitochondrial markers in sponges, researchers began using the *ribosomal internal transcribed spacer* (*ITS*), including the entire *ITS1*, 5.8S rRNA, and *ITS2* regions. For this marker, genetic diversity varied from moderate levels, e.g., 0.13–0.78 in *Hymeniacidon flavia* (Hoshino et al. 2008) and 0.18–0.79 in *Cliona vermifera* (León-Pech et al. 2015), to extremely high values, e.g., 0.87–1.00 in *Neoschrammeniella fulvodesmus* (Ekins et al. 2016). Although the use of the *ITS* was promising, the fact that they can have intra-genomic polymorphisms in some species (Duran et al. 2004a; Wörheide et al. 2008; Ekins et al. 2016) made it not ideal for population genetic studies.

Highly polymorphic microsatellite loci optimized for a number of sponges have been the most efficient markers to describe genetic diversity within species, populations, and individuals (Fig. 5b). The average genetic diversity (considered here as heterozygosity) is usually between 0.4 and 0.8 (Fig. 5b), with slightly lower

values reported when the sampling range is limited (Fig. 5); although of course, we should keep in mind that these genetic diversity values largely depend on the number of microsatellites used and their own particular variability. In *Paraleucilla magna* (Fig. 5b), higher expected heterozygosity (*He*) was detected when a geographically restricted area was studied, but this higher *He* was likely related to this calcareous sponge's invasive nature and the admixture of different genetic pools at the introduced populations analyzed (Guardiola et al. 2012). Unfortunately, there is only one population genetics study using 67 SNPs in sponges (Brown et al. 2017). In this deep-sea sponge, *He* values were lower than those observed for other sponges, ranging from 0.240 to 0.323, but the lack of other studies using these genomic markers makes it impossible to have an idea of the magnitude of these genetic diversity values within this animal group.

In general, sponges present populations that are not in HWE due in most cases to significantly lower values of observed heterozygosity (*Ho*) than *He* within populations. Whereas in some cases, this heterozygote deficiency can be explained by the presence of null alleles and technical difficulties in scoring microsatellite loci (Dailianis et al. 2011; Chaves-Fonnegra et al. 2015), in others, several non-exclusive biological factors may promote this pattern of lower heterozygosity, including high levels of inbreeding due to mating between relatives; low dispersal potential of both sperm and larvae; self-fertilization (in hermaphroditic species), selection, and spatial and temporal Wahlund effect as a consequence of overlapping age cohorts with different genetic structures within sampling sites; and the existence of different breeding subunits; but the relative contribution of each factor could not be properly tested in almost any study (Whalan et al. 2008; Wörheide et al. 2008; Duran et al. 2004a; Bell et al. 2014; Pérez-Portela et al. 2015). In some species, it is not heterozygote deficiency but heterozygote excess that resulted in HWE deviation (e.g., Blanquer and Uriz 2010), caused by an intriguing potential selection against homozygotes or mechanisms to avoid mating among relatives. There are only three studies in which sponge populations seem to be in HWE (Giles et al. 2015; Riesgo et al. 2016; Brown et al. 2017) that could be due in part to the removal of loci not meeting the HWE criteria (Giles et al. 2015; Riesgo et al. 2016), a strategy that does not provide the same results in other species (e.g., Chaves-Fonnegra et al. 2015).

2.4 Potential Factors Influencing Genetic Diversity: Clonality, Chimerism, and Hybridization

Sponges are extremely plastic animals, with astonishing regenerative capabilities, that can outlive fission (Calderon et al. 2006; Brown et al. 2017) and, for some species, even complete dissociation of their cells (Eerkes-Medrano et al. 2015). Such processes, together with the different strategies of asexual reproduction exhibited by sponges, produce small clonal propagules (reviewed in Uriz and Turon 2012). The extent of clonality has been assessed in a handful of sponge species, with contrasting

results: while some sponges showed that asexual reproduction contributed over 60% to their overall populations (Davis et al. 1996; Duran et al. 2004a; Miller et al. 2001), others showed moderate levels of clonality (Zilberberg et al. 2006; Calderon et al. 2006; Blanquer et al. 2009), and still others showed negligible levels (Guardiola et al. 2012; Chaves-Fonnegra et al. 2015; Pérez-Portela et al. 2015; Riesgo et al. 2016). Whereas some species showed clonal reproduction as a mechanism for colonization of new substrates at the scale of centimeters (Davis et al. 1996), in general for most sponges, clonal reproduction does not largely contribute to population recruitment or to their spatial genetic structuring (Miller et al. 2001; Duran et al. 2004a; Blanquer et al. 2009, and a review in Uriz and Turon 2012), a pattern that contrasts with that observed in many anthozoan species (Cnidaria) (e.g., Adjeroud et al. 2014; Gélin et al. 2017). However, in *Crambe crambe*, clonality due to fission was demonstrated to have an important effect at small spatial scales, although the short dispersal potential of the larva seemed to be more important in determining the genetic structuring of this species (Calderon et al. 2006).

Other biological phenomena, such as chimerism and hybridization, could also potentially contribute to the genetic structure of sponge populations, although they have been rarely studied at the molecular level. It has been suggested that both chimerism and hybridization might increase genetic diversity in sessile invertebrates (Santelices 2004), raising their chances of survival by acquiring adaptive advantages (Maldonado 1998). In *Scopalina lophyropoda*, Blanquer and Uriz (2011) found that more than 77% of the individuals contained at least two multilocus genotypes (MLGs), which was explained by the high number of fusion events previously observed in this species (Blanquer et al. 2009). Such fusion processes are not new for the sponge science; allogeneic (from different individuals) and isogeneic (from the same individual) fusions have been studied for decades already (e.g., Smith and Hildemann 1986; Wulff 1986). In some species, the capability of tissue fusion among different individuals of the same species is lost at the adult stage (Ilan and Loya 1990; Padua et al. 2016), while larvae and juveniles can easily fuse (e.g., Ilan and Loya 1990; Maldonado 1998; Warburton 1958). Tissue fusion is mediated by proteoglycan molecules called aggregation factors (Fernàndez-Busquets et al. 2002; Grice et al. 2017), among others, which are highly polymorphic (Grice et al. 2017). The complements of aggregation factors differ greatly among sponges, some having a very diverse tool kit, while others likely have a depauperated system for allorecognition (Grice et al. 2017).

Hybridization has also been reported for *Ircinia fasciculata* and *Ircinia variabilis* in the Mediterranean (Riesgo et al. 2016), with a predominant directionality of gene flow from *I. variabilis* to *I. fasciculate*, increasing the genetic diversity where pervasive hybridization was observed, a process that might somewhat palliate the genetic loss of *I. fasciculata* caused by population decimation due to recent mass mortality events (Riesgo et al. 2016). Perhaps the low variability in the allorecognition system of this and other sponges might be behind the discordances and incongruences in sponge phylogeny and population genetics in some cases (e.g., DeBiasse et al. 2014; Bell et al. 2015).

3 Phylum Ctenophora

Besides their natural beauty, ctenophores have attracted a lot of attention because they are essential to understand the evolution of metazoans (Shen et al. 2017) and many ecological interactions in the ocean (Purcell and Arai 2001). But almost nothing is known about the biotic and abiotic forces shaping ctenophores' populations at the genetic level, or the major drivers promoting divergence, and finally speciation (see Fig. 2). Moreover, this lack of information is hampering our understanding of the potential of ctenophores to generate negative impacts during *bloom* events.

Ctenophores are holoplanktonic organisms that spend their entire life cycle in the plankton, although they are weak swimmers (Bayha et al. 2015 and references herein). Because of their holoplanktonic nature, they are expected to disperse long distances during their life-span, mostly carried by major oceanographic currents, and show extensive gene flow among distant areas, little genetic differentiation, and low rates of speciation (Palumbi 1994). Nevertheless, this a priori expectation, based on their ecological and biological features, has never been properly tested, and scarce information is available on ctenophores' phylogeography and genetic patterns (Fig. 2). The few studies performed on population genetics of this phylum are limited to only one invasive species, the comb jelly *Mnemiopsis leidyi* (Reusch et al. 2010; Ghabooli et al. 2011, 2013; Bolte et al. 2013; Bayha et al. 2015).

Mnemiopsis leidyi was artificially introduced in the Black Sea, Aegean Sea, Caspian Sea, Adriatic Sea, Mediterranean Sea, Baltic Sea, and North Sea from the Western Atlantic (Reusch et al. 2010; Ghabooli et al. 2011; Costello et al. 2012; Bolte et al. 2013; Ghabooli et al. 2013; Bayha et al. 2015). Analyses across the native and invasive range using mitochondrial DNA sequences, the nuclear *ITS1* and *ITS2*, and microsatellite loci revealed important details about the translocation routes and number of introduction events to the invasive range, but provided little information about natural biogeographic and genetic breaks, connectivity, and distribution of genetic diversity. Following the expectation of long-dispersal potential and the absence of a benthic stage with specific requirements, which may limit the dispersal potential of the species, as, for instance, available bottom substrate, *M. leidyi* is widely distributed along several biogeographical provinces (Spalding et al. 2007) along its native range, covering a large range of environmental conditions, from the North Atlantic Coast of the USA to South Argentina (Bayha et al. 2015). However, against the initial expectation of a long-dispersal species, *M. leidyi* showed deep mitochondrial divergence in the *cytochrome b* among South America, Caribbean Sea, Gulf of Mexico and Florida, and the North Atlantic Coast of America (Bayha et al. 2015), with four highly divergent lineages allopatrically distributed (Costello et al. 2012; Ghabooli et al. 2011, 2013; Bayha et al. 2015). Genetic breaks between South (Argentina and Brazil) and North Atlantic American populations, which displayed significant differences in genetic structure among some populations based on Φ_{ST} (~0.578 for the *cytochrome b*) and F_{ST} (~0.246 for microsatellites) as a result of low levels of gene flow, can be explained by major oceanographic

circulation and well-known biogeographical breaks (Spalding et al. 2007); but additional genetic breaks between the Caribbean Sea and Gulf of Mexico cannot be easily interpreted in a vagile holoplanktonic organism mostly dispersed by dominant oceanographic currents. Actually, these levels of population divergence are over the Φ_{ST} average of widely distributed jellyfish for *COI* (see details on jellyfish below and Fig. 6a) and within the range of F_{ST} values for microsatellites in sponges (see Fig. 5a). The preferential behavior of *M. leidyi* for remaining within bays, estuaries, and near-shore habitats (Kremer and Nixon 1976; Kremer 1994; Purcell 2011) might reduce its ability to disperse long distances, as also observed in some jellyfish species (Dawson et al. 2015; Dong et al. 2015), and so limiting migration and gene flow across open waters. Nonetheless, mito-nuclear discordance was detected between markers (Bayha et al. 2015), which has turned into a relatively common pattern in marine invertebrates (e.g., DeBiasse et al. 2014; Glynn et al. 2015; Garcia-Cisneros et al. 2016; Pérez-Portela et al. 2017). Nuclear microsatellite loci revealed weaker genetic structure along the native range, with only major breaks across the Florida Strait, between the Gulf of Mexico, and the Atlantic Coast of Florida, and the North Atlantic populations found further north than Cape Hatteras (North Caroline), due to the oceanographic front generated between the Gulf Steam and the Labrador Current. Although authors discussed the lower resolution of microsatellite loci as a potential cause of this mito-nuclear discordance (Bayha et al. 2015), past divergence that occurred faster in mitochondrial DNA due to the one-quarter smaller effective population size and/or selection of some particular mitochondrial haplotypes/lineages over others under different environmental

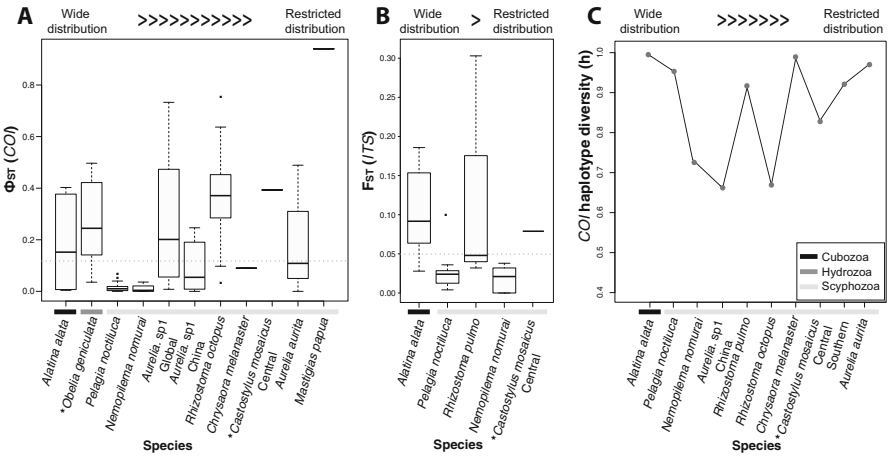

Fig. 6 Genetic divergence and diversity of medusozoans (Cnidaria) with free-swimming medusa stage. (**a**) Boxplots of genetic distances between populations based on the *COI* partition (Φ_{ST}), (**b**) genetic distances between populations based on the *ITS* (F_{ST}), and (**c**) haplotype diversity (h) based on *COI*. "Wide distribution" refers to studies including worldwide distribution, whereas "restricted distribution" refers to studies considering local areas at the scale of only few hundred kilometers. * Only one cryptic species was considered

conditions across the distribution range (Silva et al. 2014; Consuegra et al. 2015; Glynn et al. 2015) could also explain this geographical distribution of mitochondrial lineages. However, past divergence or selection hypotheses were never tested for this ctenophore, and therefore further investigations are required to clarify these particular questions.

4 Phylum Cnidaria

The phylum Cnidaria is divided in two main groups, Medusozoa and Anthozoa (Vargas and Zardoya 2012; Kayal et al. 2013), and comprises a variety of biological life cycles and ecological requirements that influence species' dispersal potential and distribution ranges (Fautin 2002; Lawley et al. 2016). Regarding population genetic studies of the phylum, we observe a bias on the investigation effort between these two groups, with over 90% of the total studies published in Anthozoa (Fig. 3) and several reviews on genetic divergence and connectivity of this invertebrate group (Benzie 1999; Selkoe et al. 2014; Coelho and Lasker 2016).

4.1 Genetic Markers for Population Genetics in Cnidarians

In cnidarians, as in sponges, allozymes were among the first markers used for population genetic analyses (e.g., Ayre et al. 1997; Miller 1997; Ayre and Hughes 2000; Goffredo et al. 2004), but more recently, highly variable markers such as the nuclear introns *ITS1* and *ITS2* (e.g., Dawson 2005b; Calderon et al. 2006; Stopar et al. 2010; Miller et al. 2012; Ramšak et al. 2012; Dong et al. 2016; Lawley et al. 2016), nuclear microsatellite loci (e.g., Glynn et al. 2015, 2016; Serrano et al. 2014, 2016; Pérez-Portela et al. 2016), and different fragments of mitochondrial DNA (e.g., Dawson 2005a, b; Govindarajan et al. 2005; Dawson et al. 2015; López et al. 2015; Lawley et al. 2016) have been extensively applied. Nevertheless, many studies have used a limited number of genetic markers, especially in Medusozoans (e.g., Dong et al. 2015; Dawson et al. 2015; López et al. 2015; van Walraven et al. 2016), a trend that is slowly shifting from few to tens and thousands of markers, thanks to the application of cost-effective isolation of microsatellites and genome-wide scans through high-throughput sequencing (e.g., Reitzel et al. 2013; Drury et al. 2016; Everett et al. 2016; Devlin-Durante and Baums 2017). Mitochondrial fragments, as explained before, are unsuitable for most cnidarian studies requiring fine intra-specific resolution, but this molecule displays appropriate substitution rates within the class Scyphozoa (Medusozoa), a group in which mitochondrial markers have been extensively used (see Shearer et al. 2002 for a review and Govindarajan et al. 2005; Dawson et al. 2015; Glynn et al. 2015; Lawley et al. 2016; van Walraven et al. 2016 for examples).

4.2 Population Genetics in Medusozoa: From Panmixia to Local Structure

Jellyfish, commonly named the planktonic adults of the group Medusozoa, play an important ecological role within marine food webs around the world, being very important due to their predatory activity on zooplankton and ichthyoplankton (Purcell and Arai 2001; Sabatés et al. 2010) and having large economical relevance. Some edible jellyfish are commercially exploited as a fishery source (Omori 1981; Arai 1996; Pitt and Kingsford 2000), but at the same time, jellyfish, which are similar to most zooplankton, form aggregations and are prone to jellyfish *blooms* (Purcell et al. 2015). Anthropogenic activities and impacts derived from them seem to contribute to increase *blooms*, although the specific role of each factor is still largely unknown (Purcell et al. 2007; Molinero et al. 2008; Purcell 2011). Jellyfish *blooms*, which in many cases also contain ctenophore species, can have strong negative impacts on commercial fishing, aquaculture, and drain systems and generate health concerns for swimmers due to their toxicity (Purcell and Arai 2001; Purcell et al. 2015; Dong et al. 2016).

Despite their ecological relevance, commercial interest, and detrimental effects for some human activities, Medusozoans have received less attention than its sibling group, Anthozoa, in terms of studies conducted on population genetics/genomics and connectivity (Fig. 2). Due to the scarce information on jellyfish's population genetics, as in ctenophores, there is still a limited understanding of the main drivers of their genetic architecture, the effect of *blooms* on effective population size, and genetic diversity at the intra-specific level, among many other questions. Within the group Medusozoa, there is also a bias in the number of genetic studies among the four different classes included in this group, and most research focused on commercially exploited species, those generating negative impacts for human activities and/or invasive species (examples in Dawson 2005a, b; Graham and Bayha 2008; Aglieri et al. 2014; Glynn et al. 2016; Lawley et al. 2016). Approximately, 60% of the studies on population genetics of Medusozoa involve species of the class Scyphozoa, 35% the class Hydrozoa, 5% the class Cubozoa (box jellyfish—with only one study published), and no studies on the class Staurozoa. For this reason, in this chapter we mostly focused on scyphozoans, with no further mention to Staurozoans.

Medusozoans have an unique and complex biological cycle that typically includes three consecutive life stages (Arai 1996). The first stage is a short-living larval stage (planula) that settles on the substrate to create a benthic asexual polyp, which gives rise to the adult medusa by strobilation (class Scyphozoa), budding (class Hydrozoa), or metamorphosis (class Cubozoa) or lacking the last phase (class Staurozoa) (Arai 1996). Hence, the distribution of most jellyfish species is limited to coastal areas where hard substrate is available for attachment of the benthic polyp (metagenic and/or meroplanktonic species), and only some groups that lost the polyp stage (holoplanktonic species) are more widely distributed across both inshore and offshore marine environments (Boero et al. 2008; Lee et al. 2013). Due to the

existence of two dispersal stages in most species, the short-lived planula and the long-lived planktonic medusa, jellyfish would be a priori expected to display genetic homogeneity over large geographical scales (Palumbi 1994, 1995). Whereas this expectation is true for some species, it has been demonstrated that jellyfish have populations genetically more structured than initially thought (Dawson 2005b; Govindarajan et al. 2005; Dawson et al. 2015; Dong et al. 2015; Glynn et al. 2015) (see Fig. 6a, b). This genetic structure is the result of a complex combination of biological, historical, and environmental factors. Population genetic studies of Medusozoa have traditionally applied different genetic markers and statistics to measure population divergence and connectivity, making the comparison among studies difficult or even controversial. We here summarize and compare the fixation indexes, Φ_{ST} and F_{ST}, in two of the most extended genetic fragments used, the mitochondrial *COI* and the nuclear *ITS*, respectively, to have a comprehensive idea about the distribution of genetic diversity across species and divergence within species at different geographical scales (see Fig. 6a, b).

4.2.1 Class Scyphozoa

Within the class Scyphozoa, the holoplanktonic species *Pelagia noctiluca* (see Fig. 4c), one of the most studied jellyfish due to its blooming nature along the European coast and which can reproduce in deeper offshore waters, displays genetic homogeneity over thousands of kilometers (Licandro et al. 2010; Stopar et al. 2010; Glynn et al. 2016). Both nuclear and mitochondrial markers revealed weak genetic structure across the whole Eastern Atlantic and Mediterranean Sea and extended gene flow and admixture between these two basins (Stopar et al. 2010; Glynn et al. 2016), with very low and no significant Φ_{ST} and F_{ST} values (Fig. 6a, b); however, significant temporal variation in genetic structure among nearby Mediterranean sites (Aglieri et al. 2014) and past divergence between NE Atlantic and South African populations (Miller et al. 2012) were noticed. Additionally, divergence of *COI* was observed across the NE Atlantic and Mediterranean basins from the sequences network, likely a result from an allopatric divergence during the Pleistocene sea-level fluctuations associated with cyclical glaciations (Patarnello et al. 2007). Nevertheless, the absence of divergence for the nuclear microsatellite loci and *ITS* suggests that after the Pleistocene divergence between the Atlantic and Mediterranean basins, there was secondary contact of lineages and complete admixture (Glynn et al. 2016). Hence, extensive gene flow across the Atlantic-Mediterranean arch in *P. noctiluca* contrasts with the common phylogeographic break found for other holozooplanktonic invertebrates (Peijnenburg et al. 2004). However, it is in concordance with the general pattern of Pleistocene vicariance during glacial events and interglacial secondary contacts (see examples in Garcia-Cisneros et al. 2016; Taboada and Pérez-Portela 2016; Pérez-Portela et al. 2017). An opposite example, also across the NE Atlantic, is offered by the metagenic and meroplanktonic species, *Rhizostoma octopus*. *Rhizostoma octopus* is a high-dispersal species that displayed significant differences in population structure at moderate scales of tens to hundreds

of kilometers along the Celtic Sea and Atlantic Coast of France, for both mitochondrial and nuclear markers, with signals of Pleistocene northward demographic expansions and southward retractions in response to ice age cycles (Lee et al. 2013; Glynn et al. 2015). Lee et al. (2013), combining two different techniques, population genetic analyses and Lagrangian modeling of oceanographic dispersal, observed that links between modern ocean currents and gene flow patterns are mixed in this species. Such results were attributed to the fact that jellyfish do not act as passive drifters driven by marine currents (Fossette et al. 2015). The strong directional swimming behavior of many jellyfish species prevents them from being advected to open waters and lets them remain in shallow coastal areas where there are available benthic substrata for the attachment of their polyps (Doyle et al. 2007).

Nevertheless, panmixia is not exclusive of holoplanktonic species. Genetic homogeneity due to contemporary gene flow is also observed in meroplanktonic species, such as *Chrysaora melanaster* across the Bering Sea (Dawson et al. 2015) and the widely distributed giant jellyfish, *Nemopilema nomurai*, along offshore coastal waters of China (Dong et al. 2016) (see Fig. 6a, b). But even for species with similar biological cycles at the same oceanographic areas and scales, there are large differences in genetic structure. A very good example is the contrasted patterns between *N. nomurai* and *Aurelia* sp. 1 along the Chinese coast. Whereas *N. nomurai* showed low and non-significant values of divergence between sites (Dong et al. 2016) (Fig. 6a, b), the inshore species *Aurelia* sp. 1 displayed significant differences over short distances of less than 100 km (Dong et al. 2015) (see "*Aurelia* sp. 1 China" and "*Aurelia* sp. 1 Global" in Fig. 6a, b). In these particular species, differences in oceanographic circulation between offshore and inshore waters at the Chinese Sea seem to enhance gene flow among *N. nomurai* offshore locations, promoting genetic admixture and homogeneity at large geographical scales, whereas eddies and gyres locally retain individuals in inshore populations of *Aurelia* sp. 1, restricting gene flow and favoring divergence among nearby sites and populations' self-recruitment (Dong et al. 2015, 2016). Hence, the interplay of this offshore/inshore preference with hydrodynamic circulation along the Chinese coast configures different gene flow patterns, and finally species' genetic architecture, in these two sympatric species (Dong et al. 2015, 2016).

Although jellyfish have, in general, lower levels of population divergence than other invertebrates (see examples in sponges and brooding anthozoans), empirical studies highlight that geographical partition of genetic diversity is commonplace in jellyfish (Dawson 2005b; Ramšak et al. 2012; Lee et al. 2013; Dawson et al. 2015), promoting divergence and speciation (Dawson and Martin 2001; Dawson and Hamner 2003; Dawson 2005a; Ki et al. 2008). There are outstanding examples of jellyfish with very restricted gene flow such as *Catostylus mosaicus*, *Aurelia aurita*, and *Mastigias papua* (Dawson 2005b; Dawson et al. 2015; van Walraven et al. 2016) (Fig. 6a, b). These three species showed large and significant values of Φ_{ST} and divergent of *COI* lineages, even cryptic speciation, at the scales of only tens of kilometers (Dawson 2005b; Dawson et al. 2015; Dawson and Hamner 2003). The most extreme example is that of *M. papua*, a species divided in three genetically distant populations, each confined into an isolated marine lake of Papua, with no sign

of recent gene flow among them (Dawson et al. 2015). Part of this divergence could result from past demographic events during Pleistocene glaciation cycles together with contemporary barriers to gene flow (Dawson 2005b).

Aurelia aurita is the only species for which the polyp stage has been considered for population genetic analyses (van Walraven et al. 2016). Although *A. aurita*'s Φ_{ST} values are similar to average jellyfish values, the van Walraven and coauthors' study (2016) was geographically restricted. The lack of connectivity among *A. aurita* populations at a geographical scale of only a few kilometers was explained by the hydrodynamic circulation across that area (van Walraven et al. 2016).

4.2.2 Classes Cubozoa and Hydrozoa

Population genetic studies of cubozoans and hydrozoans with free-swimming medusa stages do not differ from what was previously discussed for scyphozoans (Govindarajan et al. 2005; Lawley et al. 2016) (see Fig. 6a, b). Three reciprocally cryptic species (clades), allopatrically distributed, were discovered in the hydromedusa *Obelia geniculata*. Additionally, within the main clade, significant genetic divergence among populations was also observed, suggesting limited dispersal potential (Govindarajan et al. 2005). On the other hand, the worldwide distributed cubozoan *A. alata,* showed Φ_{ST} and F_{ST} distances over the average for jellyfish, but only the population from Bonaire, within the Caribbean Sea, displayed significant differences with all the other sites around the world, and all the other populations showed low and non-significant values of divergence at large geographical scales, although data obtained do not discard artificial translocation by human activities in this species (Lawley et al. 2016). Notwithstanding, recent studies from widely distributed hydrozoans of the Aglaopheniidae family, a brooding group with internal fertilization characterized by the absence of a medusa stage, showed strong patterns of genetic divergence at small spatial scales from hundreds to thousands of meters (mean F_{ST} ~0.35 and all comparisons between populations significant) and strong isolation by distance using microsatellite loci (Postaire et al. 2016, 2017a, b). They additionally displayed significantly lower values of observed heterozygosity than expected within populations, likely due to high rates of inbreeding and speciation signs. Population divergence, F_{ST} values, and inbreeding coefficients for these species (Postaire et al. 2017a, b) resembled those assessed in low-dispersal sponges, brooding corals, and gorgonians (Ayre and Hughes 2000; Lasker and Porto-Hannes 2015; Pérez-Portela et al. 2016). Weak dispersal potential of the larvae in these species, philopatric behavior, and low sperm dispersal generate patches of closely related individuals, overlapping generations, and nonrandom mating within sites. In Aglaopheniids, authors concluded that life-history traits are more important than oceanographic circulation in shaping the genetic structure of populations (Postaire et al. 2017a, b).

4.2.3 The Effect of *Blooms* on Genetic Features of Medusozoans

Regarding medusozoan *blooms*, there are still two unanswered questions: (a) Do genetically different jellyfish populations have similar demographics, and therefore, the same potential to generate *blooms*? and (b) How do demographic *blooms* affect general genetic features at the intra- and interpopulation level? A recent review by Dawson et al. (2015) that investigated population dynamics and genetic structure over time in five scyphozoan species from distant geographical areas revealed that population dynamics are not directly determined by genetic features but instead are influenced by the interplay of both genetic architecture of populations and the environmental conditions at each site. Additionally, jellyfish *blooms* have very large interannual heterogeneity (Aglieri et al. 2014; Dawson et al. 2015), and therefore long temporal monitoring is required to understand the relative effect of these two variables over time, because temporal genetic variation can, in some cases, be higher than populations' spatial differentiation, as observed in nearby populations of *Pelagia noctiluca* over a 3-year survey (Aglieri et al. 2014). This temporal genetic variation is likely related to stochastic factors during the reproduction process that create patterns of high relatedness among individuals of the same *bloom* and high levels of inbreeding, suggested by the heterozygote deficiency observed in almost all populations analyzed with microsatellites, and the significant values of the fixation index, F_{IS} (Glynn et al. 2015, 2016), so influencing the genetic composition of recruits of a particular aggregation during time (see Aglieri et al. 2014 and references herein). The little information available also suggests that recurrent and cyclical periods of *blooms* generate patterns of high mitochondrial diversity due to the existence of many low-frequency haplotypes closely related to each other (Fig. 6c). This high haplotype diversity may provide jellyfish species with "considerable potential for evolution" (Dawson and Hamner 2009).

4.3 Population Genetics of Anthozoa

The Anthozoa species, divided in Octocorallia and Hexacorallia, present only one adult stage, a benthic polyp, and their dispersal potential mostly relies on the larva. Anthozoans exhibit both solitary and colonial species and a variety of reproduction modes and life-cycle histories, including self-fertilization, swimming planula with pelagic dispersal or benthic crawling planula, and asexual reproduction for colonial growth and/or dispersal (Fautin 2002). Although broadcasting predominates in many anthozoans, internal brooding is also widespread in the group (Gerrodette 1981; Brazeau et al. 1998; Fautin 2002; Heltzel and Babcock 2002). Anthozoans have developed a variety of mechanisms for asexual propagation such as somatic/vegetative embryogenesis, budding, fission, and fragmentation, among others, but not all species reproduce asexually (Fautin 2002).

Many octocoral and hexacoral species are reef builders and founders of three-dimensional habitats. Coral reefs, which are among the most diverse ecosystems in

the world, are also among the most threatened ones (e.g., Hughes et al. 2010; Burke et al. 2011). During the last three decades, coral reef systems have passed through bleaching and mass mortality events causing extensive habitat degradation in almost all temperate and tropical seas in the world (Coma et al. 2004; Dias and Gondim 2016; Precht et al. 2016; Hughes et al. 2017). Because of their ecological relevance, corals are among the marine benthic groups attracting the most scientific interest, with a growing body of research in population genetics (see Figs. 2 and 3), mirrored by the recent development of genome-wide approaches to explore genomic diversity (Wang et al. 2009; Lundgren et al. 2013; Reitzel et al. 2013; Shinzato et al. 2015; Drury et al. 2016; Everett et al. 2016; Devlin-Durante and Baums 2017).

4.3.1 Spatial Genetic Patterns in Anthozoans

A large amount of research has already demonstrated that population genetic parameters and connectivity patterns in marine invertebrates are species-specific and locally dependent, and anthozoans are not an exception to this rule (Benzie 1999; Ayre and Hughes 2000; Jones et al. 2009; Pinzón et al. 2012; Selkoe et al. 2014; Coelho and Lasker 2016). However, different reviews on this particular topic were able to extract some generalities in terms of spatial genetic patterns associated to biological traits of the species (Benzie 1999; Selkoe et al. 2014; Coelho and Lasker 2016). It has been observed that whereas the premise of short dispersal potential and populations genetically structured at local scales (from meters to few kilometers) is well supported in brooders, although with exceptions (e.g., Serrano et al. 2016), broadcast spawners display an enormous gradient of patterns from local to moderate and broad regional connectivity (Coelho and Lasker 2016 and examples herein). Coelho and Lasker (2016) also present a comprehensive review on connectivity in corals and a discussion about the most important biological, ecological, and physical factors controlling connectivity in anthozoans, including the potential effect of bathymetry restricting gene flow between shallow and deep populations of anthozoans (e.g., Underwood 2009; Oppen et al. 2011; Serrano et al. 2014; Pérez-Portela et al. 2016), a topic of increasing interest due to the current decline of shallow coral reefs and for which deep populations may (or may not) act as a refuge and source of recruits and genetic diversity (see Bongaerts et al. 2010; Costantini et al. 2011).

4.3.2 Effect of Clonal Propagation on the Genetic Structure of Anthozoans

Populations with more genetic diversity are expected to better resist perturbations and environmental shifts (Hughes and Stachowicz 2004; Pauls et al. 2013). For this reason, applying molecular tools to assess levels of genetic diversity and to identify ecological and evolutionary processes generating and/or increasing diversity is particularly interesting in the context of coral reef management. In spite of the large number of population genetic studies performed in anthozoans (mostly in

Scleractinian corals), the impact of clonal reproduction, understood in this context as asexual propagation, on the architecture of their populations, genetic diversity, connectivity patterns, effective population sizes, and long-term persistence of populations is still unclear. There are a few studies addressing these particular questions, because most population genetic studies on asexual species are designed to avoid sampling clonal individuals; or clones, once detected, are removed from the databases, lowering in some cases the statistical power of the analyses (e.g., Adjeroud et al. 2014; Drury et al. 2016; Lukoschek et al. 2016; Serrano et al. 2014, 2016; Suzuki et al. 2016).

In organisms with mixed asexual and sexual reproductive systems, the theory predicts that asexual propagation is more advantageous to restock stable parental habitats, whereas sexual reproduction promotes dispersal and colonization of heterogeneous habitats (Williams 1975). Nevertheless, this expectation does not match with many empirical studies in corals and gorgonians that related high environmental disturbances (e.g., high hydrodynamism, exposition to waves, incidence of hurricane, trampling, fishing and anchoring, among others) with higher rates of asexual propagation and recruitment through fragmentation of the colony branches (in most cases) or post-disturbance colonization when conditions for sexual recruitment significantly decline (Gilmour 2002; Yeoh and Dai 2010; Pinzón et al. 2012; Foster et al. 2013; Adjeroud et al. 2014; Gélin et al. 2017), although disturbances do not fully explain the continual investment in asexual propagation in other cases (Gilmour 2002; Baums et al. 2006; Sherman et al. 2006).

Several studies also unraveled that levels of asexual reproduction are not species-specific, but reproductive strategies, which finally determine clonal rates, largely vary throughout the geographical range of the species (Miller and Ayre 2004; Sherman et al. 2006; Yeoh and Dai 2010; Pinzón et al. 2012; Combosch and Vollmer 2013; Adjeroud et al. 2014; Gélin et al. 2017). Within particular species, we find populations mostly maintained by clonal recruitment, with a high proportion of the colonies sharing the same multilocus genotypes (MLGs) (clonality ranging from 47 to 100%) (Adjeroud et al. 2014; Japaud et al. 2015; Gélin et al. 2017), as well as those in which asexual recruitment does not play a predominant role in local recruitment (McFadden 1997; Miller and Ayre 2004;; Sherman et al. 2006; Pinzón et al. 2012). Whereas the effect of asexual reproduction on population divergence has not been tested in most species, in *Pocillopora damicornis* from the Society Islands, the presence of shared clones seemed to homogenize genetic structure within populations and to increase genetic distances among populations due to the dominance of different MLGs (clones). Interestingly, when clones were removed from the analysis—MLGs considered only once—a pattern of panmixia came into view (Adjeroud et al. 2014). This may happen because high rates of clonal recruitment are compatible with high levels of gene flow and larvae recruitment from distant populations (Dahl et al. 2012; Adjeroud et al. 2014). Additionally, long-distance dispersal of some clones is also possible among sites across 40–200 km (Souter et al. 2009; Adjeroud et al. 2014; Gélin et al. 2017) likely as a result of parthenogenetic larvae (Fautin 2002; Gélin et al. 2017, and references herein). Although heterozygosity deficiency is common in anthozoans (e.g., Ayre and

Hughes 2000; Liu et al. 2005; Costantini et al. 2011; Pérez-Portela et al. 2016), clonality does not necessarily involve genetic diversity depletion and heterozygosity deficiency. Even under high rates of clonal propagation, gene flow can still exist, maintaining levels of genetic diversity within populations (Dahl et al. 2012; Pinzón et al. 2012). In other cases, populations mostly maintained by clonal propagation are characterized by a significant heterozygote excess (although not always) compared to the expected values under the Hardy-Weinberg equilibrium due to the long-term accumulation of mutations in clonal lineages (De Meeûs and Balloux 2005; Hellberg 2007; Adjeroud et al. 2014; García-Cisneros et al. 2017); and this genetic feature has been hypothesized by some authors as an evolutionary strategy to retain genetic diversity within individuals in asexual populations (Marriage and Orive 2012). Overall, it seems that clonal propagation might play a more relevant role in the population genetics and recruitment of anthozoans than in all the other groups discussed throughout this chapter, but studies specifically designed to measure clonality are desirable.

5 Conclusions and Further Research

The field of molecular ecology in sponges, ctenophores, and cnidarians has exponentially grown during the last two decades, thanks to the advent of multilocus approaches. However, the use of *COI* is still widespread, regardless of its very low variability in many of these taxa (e.g., Wörheide 2006; Dawson et al. 2015; Dong et al. 2015; López et al. 2015; van Walraven et al. 2016). In this sense, the future implementation of high-throughput sequencing for genome-wide scanning is extremely promising in the phyla here presented. Currently, genome-wide scanning techniques such as RAD sequencing and genotyping by sequencing can be easily applied to non-model invertebrates because previous genomic knowledge is not required (Reitzel et al. 2013). On the other hand, the large number of hypervariable genomic markers obtained (from hundreds to several hundred thousand) distributed across the whole genome provides a stronger statistical support and higher resolution than more "classic" markers (e.g., microsatellite loci or sequence fragments of only a few genes) to investigate fine- and large-scale genomic structure of populations at temporal and spatial scales, identify migrants between populations, detect clones, analyze the neutrality of the markers used, and explore the role of non-neutral processes of divergence shaping the general genetic architecture of these species, among many other questions (Garner et al. 2016). All these advantages give us the opportunity to rapidly increase our knowledge on general population genomics of unexplored marine groups. To our knowledge, only a few studies have isolated and used SNPs from genomic and transcriptomic datasets of early-splitting lineages of metazoans, most of them in anthozoans (Wang et al. 2009; Lundgren et al. 2013; Reitzel et al. 2013; Shinzato et al. 2015; Drury et al. 2016; Everett et al. 2016; Devlin-Durante and Baums 2017) and a few in jellyfish (Li et al. 2017) and sponges (Brown et al. 2017). However, the effect of environmental variation on the

distribution of particular alleles and local adaptation has been rarely explored in these animals (Reitzel et al. 2013), even though their sessile nature (in some) and widespread distribution (in others) make them ideal candidates for studying landscape genomics. In this sense, in ctenophores and jellyfish, population genomics will be fundamental to understanding the scale of their population dynamics and so to predicting the potential role of human-mediated environmental changes on *bloom* events (Glynn et al. 2015).

Most of the studies on the population genetics of sponges agree in that sponge populations are highly structured and differentiated. But one of the additional aspects of the molecular evolution of sponges that will enable our interpretation of their population genetic patterns is the elucidation of the effects of introgression on the genetic diversity and speciation patterns of sponges, which currently is virtually unknown (e.g., Riesgo et al. 2016), even though the extent of hybridization could very well be similar to that in corals. The pre-zygotic barriers of early-splitting lineages of metazoans to prevent hybridization are not well studied, although there are many reports of acrosomes and acrosomal vesicles in the literature in almost all sponge groups that could harbor specific recognition proteins (Maldonado and Riesgo 2009a). Since it has been recently suggested that hybridization could be beneficial to palliate the genetic sweeps caused by bottleneck events in sponges (Riesgo et al. 2016), and given the increasing numbers of diseases and temperature-associated mortalities and bleaching events in corals (e.g., Precht et al. 2016; Garrabou et al. 2009), this particular aspect of their biology cries for attention. But by far, one of the main issues that should be approached with accuracy in early-splitting lineages of metazoans to avoid confusion in the interpretation of genetic patterns is cryptic speciation and hybridization between recently split lineages. It is now recognized that given the morphological plasticity of these phyla, clearly divergent lineages have been treated as single species for a long time (e.g., Lazoski et al. 2001; Govindarajan et al. 2005; Xavier et al. 2010; Bayha et al. 2015; Dong et al. 2015; Postaire et al. 2016), and that could have serious effects on our understanding of the drivers of diversification and the global assessments of biodiversity for conservation purposes.

An outstanding finding is the different contribution of asexual reproduction to the local recruitment in sponges and asexual anthozoans. Whereas in some anthozoans clonal propagation plays an important role in population genetic diversity and structure (e.g., Adjeroud et al. 2014), its effect is almost negligible in sponges, although further research in this line is required because previous studies applied experimental designs, in most cases, to avoid sampling clones.

Jellyfish population genetic studies corroborate that mainly in species with large dispersal potential gene flow is not solely determined by species' life cycle and oceanographic circulation. The ability to horizontally swim against marine flows (Fossette et al. 2015), past population expansions and contractions during the Pleistocene glaciations (Dawson 2005b), vicariance and secondary contacts (Lee et al. 2013; Glynn et al. 2016), and environmental heterogeneity largely influence jellyfish genetic structure, although the relative role of each factor remains controversial (Lee et al. 2013 and references herein). For meroplanktonic species, shallow

benthic habitat availability might largely determine the dispersal potential of species, increasing the genetic structure and divergence of populations, although habitat heterogeneity might affect polyp survival and therefore drive genetic drift and divergence (Lee et al. 2013). Traditionally, genetic drift and nonrandom mating have been discarded as important factors influencing the genetic architecture of jellyfish because of their large population sizes (van Walraven et al. 2016), especially during *bloom* events (Aglieri et al. 2014), but these two factors should be directly considered in future studies. Among the most important limitations on our knowledge is the fact that most studies have only investigated the medusa stage, because polyps are difficult to locate in the field, missing out the life stage that potentially limits gene flow among populations, and that is largely affected by benthic environmental heterogeneity (van Walraven et al. 2016). In this sense, population genomic studies of staurozoans, and other species lacking the medusa stage, would be highly relevant to determine whether their spatial distribution of genetic diversity and demographic patterns are more similar to the evolutionary closer medusozoan species or instead to other benthic invertebrates with similar life cycles.

Finally, one obvious general conclusion on the patterns of population genetics in ctenophores is that an increase on the number of studied taxa within this group is fundamental. The population genetics of only one ctenophore species has been explored in depth. In this study, the unexpected divergence of *M. leidyi* indicates that despite its long dispersal potential and artificial translocation due to human activities, isolation may happen more frequently than initially thought. In most sponges and brooding cnidarians, including Aglaopheniidae medusozoans and different groups of anthozoans, the poor swimming abilities and short dispersal potential of the larvae, together with the strong influence of oceanographic circulation at local, medium, and large scales, seem to be behind the inbreeding signatures and the prevalent structure found in the populations of many studied areas of the world. Although there are still discordances and certainly mysterious results that call for the introduction of approaches that can shed light on local adaptation patterns. On the other hand, long pelagic larval duration and/or planktonic adults do not necessarily ensure connectivity at large spatial scales in cnidarians and ctenophores, as these species can display genetic structuring at local and regional scales in many cases (e.g., Ramšak et al. 2012; Dong et al. 2015).

The future development of methodologies to measure dispersal in marine invertebrates, combined with improved oceanographic circulation models and genome-wide scans, will provide us with meaningful data to understand the role of adaptation and both biotic and abiotic factors (and their interaction) driving the distribution of genetic diversity in early-splitting lineages of metazoans and so make predictions about spatial and temporal genetic trends with implications for management and conservation of marine resources.

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References

Adjeroud M, Guérécheau A, Vidal-Dupiol J, et al. Genetic diversity, clonality and connectivity in the scleractinian coral *Pocillopora damicornis*: a multi-scale analysis in an insular, fragmented reef system. Mar Biol. 2014;161:531–41.

Aglieri G, Papetti C, Zane L, et al. First evidence of inbreeding, relatedness and chaotic genetic patchiness in the holoplanktonic jellyfish *Pelagia noctiluca* (Scyphozoa, Cnidaria). PLoS One. 2014;9:e99647.

Allendorf FW, Leary RF, Hitt NP, et al. Intercrosses and the US Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? Conserv Biol. 2004;18:1203–13.

Andreakis N, Luter HM, Webster NS. Cryptic speciation and phylogeographic relationships in the elephant ear sponge *Ianthella basta* (Porifera, Ianthellidae) from northern Australia. Zool J Linn Soc. 2012;166:225–35.

Arai MN. A functional biology of Scyphozoa. Dordrecht, The Netherlands: Springer Science & Business Media; 1996.

Avise JC. Phylogeography: the history and formation of species. Cambridge, MA: Harvard University Press; 2000.

Avise JC, Arnold J, Ball RM, et al. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu Rev Ecol Syst. 1987;18:489–522.

Ayling AL. Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine demospongiae. Biol Bull. 1980;158:271–82.

Ayre DJ, Hughes TP. Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution. 2000;54:1590–605.

Ayre DJ, Davis AR, Billingham M, Llorens T, Styan C. Genetic evidence for contrasting patterns of dispersal in solitary colonial ascidians. Mar Biol. 1997;130:51–61.

de Bakker DM, Meesters EH, van Bleijswijk JD, et al. Population genetic structure, abundance, and health status of two dominant benthic species in the Saba Bank National Park, Caribbean Netherlands: *Montastraea cavernosa* and *Xestospongia muta*. PLoS One. 2016;11:e0155969.

Baums IB, Miller MW, Hellberg ME. Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. Ecol Monogr. 2006;76:503–19.

Bayha KM, Chang MH, Mariani CL, et al. Worldwide phylogeography of the invasive ctenophore *Mnemiopsis leidyi* (Ctenophora) based on nuclear and mitochondrial DNA data. Biol Invasions. 2015;17:827–50.

Becking LE, Erpenbeck D, Peijnenburg KT, De Voogd NJ. Phylogeography of the sponge *Suberites diversicolor* in Indonesia: insights into the evolution of marine lake populations. PLoS One. 2013;8:e75996.

Beger M, Selkoe KA, Treml E, et al. Evolving coral reef conservation with genetic information. Bull Mar Sci. 2014;90:159–85.

Bell JJ. Connectivity between island Marine Protected Areas and the mainland. Biol Conserv. 2008;141:2807–20.

Bell JJ, Smith D, Hannan D, Haris A, Thomas L. Isolation and characterisation of twelve polymorphic microsatellite markers for *Xestospongia* spp. and their use for confirming species identity. Conserv Genet Resour. 2014;6:105–6.

Bell JJ, McGrath E, Biggerstaff A, et al. Global conservation status of sponges. Conserv Biol. 2015;29:42–53.

Benestan LM, Ferchaud AL, Hohenlohe PA, et al. Conservation genomics of natural and managed populations: building a conceptual and practical framework. Mol Ecol. 2016;25:2967–77.

Bentlage B, Wörheide G. Low genetic structuring among *Pericharax heteroraphis* (Porifera: Calcarea) populations from the Great Barrier Reef (Australia), revealed by analysis of nrDNA and nuclear intron sequences. Coral Reefs. 2007;26:807–16.

Benzie JAH. Genetic structure of coral reef organisms: ghosts of dispersal past. Am Zool. 1999;39:131–45.

Benzie J, Sandusky C, Wilkinson C. Genetic structure of dictyoceratid sponge populations on the western Coral Sea reefs. Mar Biol. 1994;119:335–45.

Bergquist PR, Sinclair MR, Hogg JJ. Adaptation to intertidal existence: reproductive cycles and larval behaviour in Demospongiae. Symp Zool Soc Lond. 1970;25:247–71.

Blanquer A, Uriz MJ. Population genetics at three spatial scales of a rare sponge living in fragmented habitats. BMC Evol Biol. 2010;10:13.

Blanquer A, Uriz MJ. "Living together apart": the hidden genetic diversity of sponge populations. Mol Biol Evol. 2011;28:2435–8.

Blanquer A, Uriz MJ, Caujapé-Castells J. Small-scale spatial genetic structure in *Scopalina lophyropoda*, an encrusting sponge with philopatric larval dispersal and frequent fission and fusion events. Mar Ecol Prog Ser. 2009;380:95–102.

Boero F, Bouillon J, Gravili C, et al. Gelatinous plankton: irregularities rule the world (sometimes). Mar Ecol Prog Ser. 2008;356:299–310.

Bolte S, Fuentes V, Haslob H, et al. Population genetics of the invasive ctenophore *Mnemiopsis leidyi* in Europe reveal source-sink dynamics and secondary dispersal to the Mediterranean Sea. Mar Ecol Prog Ser. 2013;485:25–36.

Bongaerts P, Ridgway T, Sampayo E, Hoegh-Guldberg O. Assessing the "deep reef refugia" hypothesis: focus on Caribbean reefs. Coral Reefs. 2010;29:309–27.

Borojevic R. La ponte et le développement de *Polymastia robusta* (Démosponges). Cah Biol Mar. 1967;8:1–6.

Brazeau DA, Gleason DF, Morgan ME. Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers. J Exp Mar Biol Ecol. 1998;231:225–38.

Brown RR, Davis CS, Leys SP. Clones or clans: the genetic structure of a deep-sea sponge, *Aphrocallistes vastus*, in unique sponge reefs of British Columbia, Canada. Mol Ecol. 2017;26:1045–59.

Burke L, Reytar K, Spalding M, Perry A. Reefs at risk. Washington, DC: World Resources Institute; 2011. p. 124.

Calderon I, Garrabou J, Aurelle D. Evaluation of the utility of COI and ITS markers as tools for population genetic studies of temperate gorgonians. J Exp Mar Biol Ecol. 2006;336:184–97.

Catchen JM, Hohenlohe PA, Bernatchez L, et al. Unbroken: RADseq remains a powerful tool for understanding the genetics of adaptation in natural populations. Mol Ecol Resour. 2017;17:362–5.

Cerrano C, Bavestrello G, Calcinai B, Cattaneo-Vietti R, Sarà A. Asteroids eating sponges from Tethys Bay, East Antarctica. Antarct Sci. 2000;12:425–6.

Chaves-Fonnegra A, Feldheim KA, Secord J, Lopez JV. Population structure and dispersal of the coral-excavating sponge *Cliona delitrix*. Mol Ecol. 2015;24:1447–66.

Coelho MAG, Lasker HR. Larval dispersal and population connectivity in Anthozoans. In: The Cnidaria, past, present and future. New York, NY: Springer; 2016. p. 291–315.

Coma R, Pola E, Ribes M, Zabala M. Long-term assessment of the patterns of mortality of a temperate octocoral in protected and unprotected areas: a contribution to conservation and management needs. Ecol Appl. 2004;14:1466–78.

Combosch DJ, Vollmer SV. Mixed asexual and sexual reproduction in the Indo-Pacific reef coral *Pocillopora damicornis*. Ecol Evol. 2013;3:3379–87.

Consuegra S, John E, Verspoor E, De Leaniz CG. Patterns of natural selection acting on the mitochondrial genome of a locally adapted fish species. Genet Sel Evol. 2015;47:58.

Costantini F, Rossi S, Pintus E, et al. Low connectivity and declining genetic variability along a depth gradient in *Corallium rubrum* populations. Coral Reefs. 2011;30:991–1003.

Costello JH, Bayha KM, Mianzan HW, Shiganova TA, Purcell JE. Transitions of *Mnemiopsis leidyi* (Ctenophora: Lobata) from a native to an exotic species: a review. Hydrobiologia. 2012;690:21–46.

Cros A, Toonen RJ, Davies SW, Karl SA. Population genetic structure between Yap and Palau for the coral *Acropora hyacinthus*. PeerJ. 2016;4:e2330.

Dahl M, Pereyra R, Lundälv T, André C. Fine-scale spatial genetic structure and clonal distribution of the cold-water coral Lophelia pertusa. Coral Reefs. 2012;31:1135–48.

Dailianis T, Tsigenopoulos CS, Dounas C, Voultsiadou E. Genetic diversity of the imperilled bath sponge *Spongia officinalis* Linnaeus, 1759 across the Mediterranean Sea: patterns of population differentiation and implications for taxonomy and conservation. Mol Ecol. 2011;20:3757–72.

Davis AR, Ayre DJ, Billingham M, Styan CA, White G. The encrusting sponge *Halisarca laxus:* population genetics and association with the ascidian *Pyura spinifera*. Mar Biol. 1996;126:27–33.

Dawson MN. *Cyanea capillata* is not a cosmopolitan jellyfish: morphological and molecular evidence for *C. annaskala* and *C. rosea* (Scyphozoa: Semaeostomeae: Cyaneidae) in south-eastern Australia. Invertebr Syst. 2005a;19:361–70.

Dawson MN. Incipient speciation of *Catostylus mosaicus* (Scyphozoa, Rhizostomeae, Catostylidae), comparative phylogeography and biogeography in south-east Australia. J Biogeogr. 2005b;32:515–33.

Dawson MN, Hamner WM. Geographic variation and behavioral evolution in marine plankton: the case of *Mastigias* (Scyphozoa, Rhizostomeae). Mar Biol. 2003;143:1161–74.

Dawson MN, Hamner WM. A character-based analysis of the evolution of jellyfish blooms: adaptation and exaptation. Hydrobiologia. 2009;616:193–215.

Dawson MN, Martin LE. Geographic variation and ecological adaptation in *Aurelia* (Scyphozoa, Semaeostomeae): some implications from molecular phylogenetics. In: Jellyfish blooms: ecological and societal importance. Springer; 2001. p. 259–73

Dawson MN, Cieciel K, Decker MB, et al. Population-level perspectives on global change: genetic and demographic analyses indicate various scales, timing, and causes of scyphozoan jellyfish blooms. Biol Invasions. 2015;17:851–67.

De Goeij JM, Van Oevelen D, Vermeij MJ, et al. Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science. 2013;342:108–10.

De Meeûs T, Balloux F. F-statistics of clonal diploids structured in numerous demes. Mol Ecol. 2005;14:2695–702.

DeBiasse MB, Hellberg ME. Discordance between morphological and molecular species boundaries among Caribbean species of the reef sponge *Callyspongia*. Ecol Evol. 2015;5:663–75.

DeBiasse M, Richards VP, Shivji MS. Genetic assessment of connectivity in the common reef sponge, *Callyspongia vaginalis* (Demospongiae: Haplosclerida) reveals high population structure along the Florida reef tract. Coral Reefs. 2010;29:47–55.

DeBiasse MB, Nelson BJ, Hellberg ME. Evaluating summary statistics used to test for incomplete lineage sorting: mito-nuclear discordance in the reef sponge *Callyspongia vaginalis*. Mol Ecol. 2014;23:225–38.

DeBiasse MB, Richards VP, Shivji MS, Hellberg ME. Shared phylogeographical breaks in a Caribbean coral reef sponge and its invertebrate commensals. J Biogeogr. 2016;43:2136–46.

Devlin-Durante MD, Baums IB. Genome-wide survey of single-nucleotide polymorphisms reveals fine-scale population structure and signs of selection in the threatened Caribbean elkhorn coral, *Acropora palmata*. PeerJ. 2017;5:e4077.

Dias TLP, Gondim AI. Bleaching in scleractinians, hydrocorals, and octocorals during thermal stress in a northeastern Brazilian reef. Mar Biodivers. 2016;46:303–7.

Dong Z, Liu Z, Liu D. Genetic characterization of the scyphozoan jellyfish *Aurelia* spp. in Chinese coastal waters using mitochondrial markers. Biochem Syst Ecol. 2015;60:15–23.

Dong Z, Liu Z, Liu D, Liu Q, Sun T. Low genetic diversity and lack of genetic structure in the giant jellyfish *Nemopilema nomurai* in Chinese coastal waters. Mar Biol Res. 2016;12:769–75.

Doyle TK, Houghton JDR, Buckley SM, Hays GC, Davenport J. The broad-scale distribution of five jellyfish species across a temperate coastal environment. Hydrobiologia. 2007;579:29–39.

Drury C, Dale KE, Panlilio JM, et al. Genomic variation among populations of threatened coral: *Acropora cervicornis*. BMC Genomics. 2016;17:286.

Duran S, Pascual M, Estoup A, Turon X. Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Mol Ecol. 2004a;13:511–22.

Duran S, Giribet G, Turon X. Phylogeographical history of the sponge *Crambe crambe* (Porifera, Poecilosclerida): range expansion and recent invasion of the Macaronesian islands from the Mediterranean Sea. Mol Ecol. 2004b;13:109–22.

Eerkes-Medrano D, Feehan CJ, Leys SP. Sponge cell aggregation: checkpoints in development indicate a high level of organismal complexity. Invertebr Biol. 2015;134:1–18.

Ekins M, Erpenbeck D, Wörheide G, Hooper JN. Staying well connected-Lithistid sponges on seamounts. J Mar Biol Assoc U K. 2016;96:437–51.

Erpenbeck D, Hooper J, Wörheide G. CO1 phylogenies in diploblasts and the "Barcoding of Life"—are we sequencing a suboptimal partition? Mol Ecol Resour. 2006;6:550–3.

Everett MV, Park LK, Berntson EA, et al. Large-Scale Genotyping-by-Sequencing indicates high levels of gene flow in the deep-sea octocoral *Swiftia simplex* (Nutting 1909) on the West Coast of the United States. PLoS One. 2016;11:e0165279.

Fautin DG. Reproduction of cnidaria. Can J Zool. 2002;80:1735–54.

Fernàndez-Busquets X, Kuhns WJ, Simpson TL, et al. Cell adhesion-related proteins as specific markers of sponge cell types involved in allogeneic recognition. Dev Comp Immunol. 2002;26:313–23.

Fossette S, Gleiss AC, Chalumeau J, et al. Current-oriented swimming by jellyfish and its role in bloom maintenance. Curr Biol. 2015;25:342–7.

Foster NL, Baums IB, Sanchez JA, et al. Hurricane-driven patterns of clonality in an ecosystem engineer: the Caribbean coral Montastraea annularis. PLoS One. 2013;8:e53283.

Frankham R, Ballou JD, Briscoe DA. Introduction to conservation genetics. Cambridge, UK: Cambridge University Press; 2010.

Fuentes VL, Angel DL, Bayha KM, et al. Blooms of the invasive ctenophore, *Mnemiopsis leidyi*, span the Mediterranean Sea in 2009. Hydrobiologia. 2010;645:23–37.

Gagnaire PA, Broquet T, Aurelle D, et al. Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. Evol Appl. 2015;8:769–86.

Garcia-Cisneros A, Palacín C, Khadra YB, Pérez-Portela R. Low genetic diversity and recent demographic expansion in the red starfish *Echinaster sepositus* (Retzius 1816). Sci Rep. 2016;6:33269.

García-Cisneros A, Palacín C, Ventura R, Feital B, Paiva PC, Pérez-Portela R. Intraspecific genetic structure, divergence and high rates of clonality in an amphi-Atlantic starfish. Mol Ecol. 2017. https://doi.org/10.1111/mec.14454.

Garner BA, Hand BK, Amish SJ, et al. Genomics in conservation: case studies and bridging the gap between data and application. Trends Ecol Evol. 2016;31:81–3.

Garrabou J, Coma R, Bensoussan N, et al. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol. 2009;15:1090–103.

Gélin P, Fauvelot C, Mehn V, et al. Superclone expansion, long-distance clonal dispersal and local genetic structuring in the coral *Pocillopora damicornis* Type β in Reunion Island, South Western Indian Ocean. PLoS One. 2017;12:e0169692.

Gerrodette T. Dispersal of the solitary coral *Balanophyllia elegans* by demersal planular larvae. Ecology. 1981;62:611–9.

Ghabooli S, Shiganova TA, Zhan A, et al. Multiple introductions and invasion pathways for the invasive ctenophore *Mnemiopsis leidyi* in Eurasia. Biol Invasions. 2011;13:679–90.

Ghabooli S, Shiganova TA, Briski E, et al. Invasion pathway of the ctenophore *Mnemiopsis leidyi* in the Mediterranean Sea. PLoS One. 2013;8:e81067.

Giles EC, Saenz-Agudelo P, Hussey NE, Ravasi T, Berumen ML. Exploring seascape genetics and kinship in the reef sponge *Stylissa carteri* in the Red Sea. Ecol Evol. 2015;5:2487–502.

Gilmour JP. Substantial asexual recruitment of mushroom corals contributes little to population genetics of adults in conditions of chronic sedimentation. Mar Ecol Prog Ser. 2002;235:81–91.

Glynn F, Houghton JDR, Provan J. Population genetic analyses reveal distinct geographical blooms of the jellyfish *Rhizostoma octopus* (Scyphozoa). Biol J Linn Soc. 2015;116:582–92.

Glynn F, Houghton JDR, Bastian T, et al. High-resolution genetic analysis reveals extensive gene flow within the jellyfish *Pelagia noctiluca* (Scyphozoa) in the North Atlantic and Mediterranean Sea. Biol J Linn Soc. 2016;117:252–63.

Goffredo S, Mezzomonaco L, Zaccanti F. Genetic differentiation among populations of the Mediterranean hermaphroditic brooding coral *Balanophyllia europaea* (Scleractinia: Dendrophylliidae). Mar Biol. 2004;145:1075–83.

Govindarajan AF, Halanych KM, Cunningham CW. Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). Mar Biol. 2005;146:213–22.

Graham WM, Bayha KM. Biological invasions by marine jellyfish. In: Biological invasions. Heidelberg: Springer; 2008. p. 239–55

Grice LF, Gauthier ME, Roper KE, et al. Origin and evolution of the sponge aggregation factor gene family. Mol Biol Evol. 2017;34:1083–99.

Grosberg R, Cunningham CW. Genetic structure in the sea. In: Marine community ecology. Sunderland, MA: Sinauer; 2001. p. 61–84.

Guardiola M, Frotscher J, Uriz MJ. Genetic structure and differentiation at a short-time scale of the introduced calcarean sponge *Paraleucilla magna* to the western Mediterranean. Hydrobiologia. 2012;687:71–84.

Guardiola M, Frotscher J, Uriz MJ. High genetic diversity, phenotypic plasticity, and invasive potential of a recently introduced calcareous sponge, fast spreading across the Atlanto-Mediterranean basin. Mar Biol. 2016;163:123.

Hartl DL, Clark AG, Clark AG. Principles of population genetics. Sunderland, MA: Sinauer Associates; 1997.

Hellberg M. Footprints on water: the genetic wake of dispersal among reefs. Coral Reefs. 2007;26:463–73.

Heltzel P, Babcock R. Sexual reproduction, larval development and benthic planulae of the solitary coral *Monomyces rubrum* (Scleractinia: Anthozoa). Mar Biol. 2002;140:659–67.

Holland BS, Dawson MN, Crow GL, Hofmann DK. Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. Mar Biol. 2004;145:1119–28.

Holland LP, Jenkins TL, Stevens JR. Contrasting patterns of population structure and gene flow facilitate exploration of connectivity in two widely distributed temperate octocorals. Heredity. 2017;119:35.

Hoshino S, Saito DS, Fujita T. Contrasting genetic structure of two Pacific Hymeniacidon species. Hydrobiologia. 2008;603:313–26.

Huang D, Meier R, Todd PA, Chou LM. Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J Mol Evol. 2008;66:167–74.

Hughes AR, Stachowicz JJ. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proc Natl Acad Sci U S A. 2004;101:8998–9002.

Hughes TP, Graham NAJ, Jackson JBC, Mumby PJ, Steneck RS. Rising to the challenge of sustaining coral reef resilience. Trends Ecol Evol. 2010;25:633–42.

Hughes TP, Kerry JT, Álvarez-Noriega M, et al. Global warming and recurrent mass bleaching of corals. Nature. 2017;543:373–7.

Ilan M, Loya Y. Ontogenetic variation in sponge histocompatibility responses. Biol Bull. 1990;179:279–86.

Japaud A, Bouchon C, Manceau JL, Fauvelot C. High clonality in Acropora palmata and Acropora cervicornis populations of Guadeloupe, French Lesser Antilles. Mar Freshwater Res. 2015; 66: 847–851.

Jones G, Almany G, Russ G, et al. Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. Coral Reefs. 2009;28:307–25.

Kayal E, Roure B, Philippe H, Collins AG, Lavrov DV. Cnidarian phylogenetic relationships as revealed by mitogenomics. BMC Evol Biol. 2013;13:5.

Ki JS, Hwang DS, Shin K, et al. Recent moon jelly (*Aurelia* sp. 1) blooms in Korean coastal waters suggest global expansion: examples inferred from mitochondrial COI and nuclear ITS-5.8 S rDNA sequences. ICES J Mar Sci. 2008;65:443–52.

Kremer P. Patterns of abundance for *Mnemiopsis* in US coastal waters: a comparative overview. ICES J Mar Sci. 1994;51:347–54.

Kremer P, Nixon S. Distribution and abundance of the ctenophore, *Mnemiopsis leidyi* in Narragansett Bay. Estuar Coast Mar Sci. 1976;4:627–39.

Lasker HR, Porto-Hannes I. Population structure among octocoral adults and recruits identifies scale dependent patterns of population isolation in The Bahamas. PeerJ. 2015;3:e1019.

Lawley JW, Ames CL, Bentlage B, et al. Box Jellyfish *Alatina alata* has a circumtropical distribution. Biol Bull. 2016;231:152–69.

Lazoski C, SolÇ-Cava AM, Boury-Esnault N, Klautau M, Russo CAM. Cryptic speciation in a high gene flow scenario in the oviparous marine sponge *Chondrosia reniformis*. Mar Biol. 2001;139:421–9.

Lee PLM, Dawson MN, Neill SP, et al. Identification of genetically and oceanographically distinct blooms of jellyfish. J R Soc Interface. 2013;10:20120920.

León-Pech M, Cruz-Barraza J, Carballo J, Calderon-Aguilera L, Rocha-Olivares A. Pervasive genetic structure at different geographic scales in the coral-excavating sponge *Cliona vermifera* (Hancock, 1867) in the Mexican Pacific. Coral Reefs. 2015;34:887–97.

Li Y, Zhou Z, Tian M, et al. Exploring single nucleotide polymorphism (SNP), microsatellite (SSR) and differentially expressed genes in the jellyfish (*Rhopilema esculentum*) by transcriptome sequencing. Mar Genomics. 2017;34:31–7.

Licandro P, Conway DVP, Yahia MND, et al. A blooming jellyfish in the northeast Atlantic and Mediterranean. Biol Lett. 2010;6(5):688–91. https://doi.org/10.1098/rsbl.2010.0150.

Liu S-YV, Yu H-T, Fan T-Y, Dai C-F. Genotyping the clonal structure of a gorgonian coral, *Junceella juncea* (Anthozoa: Octocorallia), using microsatellite loci. Coral Reefs. 2005;24:352–8.

López C, Clemente S, Almeida C, Brito A, Hernández M. A genetic approach to the origin of *Millepora* sp. in the eastern Atlantic. Coral Reefs. 2015;34:631–8.

López-Legentil S, Pawlik JR. Genetic structure of the Caribbean giant barrel sponge Xestospongia muta using the I3-M11 partition of COI. Coral Reefs. 2009;28:157–65.

Lukoschek V, Riginos C, van Oppen MJH. Congruent patterns of connectivity can inform management for broadcast spawning corals on the Great Barrier Reef. Mol Ecol. 2016;25:3065–80.

Lundgren P, Vera JC, Peplow L, Manel S, van Oppen MJ. Genotype–environment correlations in corals from the Great Barrier Reef. BMC Genet. 2013;14:9.

Maldonado M. Do chimeric sponges have improved chances of survival? Mar Ecol Prog Ser. 1998:301–6.

Maldonado M. The ecology of the sponge larva. Can J Zool. 2006;84:1–20.

Maldonado M, Riesgo A. Reproduction in the phylum Porifera: a synoptic overview. Treballs de la Societat Catalana de Biologia. 2009a:29–49.

Maldonado M, Riesgo A. Gametogenesis, embryogenesis, and larval features of the oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). Mar Biol. 2009b;156:2181–97.

Marriage TN, Orive ME. Mutation-selection balance and mixed mating with asexual reproduction. J Theor Biol. 2012;308:25–35.

McFadden CS. Contributions of sexual and asexual reproduction to population structure in the clonal soft coral, *Alcyonium rudyi*. Evolution. 1997;51:112–26.

Mehbub MF, Lei J, Franco C, Zhang W. Marine sponge derived natural products between 2001 and 2010: trends and opportunities for discovery of bioactives. Mar Drugs. 2014;12:4539–77.

Miller KJ. Genetic structure of black coral populations in New Zealand's fiords. Mar Ecol Prog Ser. 1997;161:123–32.

Miller K, Ayre D. The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral Pocillopora damicornis. Heredity. 2004;92:557.

Miller K, Alvarez B, Battershill C, Northcote P, Parthasarathy H. Genetic, morphological, and chemical divergence in the sponge genus *Latrunculia* (Porifera: Demospongiae) from New Zealand. Mar Biol. 2001;139:235–50.

Miller BJ, Von der Heyden S, Gibbons MJ. Significant population genetic structuring of the holoplanktic scyphozoan *Pelagia noctiluca* in the Atlantic Ocean. Afr J Mar Sci. 2012;34:425–30.

Molinero JC, Ibanez F, Souissi S, et al. Climate control on the long-term anomalous changes of zooplankton communities in the Northwestern Mediterranean. Glob Chang Biol. 2008;14:11–26.

Moritz C. Defining "evolutionarily significant units" for conservation. Trends Ecol Evol. 1994;9:373–5.

Omori M. Edible jellyfish (Scyphomedusae: Rhizostomeae) in the Far East [Sea] waters: a brief review of the biology and fishery. Bull Plankton Soc Jpn. 1981;28:11.

Oppen V, Madeleine J, Bongaerts P, et al. The role of deep reefs in shallow reef recovery: an assessment of vertical connectivity in a brooding coral from west and east Australia. Mol Ecol. 2011;20:1647–60.

Padua A, Leocorny P, Custódio MR, Klautau M. Fragmentation, fusion, and genetic homogeneity in a calcareous sponge (Porifera, Calcarea). J Exp Zool A Ecol Genet Physiol. 2016;325:294–303.

Padua A, Cunha H, Klautau M. Gene flow and differentiation in a native calcareous sponge (Porifera) with unknown dispersal phase. Mar Biodivers. 2017:1–11.

Palumbi SR. Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst. 1994;25:547–72.

Palumbi SR. Using genetics as an indirect estimator of larval dispersal. In: McEdward L, editor. Ecology of the marine invertebrate larvae. Boca Raton, FL: CRC Press; 1995. p. 369–87.

Palumbi SR. Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl. 2003;13:146–58.

Patarnello T, Volckaert F, Castilho R. Pillars of Hercules: is the Atlantic, ÄìMediterranean transition a phylogeographical break? Mol Ecol. 2007;16:4426–4444.

Pauls SU, Nowak C, Bálint M, Pfenninger M. The impact of global climate change on genetic diversity within populations and species. Mol Ecol. 2013;22:925–46.

Peijnenburg KTCA, Breeuwer JAJ, Pierrot-Bults AC, Menken SBJ. Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. Evolution. 2004;58:1472–87.

Pérez-Portela R, Noyer C, Becerro MA. Genetic structure and diversity of the endangered bath sponge *Spongia lamella*. Aquat Conserv Mar Freshwat Ecosyst. 2015;25:365–79.

Pérez-Portela R, Cerro-Gálvez E, Taboada S, et al. Lonely populations in the deep: genetic structure of red gorgonians at the heads of submarine canyons in the north-western Mediterranean Sea. Coral Reefs. 2016;35:1013–26.

Pérez-Portela R, Rius M, Villamor A. Lineage splitting, secondary contacts and genetic admixture of a widely distributed marine invertebrate. J Biogeogr. 2017;44:446–60.

Pinzón JH, Reyes-Bonilla H, Baums IB, LaJeunesse TC. Contrasting clonal structure among *Pocillopora* (Scleractinia) communities at two environmentally distinct sites in the Gulf of California. Coral Reefs. 2012;31:765–77.

Pitt KA, Kingsford MJ. Geographic separation of stocks of the edible jellyfish *Catostylus mosaicus* (Rhizostomeae) in New South Wales, Australia. Mar Ecol Prog Ser. 2000:143–55.

Postaire B, Magalon H, Bourmaud CAF, Bruggemann JH. Molecular species delimitation methods and population genetics data reveal extensive lineage diversity and cryptic species in Aglaopheniidae (Hydrozoa). Mol Phylogenet Evol. 2016;105:36–49.

Postaire B, Gélin P, Bruggemann JH, Magalon H. One species for one island? Unexpected diversity and weak connectivity in a widely distributed tropical hydrozoan. Heredity. 2017a;118:385–94.

Postaire B, Gélin P, Bruggemann JH, Pratlong M, Magalon H. Population differentiation or species formation across the Indian and the Pacific Oceans? An example from the brooding marine hydrozoan *Macrorhynchia phoenicea*. Ecol Evol. 2017b;7:8170–86.

Precht WF, Gintert BE, Robbart ML, Fura R, Van Woesik R. Unprecedented disease-related coral mortality in Southeastern Florida. Sci Rep. 2016;6:31374.

Purcell JE. Jellyfish and ctenophore blooms coincide with human proliferations and environmental perturbations. Annu Rev Mar Sci. 2011;4:209–35.

Purcell JE, Arai MN. Interactions of pelagic cnidarians and ctenophores with fish: a review. Hydrobiologia. 2001;451:27–44.

Purcell JE, S-i U, Lo W-T. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Mar Ecol Prog Ser. 2007;350:153–74.

Purcell J, Mianzan H, Frost JR. Jellyfish blooms IV: interactions with humans and fisheries. Dordrecht, The Netherlands: Springer; 2015.

Ramšak A, Stopar K, Malej A. Comparative phylogeography of meroplanktonic species, Aurelia spp. and *Rhizostoma pulmo* (Cnidaria: Scyphozoa) in European Seas. Hydrobiologia. 2012;690:69–80.

Reitzel AM, Herrera S, Layden MJ, Martindale MQ, Shank TM. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Mol Ecol. 2013;22:2953–70.

Reusch TBH, Bolte S, Sparwel M, Moss AG, Javidpour J. Microsatellites reveal origin and genetic diversity of Eurasian invasions by one of the world's most notorious marine invader, *Mnemiopsis leidyi* (Ctenophora). Mol Ecol. 2010;19:2690–9.

Riesgo A, Novo M, Sharma PP, et al. Inferring the ancestral sexuality and reproductive condition in sponges (Porifera). Zool Scr. 2014;43:101–17.

Riesgo A, Pérez-Portela R, Pita L, et al. Population structure and connectivity in the Mediterranean sponge *Ircinia fasciculata* are affected by mass mortalities and hybridization. Heredity. 2016;117:427.

Riginos C, Douglas KE, Jin Y, Shanahan DF, Treml EA. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography. 2011;34:566–75.

Rua CP, Zilberberg C, Solé-Cava AM. New polymorphic mitochondrial markers for sponge phylogeography. J Mar Biol Assoc UK. 2011;91:1015–22.

Sabatés A, Pagès F, Atienza D, et al. Planktonic cnidarian distribution and feeding of *Pelagia noctiluca* in the NW Mediterranean Sea. Hydrobiologia. 2010;645:153–65.

Santangelo G, Abbiati M. Red coral: conservation and management of an over-exploited Mediterranean species. Aquat Conserv Mar Freshwat Ecosyst. 2001;11:253–9.

Santelices B. Mosaicism and chimerism as components of intraorganismal genetic heterogeneity. J Evol Biol. 2004;17:1187–8.

Selkoe K, Toonen RJ. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. Mar Ecol Prog Ser. 2011;436:291–305.

Selkoe KA, Henzler CM, Gaines SD. Seascape genetics and the spatial ecology of marine populations. Fish Fish. 2008;9:363–77.

Selkoe KA, Gaggiotti OE, Bowen BW, Toonen RJ. Emergent patterns of population genetic structure for a coral reef community. Mol Ecol. 2014;23:3064–79.

Serrano X, Baums I, O'Reilly K, et al. Geographic differences in vertical connectivity in the Caribbean coral *Montastraea cavernosa* despite high levels of horizontal connectivity at shallow depths. Mol Ecol. 2014;23:4226–40.

Serrano XM, Baums IB, Smith TB, et al. Long distance dispersal and vertical gene flow in the Caribbean brooding coral *Porites astreoides*. Sci Rep. 2016;6:21619.

Setiawan E, de Voogd NJ, Swierts T, et al. MtDNA diversity of the Indonesian giant barrel sponge *Xestospongia testudinaria* (Porifera: Haplosclerida)-implications from partial cytochrome oxidase 1 sequences. J Mar Biol Assoc U K. 2016;96:323–32.

Shearer TL, Van Oppen MJH, Romano SL, Wörheide G. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol Ecol. 2002;11:2475–87.

Shen X-X, Hittinger CT, Rokas A. Contentious relationships in phylogenomic studies can be driven by a handful of genes. Nat Ecol Evol. 2017;1:0126.

Sherman CDH, Ayre DJ, Miller KJ. Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia. Coral Reefs. 2006;25:7–18.

Shinzato C, Mungpakdee S, Arakaki N, Satoh N. Genome-wide SNP analysis explains coral diversity and recovery in the Ryukyu Archipelago. Sci Rep. 2015;5:18211.

Silva G, Lima FP, Martel P, Castilho R. Thermal adaptation and clinal mitochondrial DNA variation of European anchovy. Proc R Soc Lond B Biol Sci. 2014;281:20141093.

Smith L, Hildemann W. Allograft rejection, autograft fusion and inflammatory responses to injury in *Callyspongia diffusa* (Porifera; Demospongia). Proc R Soc Lond B Biol Sci. 1986;226:445–64.

Souter P, Henriksson O, Olsson N, Grahn M. Patterns of genetic structuring in the coral *Pocillopora damicornis* on reefs in East Africa. BMC Ecol. 2009;9:19.

Spalding MD, Fox HE, Allen GR, et al. Marine ecoregions of the World: a bioregionalization of coastal and shelf areas. Bioscience. 2007;57:573–83.

Stopar K, Ramšak A, Trontelj P, Malej A. Lack of genetic structure in the jellyfish *Pelagia noctiluca* (Cnidaria: Scyphozoa: Semaeostomeae) across European seas. Mol Phylogenet Evol. 2010;57:417–28.

Suzuki G, Keshavmurthy S, Hayashibara T, et al. Genetic evidence of peripheral isolation and low diversity in marginal populations of the *Acropora hyacinthus* complex. Coral Reefs. 2016;35:1419–32.

Taboada S, Pérez-Portela R. Contrasted phylogeographic patterns on mitochondrial DNA of shallow and deep brittle stars across the Atlantic-Mediterranean area. Sci Rep. 2016;6:32425.

Topsent E. Sur les larves cuirassées de Thoosa armata. Arch Zool Exp Gén. 1903;1:1–3.

Trégouboff G. Sur les larves planctoniques d'éponges. Comptes Rendus de l'Academie des Sciences du Paris. 1939;208:1245–6.

Trégouboff G. Contribution à la connaissance des larves planctoniques d'éponges. Archives de Zoologie Expérimentale. 1942;82:357–99.

Underwood JN. Genetic diversity and divergence among coastal and offshore reefs in a hard coral depend on geographic discontinuity and oceanic currents. Evol Appl. 2009;2:222–33.

Uriz MJ, Turon X. Sponge ecology in the molecular era. In: Advances in marine biology. 2012;61:345–410. Academic Press.

Vacelet J. Planktonic armoured propagules of the excavating sponge *Alectona* (Porifera: Demospongiae) are larvae: evidence from *Alectona wallichii* and *A. mesatlantica* sp. nov. Memoir Queensl Mus. 1999;44:627–42.

Vargas P, Zardoya R. In: Vargas P, Zardoya R, editors. El árbol de la vida: sistemática y evolución de los seres vivos. Madrid; 2012.

Villamor A, Costantini F, Abbiati M. Genetic structuring across marine biogeographic boundaries in rocky shore invertebrates. PLoS One. 2014;9:e101135.

Voigt O, Eichmann V, Wörheide G. First evaluation of mitochondrial DNA as a marker for phylogeographic studies of Calcarea: a case study from *Leucetta chagosensis*. Hydrobiologia. 2012;687:101–6.

van Walraven L, Driessen F, van Bleijswijk J, et al. Where are the polyps? Molecular identification, distribution and population differentiation of *Aurelia aurita*. Mar Biol. 2016;163:1–13.

Wang S, Zhang L, Meyer E, Matz MV. Construction of a high-resolution genetic linkage map and comparative genome analysis for the reef-building coral *Acropora millepora*. Genome Biol. 2009;10:R126.

Waples RS. Definition and estimation of effective population size in the conservation of endangered species. Population viability analysis. 2002. p. 147–68

Warburton FE. Reproduction of fused larvae in the boring sponge, *Cliona celata* Grant. Nature. 1958;181:493–4.

Weersing K, Toonen RJ. Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser. 2009;393:1–12.

Whalan S, de Nys R, Smith-Keune C, et al. Low genetic variability within and among populations of the brooding sponge *Rhopaloeides odorabile* on the central Great Barrier Reef. Aquat Biol. 2008;3:111–9.

Williams GC. Sex and evolution. Princeton, NJ: Princeton University Press; 1975.

Wörheide G. Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge *Astrosclera willeyana* across the Indo-Pacific. Mar Biol. 2006;148:907–12.

Wörheide G, Hooper JN, Degnan BM. Phylogeography of western Pacific *Leucetta* "*chagosensis*" (Porifera: Calcarea) from ribosomal DNA sequences: implications for population history and conservation of the Great Barrier Reef World Heritage Area (Australia). Mol Ecol. 2002;11:1753–68.

Wörheide G, Solé-Cava AM, Hooper JN. Biodiversity, molecular ecology and phylogeography of marine sponges: patterns, implications and outlooks. Integr Comp Biol. 2005;45:377–85.

Wörheide G, Epp LS, Macis L. Deep genetic divergences among Indo-Pacific populations of the coral reef sponge *Leucetta chagosensis* (Leucettidae): founder effects, vicariance, or both? BMC Evol Biol. 2008;8:24.

Wulff JL. Variation in clone structure of fragmenting coral reef sponges. Biol J Linn Soc. 1986;27:311–30.

Xavier JR. Chapter 6: phylogeography of the haplosclerid sponge Petrosia ficiformis (Porifera, Petrosiidae) reveals a deep-water affinity of some shallow-water populations. Amsterdam: University of Amsterdam; 2009.

Xavier JR, Soest R, Breeuwer JA, Martins AM, Menken SB. Phylogeography, genetic diversity and structure of the poecilosclerid sponge *Phorbas fictitius* at oceanic islands. Contrib Zool. 2010;79:119–29.

Yeoh S-R, Dai C-F. The production of sexual and asexual larvae within single broods of the scleractinian coral, *Pocillopora damicornis*. Mar Biol. 2010;157:351–9.

Zilberberg C, Solé-Cava A, Klautau M. The extent of asexual reproduction in sponges of the genus *Chondrilla* (Demospongiae: Chondrosida) from the Caribbean and the Brazilian coasts. J Exp Mar Biol Ecol. 2006;336:211–20.

Population Genomics and Biogeography of the Northern Acorn Barnacle (*Semibalanus balanoides*) Using Pooled Sequencing Approaches

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Abstract The northern acorn barnacle (*Semibalanus balanoides*) is a robust system for the study of evolutionary processes in the intertidal. *S. balanoides* has a well-characterized ecology, a wide circumboreal distribution, and a life history characterized by tractable environmental stressors at various ecological scales. In this chapter, we discuss a variety of topics concerning the development of *S. balanoides* as a model in ecological genomics as well as inferences of demography and historical phylogeography. In addition, we introduce two novel genomic tools for *S. balanoides*: the complete mtDNA sequence and the second draft of the nuclear genome (*Sbal2*). Using these tools, we conducted a reanalysis of previously described mtDNA haplotypes, *a* and *b*, as well as genome-wide levels of variation and population structure across the North Atlantic using pooled sequencing approaches. Analyses of sequence data from older and more recent Illumina platforms revealed the effects of technical bias in the estimates of population genomic metrics. We found concordant levels of nuDNA and mtDNA genetic variation with no evidence of demographic bottlenecks. We observed low genome-wide F_{ST} values across the Atlantic, suggesting a large number of ancestral polymorphisms and shared standing variation across the basin. Comparisons of genome-wide estimates of F_{ST} with those derived from a discriminant analysis of principal components uncovered population-structure-informative SNPs. This suggests the existence of latent population structure across broad scales, despite the capacity for extensive

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planktonic dispersal. Noticeably, our samples collected in Iceland displayed higher similarity to North American populations than to the rest of Europe. We hypothesize this is consistent with a periglacial refugium in Iceland concomitant with a barrier to gene flow caused by the North Atlantic current. Lastly, we discuss challenges and opportunities for the improvement of genomic tools in barnacles. Our reflections in this area are easily generalizable to most natural populations.

Keywords Barnacles · Ecological genomics · Genome assembly · Mitochondria · Pooled sequencing · Population genetics · *Semibalanus balanoides*

1 Barnacles in the Era of Genomics

In the past decade, the development of high-throughput DNA sequencing technologies has opened new opportunities to quantify levels of variation in natural populations at remarkable resolution (Luikart et al. 2003). This technological revolution has greatly improved our ability to quantify genetic variation in non-model organisms, particularly marine systems (Crawford and Oleksiak 2016). Most marine organisms exhibit large *effective populations sizes* (N_e) and high dispersal potential; as such, they experience ample opportunities for selection to act effectively on genes that respond to ecological stress. Many marine ecosystems can be thought of as *natural laboratories* for natural selection with well-defined, tractable scales of repeatable clinal ecological stresses, such as latitudinal clines, variation within estuaries, and microhabitat variation at different levels of tidal heights (Schmidt et al. 2008). However, despite the good fit of marine systems for evolutionary studies and the advances of short- and long-read platforms of *high-throughput sequencing* (HTS), the number of reference genomes available for marine systems represents only a minuscule proportion of the taxonomic diversity of marine ecosystems (Ellegren 2014).

While there has been considerable improvement in the number of whole genomes available for natural populations, funding constraints remain the great limiting step for researchers interested in questions relating to genome-scale patterns of variation and evolution. These constraints are associated with the technical requirements for de novo *genome assembly* of complex genomes. HTS methods (e.g., Illumina) output a high volume of *short reads*, which must be assembled into novel reference sequences or mapped to existing genomes before useful information can be extracted. In the case of most natural populations, high levels of *standing genetic variation* combined with complex genome architectures make the assembly of a reference genome a daunting task. The advent of long-read sequencing platform technologies (e.g., PacBio or Oxford Nanopore; see Rhoads and Au (2015)) comes with the promise of better genome assemblies afforded by individual sequences that can span repetitive sequences that confound traditional assembly algorithms. However, long-read methods come with trade-offs compared to short-read methods,

as the increase in length (~100 bp in vs. 5,000–40,000 bp) comes with the cost of significantly higher error rates (on the order of 10% *per*-base error for long reads vs. 0.1% *per*-base error for short reads) (Quail et al. 2012). Long-read error rates can be mitigated through the sequencing of samples to very high *coverage*. However, the current cost of long-read sequencing makes this approach prohibitively expensive for investigators aiming to characterize genome-wide genetic variation in non-model systems without big funding. A natural strategy for researchers working on ecological genomics questions is to use cost-effective sequencing strategies (e.g., *pool-seq*; see Schlotterer et al. (2014)), which generate highly comprehensive genomic datasets for both de novo assembly and characterizing genetic variation.

The northern acorn barnacle (*Semibalanus balanoides*) is a robust system for the study of adaptation in the intertidal. *S. balanoides* has a well-characterized ecology, wide circumboreal distribution, and a life history characterized by tractable environmental stressors at various ecological scales in the rocky intertidal (Schmidt and Rand 2001; Schmidt et al. 2008; Flight et al. 2012; Flight and Rand 2012). This system displays large N_e, high levels of gene flow at the swimming larval stages, and adult reproductive stages committed to a sessile habit in the intertidal. This dichotomy between high gene flow during the early larval stages and commitment to specific microhabitats in a highly heterogeneous ecosystem at the terminal larval stage, with subsequent physiological stressors arising in the sessile adult stages, provides a unique opportunity to dissect the interplay of fine-scale natural selection in the context of ample gene flow. Nevertheless, few genomic datasets for barnacle populations exist, consisting mostly of a handful of allozymes, microsatellites, and single mitochondrial (mtDNA) markers. Motivated by this lack of genomic resources, Flight and Rand (2012) used a cost-effective pool-seq approach to construct a de novo reference genome while simultaneously characterizing genetic variation in barnacle populations. The first genome of *S. balanoides* (hereby referred to as *Sbal1*) was assembled using whole genome pooled sequences from three barnacle populations around the North Atlantic (Bristol, Maine (ME-2011), Narragansett, Rhode Island (RI-2011), and Southwold, England (SW-2011)). The authors used the combined output of these pool-seq experiments (a *pool of pools*) to assemble the first genome for the species. Results from *remapping* ME-2011, RI-2011, and SW-2011 reads back to *Sbal1* provided a comprehensive characterization of genome-wide levels of genetic variation across the North Atlantic. Their results showed that genome-wide levels of genetic differentiation (F_{ST}) are low among all populations surveyed (RI-2011 vs. SW-2011 = 0.0408, ME-2011 vs. SW-2011 = 0.0362, ME-2011 vs. RI-2011 = 0.0243). However, they noted the existence of particular loci throughout the genomes of populations with high F_{ST}. These loci, the authors argued, could be candidates for local adaptation.

In this chapter, we recapitulate the state of the *S. balanoides* genomic resources and describe current and future improvements. We introduce the complete assembly of the mitochondrial genome of *S. balanoides* as well as the second draft of *S. balanoides'* nuclear genome (nuDNA), *Sbal2*. We use this improved draft genome to characterize genetic variation across populations in North Atlantic range of *S. balanoides* habitat using pool-seq data. We included pooled samples from

ME-2011, RI-2011, SW-2011 (from Flight and Rand (2012)), as well as new pool-seq data from Iceland, and two locations in the United States (Maine and Rhode Island). We use these genomic tools to revisit questions of biogeography and population structure across the North Atlantic. Our results from these analyses show concordance in levels of genetic variation between mitochondrial and nuclear genomes at population-structure-informative markers. Levels of genetic differentiation, however, indicate that populations from Iceland are more similar to North America than to the rest of Europe. Based on these data, we hypothesize that current population structure and gene flow in North Atlantic *S. balanoides* are driven by oceanographic processes associated with the North Atlantic current. This, combined with estimates of deep divergence times from Wares and Cunningham (2001), suggests that Icelandic barnacles may have survived in periglacial refugia during the events of the Last Glacial Maxima (LGM; ~20,000 years ago) (Vermeij 1991; van Oppen et al. 1995; Wares and Cunningham 2001; Flight et al. 2012). Finally, we provide an outlook on the future of the barnacle as a tool for marine ecological genomics as well as other challenges involved in the process of genome assembly for natural populations.

2 Current Genomic Resources for *S. balanoides*

While the assembly of *Sbal1* provided access to new avenues for barnacle research, genome completeness statistics for *Sbal1* reveal it to be a fairly incomplete draft genome. Based on our estimates of haploid genome size (1.6 Gbp; see Box 1 for technical considerations on *Sbal1* and *Sbal2*), *Sbal1* only recovers 3% (31 Mbp) of the total barnacle genome. *Sbal1* is also a highly fragmented reference showing low *N50* (250 bp) and a high number of *contigs* (22,986). Moreover, the number of universal single-copy orthologue (USCO) genes (Simao et al. 2015), a common metric for genome completeness, show a large proportion of missing (77%) USCO genes and only a small proportion of complete (12%) or fragmented (9%) USCO genes. Nevertheless, 6,239 functional genes were predicted using gene prediction models. In this chapter, we introduce two novel genomic resources: the complete mitochondrial sequence of *S. balanoides* (NCBI GeneBank number: MG010647, MG010648, and MG010649), as well as the second draft barnacle genome (*Sbal2;* this whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession PHFM00000000. The version described in this chapter is version PHFM01000000). Both of these resources were generated de novo using the same raw data as *Sbal1*. We hope to showcase how advances in bioinformatics algorithms can improve extant genomic resources for natural populations without big funding.

Box 1 Technical Considerations on *Sbal2* De Novo Assembly

We constructed *Sbal2* de novo using the same raw reads used for the assembly of *Sbal1* (Maine (ME-2011), Rhode Island (RI-2011), and England (SW-2011); see Flight and Rand 2012); however, we employed a completely different pipeline using software better suited for the construction of diploid genomes with *de Bruijn* graphs. Prior to assembly, we filtered and trimmed reads to maximize quality. We used BBMap tools v. 33.92 (Bushnell 2016) to perform a first round of filtering by lightly trimming read edges and throwing out all reads whose minimum Phred quality score was less than 20. A second round of quality control was performed using the filtering algorithm available in the SGA aligner suite v. 0.10.15 (Simpson and Durbin 2012). This step removed homopolymeric as well as low complexity sequences. A final step depleted the read pools from duplicated reads using the software FastUniq v. 1.1 (Xu et al. 2012).

Read error correction is a common practice performed in many de novo assembly pipelines prior to assembly. Recent work, however, has shown that it may have negative effects when applied to highly heterogeneous read pools (i.e., our case), via the introduction of chimeric reads (Fujimoto et al. 2014). We thus opted to avoid error correction algorithms. Only 50% of the data passed our quality control pipeline. These reads were used for the final assembly. We used the FastQC v. 0.11.4 program (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) at all steps of quality control. Finally, prior to assembly, we used the SGA preQC module to visualize various aspects of genome assembly (sequence coverage, per-base error rates and genome size, heterozygosity and repeat content) under various *k*-mer sizes and various pooling and filtering strategies (i.e., using only one pool vs. combining all three pools). Based on the SGA preQC report, we decided to perform de novo assembly on a combination of all three pools. For all other cases, coverage was too low to successfully assemble an improved genome, relative to *Sbal1*. We tried a variety of diploid-compatible *de Bruijn* assemblers such as spades v. 3.9.1 (Bankevich et al. 2012; Safonova et al. 2015), Meraculous v. 2.0 (Chapman et al. 2011), among others. However, due to the pooled nature of our dataset (which is not ideal for de novo assembly in most cases), neither software produced successful improvements over *Sbal1*. The assembly of *Sbal2* was completed using *SparseAssembler* ($k = 37$), a software which uses an evolved *de Bruijn graph* structure called the sparse graph (Ye et al. 2012). This software is available with the 2017 release of the genome assembler DBG2OLC (Ye et al. 2015). *SparseAssembler* requires an estimate of genome size. However, instead of using the estimated size from *S. cariosus* (1.40 Gbp (Bachmann and Rheinsmith 1973)), we decided to estimate *S. balanoides'* genome size using Lander-Waterman theory (Lander and Waterman 1988) and *k*-mer counting with jellyfish v. 1.1.6 (Marcais and Kingsford 2011). Our estimates of haploid genome size

(continued)

Box 1 (continued)
approximated 1.63 Gbp. In terms of overall architecture, the resulting *Sbal2* assembly was similar to *Sbal1* insofar they are composed primarily of short ~1,000 bp long contigs with low N50 (~900 bp). *Sbal2*, however, is composed of 67,133 contigs amounting to a total length of 100 Mbp, thus tripling the amount of sequence reconstructed from the total estimated genome size (1.90% *Sbal1* vs. 6.13% *Sbal2*; total genome size = 1.63 Gbp). The BUSCO 3.0 pipeline for SCO assessment (Simao et al. 2015) against the eukaryotic database (odb9) revealed considerable improvement of *Sbal2* relative to *Sbal1* (see Sect. 2). For both genomes, gene predictions were conducted using AUGUSTUS v. 3.0.2 (Stanke and Waack 2003) on hard-masked genomes using RepeatModeler (1.0.8) and RepeatMasker (4.0.7) (Smit et al. 2013–2015). AUGUSTUS gene predictions were based on a *Drosophila melanogaster* model. RNA-seq reads used for contig validation were sequenced in 2017 from Maine barnacles. Sequencing was done in an Illumina HiSeq machine by GENEWIZ LLC, using a 2 × 150 bp PE configuration.

In most cases, genome assembly algorithms operate under a collection of biological assumptions about genome complexity and genetic variation. These assumptions are usually not met by most natural populations, including the barnacle. This occurs because natural populations tend to have more genetic variation and more sequence repetition than most algorithms are designed to handle (we provide an expanded discussion on this matter in Sect. 6). An exception to this is the mitochondrial genome of most animal species. The mitochondrial genome is small (~16,500 bp) and gene-rich, containing a low number of repeat regions. This makes assembly of the mitochondrial genome relatively straightforward compared to the more complex nuclear genome. For *S. balanoides*, we utilized the method implemented by Hahn et al. (2013) to produce a fully assembled mitochondrial reference genome (see Box 2).

The task of improving the nuclear reference of *S. balanoides* was more daunting due to the nature of our starting dataset. The combination of multiple individuals in a *pool of pools* introduces extra heterogeneity, making the genome assembly process more difficult. We generated the draft nuclear genome, *Sbal2*, using the assembly algorithm implemented by Ye et al. (2012). A detailed description of our de novo genome assembly pipeline is highlighted in Box 1. Relative to *Sbal1*, *Sbal2* constitutes a major improvement in terms of length (100 Mbp vs. 31 Mbp) and overall levels of gene content completeness. Over half (54%) of USCO searches resulted in either complete (19%) or fragmented (35%) gene calls, compared to 21% in *Sbal1*. Unfortunately, *Sbal2* remains highly fractured, consisting of 67,133 contigs with N50 of 1,475. After masking the genome for repetitive and transposable elements (Smit et al. 2013–2015), an AUGUSTUS (Stanke and Waack 2003) *Drosophila* model predicted 12,835 protein coding genes. Out of these, 5,976 (46%) had high-

Box 2 De Novo Assembly of the Whole Mitochondrial Molecule
Mitochondrial genomes were assembled de novo from sequencing reads: for *S. balanoides* these reads were obtained bioinformatically from the Flight and Rand (2012) read pool (only reads from ME-2011 pool were used). MtDNA assembly was performed using a bait and iteration method (bim) using MITObim v. 1.8 (Hahn et al. 2013) and MIRA 4.0 (Chevreux et al. 1999). Initial runs for *S. balanoides* used the whole mtDNA of *Balanus balanus* (GenBank: KM660676.1) as a seed for the bait/iteration process. This species was chosen due to its phylogenetic proximity to *S. balanoides* (Perez-Losada et al. 2014). Recent studies, however, have shown the occurrence of gene order inversions in the mitochondrial genome of some barnacle species such as *Megabalanus volcano* (Shen et al. 2014a, b). In order to account for these possible rearrangements, we repeated the bait/iteration process with *M. volcano's* mtDNA (GenBank: NC_006293.1) as a seed. All resulting sequences were annotated using MITOS (Bernt et al. 2013) and curated manually. All sequences generated were compared against the NCBI database of the subphylum *Crustacea* (taxid:6657), using the Basic Local Alignment Search Tool (nBLAST). The *S. balanoides* assembly produced using *M. volcano* as seed resulted in better BLAST identity scores and annotation and was used as the initial draft genome. MITOS annotation of the mtDNA final genome identified all 13 mtDNA-encoded Oxidative Phosphorylation (OXPHOS) pathway proteins, 2 rRNAs, and 22 mtDNA tRNAs with high levels of statistical confidence. Manual curation for protein coding regions was conducted via MAFFT (v. 7.017) alignments of the de novo *S. balanoides* mtDNA with other barnacle specimens available in NCBI: *Balanus balanus* (NC_026466), *Megabalanus volcano* (NC_006293), and *Megabalanus ajax* (NC_024636).

quality BLAST hits to the UniProt database (the UniProt consortium 2017). Despite the high level of fragmentation of *Sbal2*, AUGUSTUS predicted various coding and regulatory features for individual genes (Fig. 1a). Exons and coding sequences (CDS) were the most abundant classes predicted by the algorithm. AUGUSTUS reports posterior probabilities for introns and CDS as well as whole genes/transcripts. For *Sbal2*, most individual introns and CDS have high posterior probabilities. Regions with high-quality UniProt hits have even higher probabilities (Fig. 1b). Despite high probabilities for individual features, whole gene posteriors are low. This showcases a need to improve both the assembly as well as the prediction model. To further validate these contigs, we conducted an RNA-seq from whole barnacle individuals and mapped the reads onto *Sbal2*. RNA-seq reads mapped onto 10,340 contigs with mean coverage of 187-fold and median of 38-fold. Most mapping reads

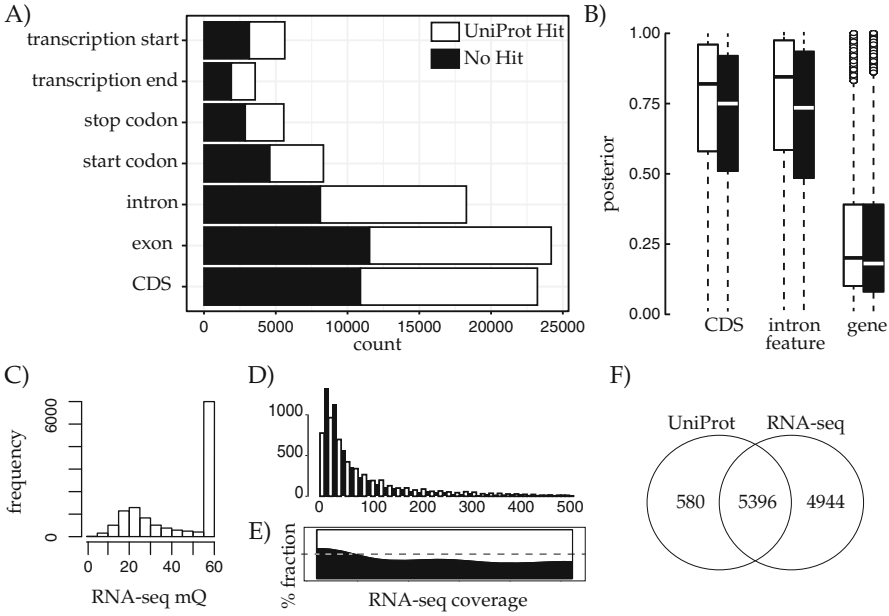

Fig. 1 Genomic features and quality of *Sbal2*. In all cases, white indicates the fraction of predictions with high-quality UniProt BLAST hits. (**a**) Number of genomic features predicted by AUGUSTUS. (**b**) AUGUSTUS posterior probabilities. (**c**) Mapping quality (mQ) of RNA-seq reads mapping to *Sbal2*. (**d**) RNA-seq coverage across the genome for contigs with and without UniProt hits. (**e**) Relative number of contigs with RNA-seq coverage. The dashed line represents the 50% fraction. (**f**) Venn diagram of number of contigs with RNA-seq reads mapping intercepted to contigs with genes having UniProt hits

mapped with good quality scores (mQ; mean = 43, median = 58; Fig. 1c). Notably, most reads mapping onto contigs with UniProt hits showed higher coverage relative to those that did not have UniProt hits (Fig. 1d, e). Out of the 67,133 total contigs assembled, 5,396 (~8%; Fig. 1f) contained loci with both UniProt hits and RNA-seq coverage. These represent candidates for subsequent studies of biological variation. Though still incomplete, *Sbal2* is a clear improvement over *Sbal1*. The assembly not only tripled the proportion of genome assembled, it also doubled the amount of informative contigs coding for putatively functional genes. It is clear, however, that the task of assembling contiguous sequences of the barnacle genome cannot be overcome using bioinformatic techniques alone. It requires the acquisition of new datasets optimized for genome assembly, e.g., a combination of long- and short-read technology and mate-pair or Hi-C sequence reads (Belton et al. 2012). However, despite the lack in contiguity, both the *Sbal1* and *Sbal2* draft genomes are robust enough to conduct population genetic analyses based on *single-nucleotide polymorphisms* (*SNPs*) data while providing links to functional annotation of a respectable sample of protein coding genes.

3 The Historical Biogeography of *S. balanoides* in the North Atlantic: As Told by mtDNA Markers

As with many other natural systems, our understanding of barnacle genetic variation and biogeography has greatly benefited from mtDNA markers (e.g., *COX I* and *D-loop*). Studies have shown that populations in the North Atlantic are composed of two mitochondrial clades: *a* and *b* (Fig. 2a; Brown et al. 2001; Wares and Cunningham 2001; Flight et al. 2012). Many authors have suggested that the current distributions of these clades are related to changes in intertidal substrate availability resulting from postglacial expansion and colonization events after the LGM (Vermeij 1991; van Oppen et al. 1995). Both Wares and Cunningham and Flight et al. investigated the hypothesis that LGM impacts were greater in North American populations relative to European ones. The most extreme interpretation of this hypothesis is a West Atlantic *tabula rasa* scenario in which rocky intertidal species went locally extinct. Consequentially, extant taxa are descendant of postglacial European recolonizers. Analyses performed with *COX I* have suggested that clade *b* is an ancient clade which may predate the LGM. Clade *a*, conversely, appears to be younger with a time to most recent common ancestor (TMRCA) of 3.3×10^4 years (Wares and Cunningham 2001). These concomitant signatures of permanence and absence in North America during LGM preclude a unified hypothesis for the barnacle LGM historical biogeography.

We revisited the historical biogeography of the species by investigating 200 *COX I* sequences from various North Atlantic populations in the United States (Rhode Island and Maine), Canada, Iceland, as well as the British Isles and Continental Europe (collectively named eastern Atlantic; samples are a combination

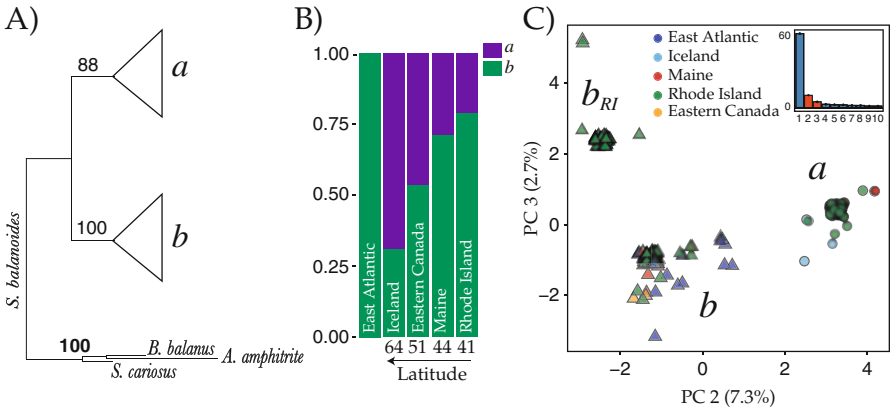

Fig. 2 Clades *a* and *b* in *COX I*. (**a**) Maximum likelihood phylogenetic tree of *S. balanoides* and closer taxa using *COX I*. (**b**) Proportions of clade *a* and *b* in the east and west North Atlantic Ocean. East Atlantic populations consist of Continental Europe and the British Isles. (**c**) Second and third dimensions of the *S. balanoides COX I* haplotypes PCA. The first dimension (not shown) of the PCA captures variation among individuals. Scree plot is shown in the top-right inset. Individuals are colored according to their population of origin

of previously published data by Wares and Cunningham (2001) and new sequences; see GeneBank accessions: MG925538–MG925662, and MG928281–MG928323). Our reanalysis of the data is consistent with previous findings of *COX I*. Clade *a* is seen only in the western Atlantic and Iceland and harbors less genetic variation ($\pi = 8.3 \times 10^{-4}$) relative to *b*. Clade *b*, on the other hand, is seen throughout the North Atlantic and shows high genetic variation among haplotypes ($\pi = 4.5 \times 10^{-3}$). The distribution of clades *a* and *b* shows a conspicuous latitudinal cline in Iceland and North America (Fig. 2b) with clade *a* showing its highest abundance in higher latitudes. The converse is true for *b* at lower latitudes. Principal component analysis (PCA) on *COX I* data reveals three primary axes of variation (Fig. 2c inset). The first axis of variation primarily captures variation among individuals (66.1% of variation). These trends are echoed by an analysis of molecular variance (AMOVA) based on geographic location: 83% of variance is found within samples and 16% between samples (Monte Carlo test $p << 0.01$; 10,000 replicates). The *a* and *b* clades are captured in the second principal component (7.3% variation explained; Fig. 2c). The third axis of variation (2.7% variation explained) suggests a subclade of *b* exclusive to RI populations. This sub-clade, b_{RI}, is defined primarily by two synonymous transitions (T/C) at haplotype positions 189 and 554 (Pearson's correlation, $|\rho| > 0.8$, $p << 0.01$). There is not enough information to assess the evolutionary relevance, if any, of clade b_{RI}. Table 1 shows various summary statistics from the *COX I* data. In general, samples from the eastern Atlantic show high levels of genetic variation ($\pi = 8.5 \times 10^{-3}$) concomitant with high levels of haplotype diversity ($h_d = 0.99$). Samples from North America show similar levels of genetic variation ($\pi = 7 \times 10^{-3}$–8×10^{-3}) but lower haplotype diversity. Samples from Iceland show the lowest amounts of genetic and haplotype variation. The Fu and Li's *D* statistic was only significantly negative for the Rhode Island populations.

The postglacial expansion hypothesis discussed by Wares and Cunningham has two central corollaries. First, *S. balanoides* clades shows amphi-Atlantic distributions (i.e., present on both west and east coast of the North Atlantic) corresponding to multiple colonization events. Second, the putatively ancestral populations (i.e., Europe) display demographic equilibrium and harbor higher levels of haplotype diversity relative to North American populations. We argue, however, that these data are not fully conclusive on the demographic history of the barnacle.

Table 1 Population genetic estimates from *COX I*

Location	*S*	h_d	π	FL-*D*	*P*(FL-*D*)
Maine	17	0.81	7.4×10^{-3}	−0.175	NS
Rhode Island	40	0.813	8.2×10^{-3}	−3.866	$p < 0.02$
Eastern Canada	12	0.731	8.2×10^{-3}	0.479	NS
Iceland	14	0.623	6.9×10^{-3}	−0.282	NS
East Atlantic	26	0.995	8.5×10^{-3}	−1.547	NS

S number of segregating sites, h_d haplotype diversity, π nucleotide diversity, *FL-D* Fu, and Li's estimate of *D* and its *p*-value

Objectively, the *COX I* clades in *S. balanoides* are hardly amphi-Atlantic: clade *a* is seen only in North America and Iceland (where they show a latitudinal cline; Fig. 2b), with no observed occurrences in Continental Europe or the British Isles. This clade, moreover, is estimated to be much younger than its counterpart (notice that *b* is thought to predate the LGM). As such, we believe the data does not lend strong support to a postglacial colonization of clade *a* from Continental Europe, especially when one considers that glaciers in Continental Europe and the British Isles have different deglaciation stories than Iceland (see Ruddiman and Mcintyre 1981). To further investigate this conundrum, we characterized mtDNA-wide patterns of genetic variation in the North Atlantic. We centered our attention on two questions: (1) Are the patterns of genetic variation estimated from *COX I* recapitulated at the whole mtDNA level? (2) Are the patterns of genetic variation estimated from mtDNA recapitulated in the nuclear genome? We also investigated whether the *a*/*b* latitudinal cline could be explained by processes other than demography. For instance, are there any nonsynonymous mtDNA variants showing correlations to the *a*/*b* latitudinal cline? Put simply, is there evidence of selection driving the *a*/*b* cline in the west Atlantic? At their core, these questions seek to gain a more comprehensive (i.e., beyond single markers) understanding of how demographic events shape genetic variation in natural populations.

Do whole mtDNA estimates recapitulate *COX I*? The presence of the *a*/*b* latitudinal cline in North America is suspect and could indicate that forces other than demography have shaped genetic variation in mtDNA clades (see Rand 1994; Silva et al. 2014; Camus et al. 2017). To further explore this issue, we mapped pool-seq datasets to our de novo mitochondrial reference genome to characterize patterns of clinality in mtDNA mutations across the western Atlantic. While pooled sequencing is a powerful tool to discover genetic variation in natural populations, it does not provide haplotype information. This is a major drawback when investigating linked markers such as mtDNA. However, we can still investigate key aspects of genetic variation across locations, as well as potential SNPs associated to the latitudinal cline. In this sense, pool-seq works as a SNP discovery tool. Downstream validation candidates can be done with Sanger sequencing or other targeted approaches (e.g., Nunez and Oleksiak 2016).

We analyzed six pool-seq datasets: the three original datasets used to assemble *Sbal2*, ME-2011, RI-2011, and SW-2011 and new pool-seq datasets from Reykjavik, Iceland (ICE-2017); Damariscotta, Maine, USA (ME-2017); and Jamestown, Rhode Island, USA (RI-2018), sequenced in 2017/2018. We discovered 2,157 (13% polymorphic sites) SNPs across all pools. These SNPs occur at different mtDNA locations as follows: 1,761 (81%) SNPs occurred in mtDNA genes, 49 (2.2%) in the *D-loop*, 181 (8.4%) in rRNAs, and 119 (5.5%) in tRNAs. One thousand five hundred ninety-four (74%) were transitions and 563 (26%) are transversions. Consistent with the predictions of strong mtDNA purifying selection (Rand 2008), 1,830 (85%) of coding SNPs were synonymous and 327 (15%) nonsynonymous. We did not discover any nonsynonymous SNPs significantly associated with the *a*/*b* latitudinal cline. We note, however, that our approach is hindered by the lack of haplotypic data. MtDNA-wide estimates of genetic

Table 2 Technical summaries and population genetic estimates from pool-seq datasets

Population	Year	HiSeq Tech.	Pool size	Mean coverage	π mtDNA	π nuDNA
Bristol, Maine, USA	2011	2000	20	13.3×	6.02×10^{-3}	5.0×10^{-3}
Damariscotta, Maine, USA	2017	2500	37	52.2×	8.86×10^{-3}	5.8×10^{-3}
Narragansett, Rhode Island, USA	2011	2000	20	11.31×	7.40×10^{-3}	4.8×10^{-3}
Jamestown, Rhode Island, USA	2018	2500	37	37.2×	7.22×10^{-3}	5.5×10^{-3}
Reykjavík, Iceland	2017	2500	20	46.5×	5.17×10^{-3}	5.6×10^{-3}
Southwold, UK	2009–2011	2000 + GAIIx	20	6×	7.21×10^{-3}	5.2×10^{-3}

Genetic variation (π) was estimated for both mtDNA and nuDNA

variation (π) are relatively consistent with *COX I* observations (Table 2). Figure 3a shows levels of mtDNA-wide genetic differentiation (F_{ST}) between populations. In general, geographic distance is an appropriate predictor of linearized F_{ST} values (Slatkin 1995) (Adj. $R^2 = 0.3938$, F-statistic $= 10.09$ on 1 and 13 DF, *p-value* < 0.01). Iceland, however, appears an outlier in the analysis, showing higher similarity to ME and RI relative to SW, despite closer geographical proximity to Southwold (Fig. 3b). This is likely driven by the high abundance of haplotype *a* in Iceland contrasted to its complete absence in other European samples (Fig. 2b). Regarding differences between the east and west coasts of the Atlantic, one mtDNA variant occurring in the *NADH* dehydrogenase subunit 4 (*NADH 4*) appears fixed for a nonsynonymous codon only seen in Southwold. This SNP results in a conservative change of phenylalanine to leucine. Future validation of this marker is required. Overall, there seems to be a satisfactory level of concordance between the *COX I* estimates of variation and those of whole mtDNAs. Patterns of genetic variation in Iceland are more similar to those in Rhode Island and Maine – 4,000 km away – than to patterns in the British Isles or Norway. This is puzzling because Iceland is located much closer to European shores than to American ones.

4 Characterizing Genetic Variation in the North Atlantic: What Stories Does the Nuclear Genome Tell?

Are estimates of genetic variation from mtDNA informative about *S. balanoides* evolution, or are they idiosyncratic to the molecule? Over the last decade, multiple studies have highlighted issues with using mtDNA as a demographic estimator (Toews and Brelsford 2012; Bazin et al. 2006; Meiklejohn et al. 2007). Most of these issues are related to evolutionary processes that generate discordances between mtDNA and nuDNA such as mtDNA adaptive introgression, sex-biased asymmetries, hybrid zones, human introductions, etc. Inferences from nuDNA allow

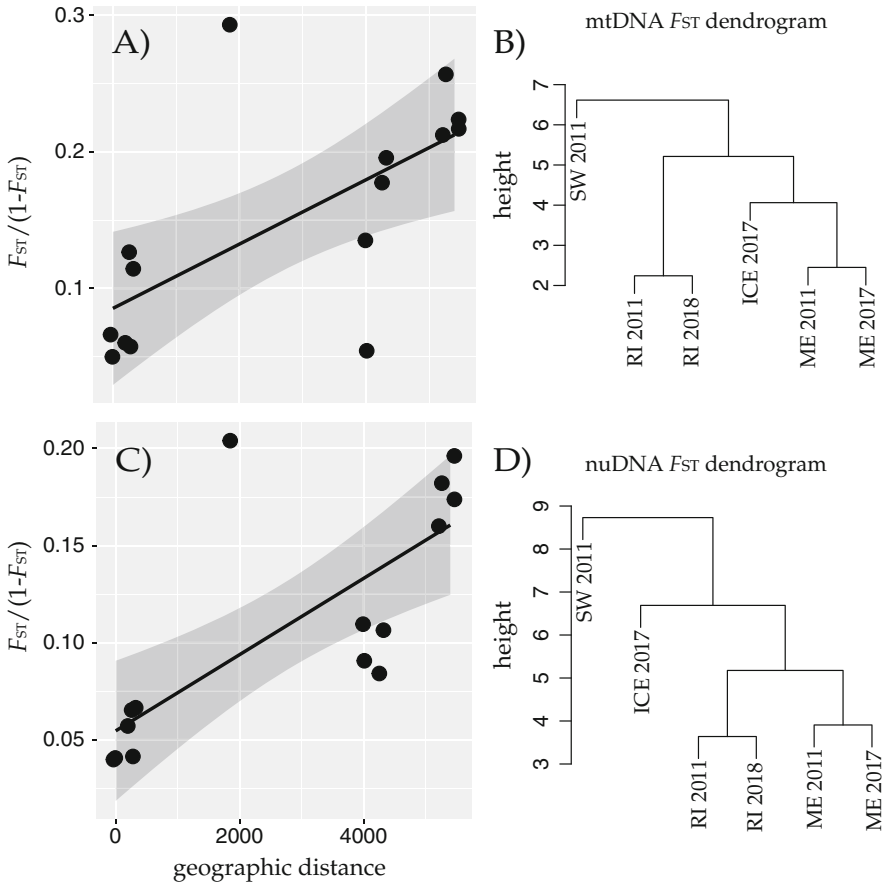

Fig. 3 Genetic differentiation across barnacle populations. Geographic distance vs. linearized F_{ST} ($F_{ST}/(1-F_{ST})$) for whole (**a**) mtDNA and (**c**) nuDNA. In both cases, the outlier F_{ST} in the top corner of the plot corresponds to Iceland vs. Southwold comparisons. The outliers in the lower corners correspond to comparisons of Iceland vs. North American populations. F_{ST} based cluster dendrogram for (**b**) mtDNA and (**d**) nuDNA estimated using structure-informative SNPs only

testing new hypotheses about demography and selection in barnacle populations. In order to investigate genetic variation in the barnacle's nuDNA, we remapped all six pool-seq datasets from ME-2011 and 2017, RI-2011 and 2018, ICE-2017, and SW-2011 to *Sbal2* (technical details in Box 3). Levels of genetic diversity in the nuclear genomes are similar across all populations (see Table 2). The only noticeable difference is produced by sequencing platform (see below), where more advanced technologies produce slightly higher estimates of variation. Analysis of genetic diversity at synonymous (π_S), nonsynonymous (π_{NS}), and neutral ($\pi_{neutral}$) sites revealed no significant trends (Fig. 4; Adj. $R^2 = 0.2$, F-statistic $= 1.7$ on 1 and 4 DF, *p-value* > 0.1). The π_{NS}/π_S-to-$\pi_{neutral}$ analysis is a powerful tool to detect

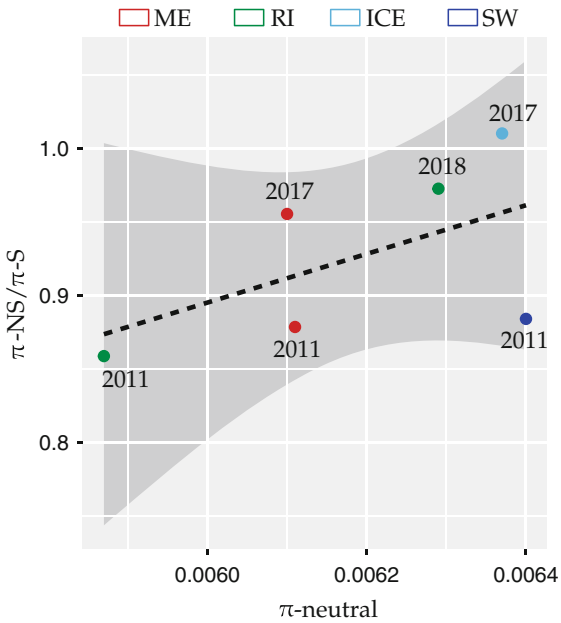

Fig. 4 Patterns of functional genetic variation (π nonsynonymous to π synonymous ratio) relative to neutral genetic variation. The linear regression shown in dashed lines is not statistically significant

genetic load associated with demographic contractions (Marsden et al. 2016; Elyashiv et al. 2010; Akashi et al. 2012). For this metric, we considered the ratio of nonsynonymous to synonymous variation within each gene. Neutral variation was estimated from the noncoding (i.e., excluding regulatory and promoter regions) neighborhood of each gene. The metric is reported as average per sample. Flight et al. (2012) had discussed the possibility of bottlenecks occurring during post-glacial expansion in barnacles. After a severe bottleneck, the reduction in population size may result in a reduction of purifying selection's ability to purge out deleterious variation (Kimura and Ohta 1971). This would result in a relative excess of nonsynonymous variation and negative relationship between π_{NS}/π_S to neutral variation, but such signal is not detected in our analysis.

We characterized locus specific differences in the barnacle's nuDNA by using the *PoPoolation2* pipeline on the six pool-seq datasets. Quality filtering (see Box 3) resulted in 495,275 genome-wide SNPs (these exclude SNPs happening in the neighborhood of *indels*). Retaining biallelic SNPs occurring only at validated contigs (see Sect. 2) results in 51,499 variants. Setting coverage limits, with a lower limit of 10× and upper limit to 100×, results in 11,775 SNPs. Finally, we trimmed some linkage associations by removing SNPs 500 bp apart from each other. This results in 3,177 high-quality SNPs for a first-pass analysis. PCA on these first-pass SNPs revealed that the primary signal in the data was driven by

Box 3 Individual Pool Read Mapping, SNP Calling, Allele Frequency Estimation, and Other Analysis

Before mapping, reads from all 6 *S. balanoides* pools were sanitized using various quality metrics assessed with FastQC v.0.11.4. Samples from ME-2011, RI-2011, and SW-2011 were obtained from Flight and Rand (2012). ICE-2017, ME-2017, and RI-2018 samples were sequenced by GENEWIZ LLC with a 2 × 150 bp PE configuration using an Illumina HiSeq 2500 machine. Read mapping for both mitochondrial and nuclear genomes inferences was done using BWA-MEM v. 0.7.12 (Li 2013). Variants for all pools were called with SAMtools v. 1.3.1 (Li et al. 2009). Population genetic analyses were conducted using the software tools in *PoPoolation* and *PoPoolation2* (Kofler et al. 2011). Both these programs estimate genetic parameters correcting for the coverage and size of the pools. Downstream data analyses were conducted in R v. 3.3.2 (https://cran.r-project.org) using various packages contained in the *'tidyverse'* v. 1.1.1 R library (http://tidyverse.org). For all analyses, we removed singleton SNP calls as well as those with a minimum allele frequency of 5%.

Other analyses presented in this chapter: Phylogenetic analyses were conducted with RAxML (Stamatakis 2014).Clustering analysis and principal component analysis were performed using the R packages factoextra v.1.0.5 (http://www.sthda.com/english/rpkgs/factoextra/) and FactoMineR v.1.36 (Lê et al. 2008). Gene homology searches were done via BLASTp searches (UniProt/Swiss-Prot database (The UniProt Consortium 2017). Version 2.6.0).

technology differences (Fig. 5a). This technology-driven bias is alarming as it may completely obscure the biological signal in the data. We utilized a discriminant analysis of principal components approach (DAPC, (Jombart et al. 2010)) to correct for technology bias while enriching for SNPs informative to population structure (also known as PCA-informative markers; see Paschou et al. 2007; Patterson et al. 2006; Price et al. 2006). We retained SNPs with loadings greater than the 90th percentile of the DAPC's first and second discriminant functions. This reduces the dataset to 650 high-quality/structure-informative SNPs. These variants occurred in introns (348; 53%), coding regions (178; 27%), 5′UTRs (29, 4%), 3′UTRs (22, 3%), and noncoding regions (74, 13%). Of the coding variants, 104 were synonymous and 74 were nonsynonymous.

PCA on these SNPs results in three principal axes of variation (Fig. 5b, c). The first axis (47% variation) captures differences between Southwold, the United Kingdom, and all other populations. The second axis (27% variation) partitions North America from Iceland. Finally, the third axis captures differences within North America (13% variation). Correlation analysis revealed 422 SNPs with significant correlations to PC1 (Pearson correlation coeff. $|\rho| > 0.8$, $p < 0.05$),

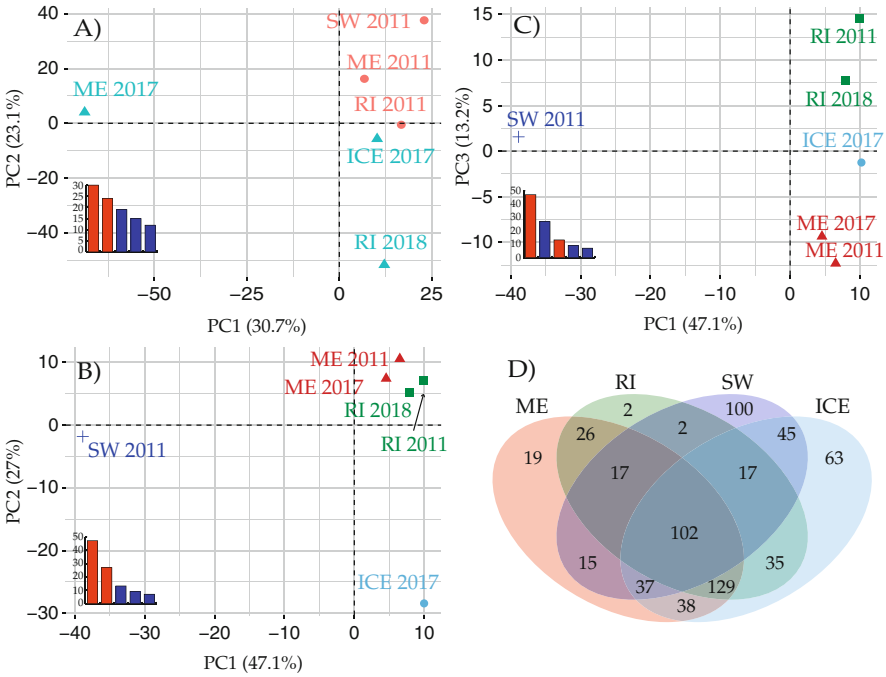

Fig. 5 Principal components analyses across six pool-seq datasets. (**a**) Technology bias. Light red indicates samples sequenced in an Illumina HiSeq 2000 machine. Light green samples indicate samples sequenced in an Illumina HiSeq 2500 machine. (**b**, **c**) Population structure-informative SNPs. In each PCA plot, a scree plot is shown in the inset with the first five PCA dimensions. Displayed dimensions are highlighted in red. (**d**) Venn diagram of private alleles *per* population

162 to PC2, and 20 to PC3. To ensure that these SNPs primarily capture signals of "neutral" evolution, we conducted a final filtering step consisting of a selection outlier scan with PCAadapt (Luu et al. 2017) on our 650 post-filtering SNPs. This approach takes advantage of the PCA inferred population structure to control for demographic differences. PCAadapt simulates individual genotype calls by sampling allele frequencies from pool-seq data using binomial random draws. For the analysis, we controlled for demography using the first three components of the PCA (same as in Fig. 5b, c). Using the Mahalanobis distance approach and a false discovery rate (Benjamini and Hochberg 1995) of 10%, we found ten outlier loci (1.5% of loci) in our dataset. These SNPs were removed from all downstream analyses.

Similar to the estimates of genetic variation (π), population-specific expected heterozygosity (H_E) is similar across all samples (ME-2017 = 0.2805, ME-2011 = 0.2427, RI-2018 = 0.2681, RI-2011 = 0.2137, ICE-2017 = 0.2804, SW-2011 = 0.2322). ME and RI analysis also revealed a small number of private alleles (2 and 19, respectively; Fig. 5d). The number of private alleles in ICE and SW, however, was much larger (63 and 100, respectively). Finally, we estimate

nuDNA F_{ST} for all pairwise population comparisons using our allele frequency estimates of structure-informative markers. MtDNA and nuDNA F_{ST} values are similar and highly correlated (Pearson correlation coeff. $\rho = 0.905$, $p << 0.01$). As such, geographic distance appears to be an appropriate predictor of population structure (Fig. 3c; Adj. $R^2 = 0.532$, F-statistic = 16.95 on 1 and 13 DF, *p-value* <0.01). As before, differentiation estimates relative to Iceland appear to be outliers. For nuDNA, however, Iceland no longer appears closer to ME than to SW (see Fig. 3b). Nonetheless, it still displays higher similarity to North American populations than to European ones (Fig. 3d), with remarkably higher F_{ST} values relative to SW, despite geographical proximity. These analyses suggest the existence of latent population structure across North Atlantic populations.

5 Population Structure, Dispersal, and Standing Variation in the North Atlantic: Technology Bias vs. Biological Signal

It is clear that *S. balanoides'* historical biogeography is fairly complex. Even with mtDNA markers and a clear hypothetical model of postglacial expansion, the biological signal captured in this haploid marker is not fully conclusive. We had hoped that analyses of nuDNA SNP markers would shine a clearer light on the issue. However, the challenges associated with genome assembly, as well as technical idiosyncrasies of different sequencing platforms, added unexpected layers of complexity. The PCA in Fig. 5a captures this issue. This is a cautionary tale about combining datasets of varying depth of coverage and sequencing platforms without controlling for inherent biases. In this chapter, we controlled for sequencing effects by using a DAPC approach. This approach uses grouping priors to find linear combination of SNPs whose variance minimize the sequencing effects while maximizing the variance explained by the geographically defined populations (e.g., Maine, Rhode Island, etc.). The resulting dataset is comprised primarily of structure-informative SNPs (Fig. 5b, c).

In their *Sbal1* analyses, Flight and Rand (2012) showed that genome-wide F_{ST} values are low across the North Atlantic (RI-2011 vs. SW-2011 = 0.0408, ME-2011 vs. SW-2011 = 0.0362, ME-2011 vs. RI-2011 = 0.0243). Our estimates of genetic differentiation considering all 3,177 high-quality/first-pass nuDNA SNPs markers in *Sbal2* are concordant with these estimates (Fig. 6). We estimated F_{ST} avoiding cross-sequencing platforms comparisons to mitigate technical bias. Figure 6 also highlights the F_{ST} values for two types of SNP markers, structure-uninformative and structure-informative. Structure-uninformative markers capture genome-wide levels of differentiation with varying neutral evolutionary dynamics (e.g., *homoplasies*, *incomplete lineage sorting*, *ancestral polymorphisms*, standing genetic variation, etc.) Structure-informative markers, on the other hand, capture demographic stratification resulting primarily from isolation by distance and limited dispersal (Wright

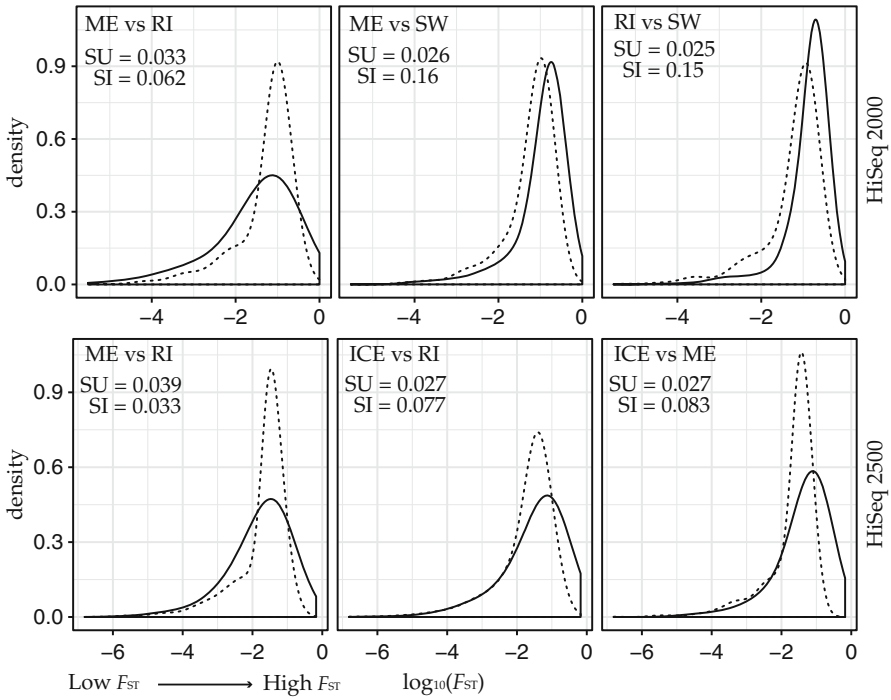

Fig. 6 Analyses of $\log_{10}$ transformed F_{ST} distributions based on two classes of nuDNA SNPs: only structure-informative markers (SI; solid lines) and only structure-uninformative markers (SU; dashed lines). All comparisons were done among samples of the same sequencing technology family: the top row corresponds to Illumina HiSeq 2000 and the bottom row to Illumina HiSeq 2500

1943; Malécot and Blaringhem 1948; Aguillon et al. 2017). As a result, they provide an inflated estimate of F_{ST} (e.g., Kidd et al. 2011). We centered our attention on these structure-informative markers, however, because of their strong correlation with mtDNA F_{ST} estimates (Pearson correlation coeff. $\rho = 0.905$; see Fig. 3c). In the past, some authors had argued that the discrepancy between whole-genome F_{ST} and high mtDNA F_{ST} warranted a non-neutral hypothesis (Flight and Rand 2012). However, here we show that mtDNA estimates of differentiation are consistent with neutrality once population structure is considered. Thus, for *S. balanoides*, mtDNA appears to behave as a structure-informative marker, albeit a weak one in which rapid substitution rates may mask important demographic signals (Eytan and Hellberg 2010).

How might these data contribute to our understanding of barnacle phylogeography? For *S. balanoides*, most genome-wide SNPs have low F_{ST} values. We first considered whether or not these patterns could be the result of errors during SNP identification. However, we doubt this to be the case as our SNP discovery pipeline was conservative with several quality filters (see Sect. 2 and Box 3). Moreover, the sampling design of pool-seq experiments is robust against stochastic sampling as multiple individuals are

pooled into single sequencing experiment (Schlotterer et al. 2014). These low levels of nuDNA F_{ST} suggest two possible scenarios. First, not enough time has passed for the sorting of standing variation common to all North Atlantic populations. In other words, standing genetic variation across North Atlantic populations has been conserved even in the face of drastic shifts in climate and substrate availability. Alternatively, modern mercantile shipping may generate high levels of connectivity across the Atlantic. In general, we favor the former interpretation of the structure-uninformative SNPs. A hypothesis of mercantile dispersal would signify a quasi-*panmictic* demographic model. This is not compatible with the signature of structure inferred from the structure-informative SNPs. Moreover, multiple laboratory experiments and reciprocal transplants have identified fundamental differences in the ecology and physiology of barnacle populations across the Atlantic (Crisp 1964, 1968b). For instance, North American barnacles show higher susceptibility to infection by the parasite *Hemioniscus balani* (Crisp 1968a). Even if one could imagine barnacle larvae from different regions traveling large distances via mercantile ships, the ecological challenges unique to each region may pose barriers to the establishment of true trans-Atlantic panmixis. A more comprehensive characterization of barnacle population structure, including populations from the Pacific Northwest, could provide new insights into these hypotheses as it relates to the trans-Arctic interchange of marine taxa (Vermeij 1991).

The Iceland population sample shows puzzling levels of genetic variation and differentiation in both mtDNA and nuDNA. As a land mass, Iceland is located in the northeast side of the Atlantic (~64° N, ~18° W), ~1,100 km from the British Isles and ~1,500 km from Norway. Despite geographical proximity, ICE shows higher similarity to North American populations (>4,000 km away) than to European ones. What process may explain this pattern? One possibility is that Iceland populations of mtDNA clade *a* are descended from a periglacial refugia similar to those suggested for the isopod *Idotea balthica* and the green crab *Carcinus maenas* (Wares 2001; Roman and Palumbi 2004). Thus, the current distribution of *S. balanoides* resulted from the dispersal and *admixture* between the expansions of clades *a* and *b*. However, this would not explain why clade *a* is not found in Continental Europe or the British Isles. In other words, why would clade *a* only expand to the southwest but not to the southeast? We hypothesize that the oceanographic dynamics of the North Atlantic current (NAC) system may pose a barrier for barnacle dispersal across the North Atlantic (Fig. 7; see Orvik and Niiler 2002; Fratantoni 2001). In addition, the Irminger and Greenland currents may boost barnacle dispersal toward North America. Testing these hypotheses is beyond the scope of this chapter, but they present an ideal opportunity for the integration of population genetic data with oceanographic data. Specifically, they would require extensive sampling across potential phylogenetic breaks (e.g., the Faroe Islands, the Shetland Islands, Greenland, etc.). We note that current analyses lack samples from south of the English Channel, and some evidence for different mtDNA haplotypes exists in samples from the French coast (N. Bierne, *pers. comm.*), so it will be helpful to broaden the scope of sampling to clarify these hypotheses.

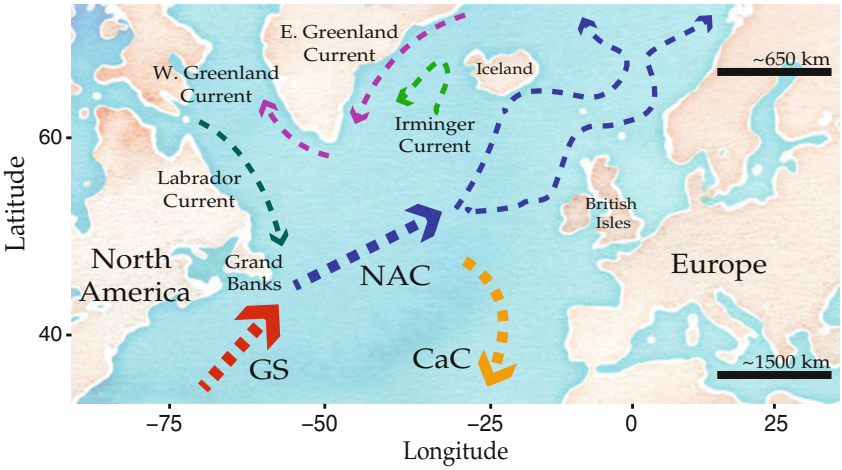

Fig. 7 A simplified diagram of surface currents in the North Atlantic (see Orvik and Niiler 2002; Fratantoni 2001). Notice that the spatial scale changes with latitude. *GS* Gulf Stream. *NAC* North Atlantic Current. *CaC* Canary Current

6 Population Genomics Challenges for Natural Populations

Like most natural populations, many processes drive the evolution of *S. balanoides*. In the North Atlantic, these are defined by a dynamic historical phylogeography shaped by the events of the LGM (Wares and Cunningham 2001; Flight et al. 2012), as well as by selection at both microhabitat (Schmidt and Rand 1999, 2001; Schmidt et al. 2000; Flight et al. 2010) and macrohabitat scales (Flight and Rand 2012). As such, gaining a comprehensive understanding of the genomic landscape of this system will provide insight into how adaptive and demographic processes shape genetic variation in the wild. This understanding will facilitate the prediction of the evolutionary potential of natural populations in the face of new ecological challenges, e.g., global climate change (Helmuth et al. 2006). However, performing such analyses on diploid organisms, such as the barnacle, can be prohibitively challenging. The acorn barnacle genome displays high levels of genetic variation (see Sect. 4). This makes SNP detection more difficult, especially when present at low frequency in a population, often requiring the organism to be sequenced at much higher coverage (Ellegren 2014). This challenge is a common one as several studies have identified particularly high rates of heterozygosity present in marine invertebrate species, including the sea urchin *Strongylocentrotus purpuratus* with 4–5% heterozygosity and the urochordate *Ciona savignyi* with 16.6% heterozygosity (Sea Urchin Genome Sequencing Consortium et al. 2006; Small et al. 2007). For some systems, the sequence analysis problem of high levels of heterozygosity can be circumvented through inbreeding. This will not work for species such as *Semibalanus* which are

obligate outcrossers with annual generation times. Even for species in which this strategy is possible, the process can be costly, time consuming, and interfere with studies of fitness and selection (Ellegren 2014).

To obtain allele frequencies at a population scale, the genomes of individuals within the same population may be pooled and sequenced (Schlotterer et al. 2014). For species without a reference genome, determination of allele frequencies from a pool-seq experiment can be difficult due to confounding factors such as repeats, paralogs, sequencing errors, PCR errors, and microbial contamination. Sequencing data can be aligned to a genetically similar species with a well-annotated reference genome to facilitate SNP discovery; however even closely related species often contain diverged genomic regions that may result in inferior alignments (Schlotterer et al. 2014). Thus, when possible, de novo genome assembly is recommended.

De novo assembly of diploid organisms from natural populations has historically been difficult and costly, but improvements in computational methods as well as sequencing technologies have made de novo assembly a tractable tool to facilitate population genomics studies. Reductions in sequencing costs have enabled genomes to be sequenced to higher coverage, resulting in fewer gaps in the assembled genome. Additionally, the combination of traditional HTS methods with new sequencing technologies such as Hi-C (which uses spatial distance between genome-wide loci to improve assembly) can produce chromosome length reference genomes, even for non-model species (Oddes et al. 2018; Dudchenko et al. 2017). Computational methods have also been developed to leverage these sequencing approaches to assemble a complete reference genome. These methods can be strung together to form an assembly pipeline. One example of an assembly pipeline is (filtering → error correction (but see Fujimoto et al. (2014)) → duplicate read removal → assembly → scaffolding → gap filling). Several commonly used software tools for assembly, scaffolding, and gap filling are described in Table 3.

7 Summary: Where Do We Go from Here?

In the present chapter, we have covered a variety of topics concerning tools and characterizations of genetic variation in barnacle populations in habiting the North Atlantic. First, we provided a mtDNA-wide characterization of previously described mtDNA haplotypes, *a* and *b*. We also introduced the second iteration of the *S. balanoides'* reference genome (*Sbal2*) in which we characterized genome-wide levels of variation and population structure. Our preliminary results revealed a cautionary tale on the effect of technical bias in sequencing experiments. We accounted for these biases by focusing our efforts on structure-informative markers consistent across technologies. Our analysis revealed concordant levels of nuDNA and mtDNA genetic variation. This suggests that the demographic inferences done with mtDNA have been informative, despite the weak signal characteristic of the marker. Patterns of variation in nuDNA provided no evidence of demographic bottlenecks. The large number of structure-uninformative SNPs in the nuclear

Table 3 Description of bioinformatic tools for de novo genome assembly

Software (references)	Description	Data type
Quality control		
Jellyfish (Marcais and Kingsford 2011)	*K*-mer counter. Can be used to filter reads containing errors, estimate genome size, and estimate genomic variance	Raw reads
Genome assembly		
dipSPAdes (Bankevich et al. 2012; Safonova et al. 2015)	DBG assembler designed for diploid, highly polymorphic genomes (an extension of the SPAdes assembler)	Short reads
Meraculous-2D (Chapman et al. 2011)	DBG assembler designed to assembly highly heterozygous diploid genomes (an extension of the Meraculous assembler)	Short reads
Platanus (Kajitani et al. 2014)	DBG assembler designed for highly heterozygous genomes. Includes a scaffolding step	Short reads
DBG2OLC (Ye et al. 2015)	Hybrid assembler. Combines DBG and OLC approaches	Short and long reads
MaSuRCa (Zimin et al. 2013)	Hybrid assembler. Combines DBG and OLC approaches	Short reads or both short and long reads
Falcon (Chin et al. 2016)	Hierarchical assembly process that uses and overlap graph and includes an error correction step	Long reads
Genome scaffolding		
SSPACE (Boetzer et al. 2011)	Uses read pairs to assess the order, distance, and orientation of contigs	Assembled contigs, paired-end/mate-pair reads
OPERA-LG (Gao et al. 2016)	Uses read pairs to assess the order, distance, and orientation of contigs. Designed for large, repeat-rich genomes	Assembled contigs, paired-end/mate-pair reads
Gap filling		
Gapfiller (Boetzer and Pirovano 2012)	Uses distance information from read-pairs to fill gaps	Assembled scaffolds, paired-end reads
Sealer (Paulino et al. 2015)	Designed for large genomes. Uses a DBG approach to fill gaps	Assembled scaffolds, paired-end/mate-pair/RNA-seq reads

OLC overlap-layout-consensus method. *DBG De Bruijn* graph method

genome with low F_{ST} values across the Atlantic suggests a large number of ancestral polymorphisms and shared standing variation in populations. The Icelandic population displayed higher similarity to North American populations than to the rest of Europe. We hypothesize this is consistent with a periglacial refugium in Iceland concomitant with a barrier to gene flow caused by the NAC. Lastly, we discussed challenges and opportunities for the improvement of genomic tools in the barnacle. Our reflections in this area are easily generalizable to most natural populations.

So where do we go from here? From a phylogeographic perspective, the biggest challenge is decoupling the roles of selection and demography (both current and historical) in determining the patterns of genetic variation in the species. In order to tackle questions related to barnacle demography (including the postglacial expansion), we need to (a) propose robust null demographic models from which to derive testable hypothesis (e.g., Maggs et al. (2008)), (b) generate datasets with robust genetic signals and haplotype information, and (c) ensure adequate sample sizes in order to maximize the power and reliability of demographic inferences. Questions regarding the role of selection require identifying well-defined and repeatable gradients of environmental stresses and performing genomic characterizations in order to identify potential candidates of selection while at the same time accounting for the underlying demography (Endler 1986; Crawford and Oleksiak 2016; Schmidt et al. 2008). These types of studies would be facilitated by a better assembly of the *S. balanoides* genome. We note, however, that the decision of investing resources into the production of high-quality reference assemblies depends ultimately on research goals and priorities. Regarding the types of datasets required to perform these inferences, it is clear that single marker approaches (i.e., mtDNA markers) cannot provide a comprehensive picture of the demographic processes shaping genetic diversity in the intertidal (see Bazin et al. (2006)). Thus, in order to test our biogeographic hypotheses, we require a comprehensive dataset of genome-wide markers for a large number of individuals across the North Atlantic, preferably with haplotype information (i.e., pool-seq approaches may not be ideal). These data could be used to test a variety of hypotheses such as ancestry inferences or demographic reconstructions using coalescent theory approaches. Moreover, testing our assumptions of shared standing variation preceding the most recent glaciation could greatly benefit from collecting information about the Pacific populations of *S. balanoides*. We acknowledge that generating robust datasets with numerous individuals and hundreds of thousands of genome-wide markers could prove difficult or impossible due to funding constraints. However, as we improve the quality of the *S. balanoides* reference genome, approaches such as whole genome sequencing of multiple individuals at low coverage (Le and Durbin 2011) would be ideal to generate our desired dataset.

Finally, we would like to highlight the idea that for marine organisms without a reference genome, cost-effective HTS approaches, such as pool-seq, combined with modern genome assembly algorithms can produce practical tools to conduct population genetic inferences at the genome-wide level. These inferences should be robust as long as the drawbacks and limitations of the approach are understood. We would also like to recognize other viable approaches, such as *reduced representation libraries* (GBS or ddRAD (Baird et al. 2008; Elshire et al. 2011; Peterson et al. 2012)). These approaches normally allow for the genotyping of hundreds of individuals with the trade-off of losing genomic information for a majority of genes. Nevertheless, these approaches can provide novel insights into general levels of variation and population structure as well as provide some hints on local adaptation and demographic estimations, even for species without a reference genome (e.g., (Narum et al. 2013; Nunez et al. 2015; Emerson et al. 2010)).

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Data Availability

Companion to this book chapter, we have made available the reference sequences for *Sbal2* (DDBJ/ENA/GenBank accession PHFM00000000) and the complete mtDNA of *S. balanoides* (GeneBank accessions MG010647, MG010648, MG010649). Other genomic resources have been made available in FigShare (figshare.com). The gene feature file (GFF, v3) of *Sbal2* from the *Drosophila* AUGUSTUS model, the UniProt BLAST hits file, and the nuDNA and mtDNA SNP tables are available at DOI: 10.6084/m9.figshare.6682995. The additional *COX I* sequences from our analyses can be found in GeneBank accessions MG925538–MG925662 and MG928281–MG928323. We note, however, that files corresponding to the 2017/2018 pool-seq datasets from Iceland, Maine, and Rhode Island, as well as the transcriptome, form part of larger analyses which are currently active in our research group. These datasets will be released in the future with their corresponding publications.

Glossary

Admixture Refers to the process in which previously isolated populations begin interbreeding.

Ancestral polymorphisms Genetic variation present in two (or more) species, subspecies, or populations that appeared prior to divergence.

Assembly graph A graph that can be traversed to create an assembled DNA sequence. The most commonly used assembly graph is a *De Bruijn* graph.

Contig DNA or RNA sequence, typically assembled from multiple overlapping short sequence reads.

Coverage Indicates the number of times that a particular genomic region was sampled by mapped reads produced by a sequencing experiment.

COX I Cytochrome c oxidase I, a gene encoded in mtDNA involved in the electron transport chain. This gene is commonly used in population genetic studies and species identification or DNA barcoding.

D-loop The mitochondrial DNA control region, also known as the displacement loop. It contains the sequences for the origin of replication and transcription of the

mtDNA molecule. Its high rate of DNA substitution makes it suitable for analyses of closely related populations and species.

De Bruijn graph A directed graph representing overlaps between *k*-mers present in a set of reads. Nodes are represented by *k*-mers and edges by $(k - 1)$-mers.

De novo genome assembly The process of stringing together overlapping DNA sequence reads to make longer DNA sequences, called contigs. Perfect genome assembly would produce 1 contig for each chromosome.

Effective population sizes (N_e) The effective number of breeding individuals in a population, equivalent to the idealized population size in which the effects of stochastic sampling on allele frequencies (i.e. genetic drift) are similar to the real population of interest.

High-throughput sequencing (HTS) Highly parallelized DNA sequencing that produces millions to 100s of millions of DNA sequences of varying length (50–250 bp for the Illumina platform; 1,000 to >20,000 bp for the Pacific Biosystems (PacBio) and Oxford Nanopore platforms.)

Homoplasy A condition where a character is shared by a set of species or populations that is not shared by their common ancestor. In DNA terminology, it may refer to the independent mutation (or back-mutation) to the same nucleotide state in two populations.

Incomplete lineage sorting The process by which a phylogenetically informative marker is shared among species or populations in which other markers have diverged to fixation in each population.

Indels An insertion or deletion in a DNA sequence.

K-mer A DNA sequence of length *k*. In genome assembly, *k*-mers are generated by splitting reads into smaller pieces of length *k*.

Long reads DNA-sequences longer than 1,000 bp.

Mapping reads to a reference The process of identifying a subsequence or multiple subsequences in the reference genome that matches or approximately matches a read.

N50 A statistical measure of the average length of a set of sequences (or contigs). N50 measures the length N such that 50% of all bases are contained within sequences with length less than or equal to N.

Panmictic An idealized demographic model in which all members of a population mate randomly, resulting in panmixia.

Pool-seq An experimental approach for the quantification of genetic variation in populations through the pooling and subsequent sequencing of multiple individuals.

Reduced representation libraries An experimental approach to quantify genetic variation in populations by sampling a reduced (~10%) portion of the genome to high coverage.

Reference genome A set of genomic sequences that represents the genome of a population or species. These sequences may include DNA from multiple individuals.

Sbal1 The first generation of the *Semibalanus balanoides* genome.

Sbal2 The second generation of the *Semibalanus balanoides* genome.

Sequencing bias The introduction of sequencing artifacts by the unequal sampling of DNA sequences due to characteristics of the target sequence, such as GC content.

Short reads DNA sequences with lengths ranging from 50 to 200 bp.

Single-nucleotide polymorphisms (SNPs) A genomic variant occurring at a single-nucleotide position in genomic sequences.

Standing genetic variation Allelic variation that currently exists within populations as opposed to new variants arising by *de novo* mutation.

References

Aguillon SM, Fitzpatrick JW, Bowman R, Schoech SJ, Clark AG, Coop G, Chen N. Deconstructing isolation-by-distance: the genomic consequences of limited dispersal. PLoS Genet. 2017;13:e1006911.

Akashi H, Osada N, Ohta T. Weak selection and protein evolution. Genetics. 2012;192:15–31.

Bachmann K, Rheinsmith EL. Nuclear DNA amounts in Pacific Crustacea. Chromosoma. 1973;43:225–36.

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One. 2008;3(10):e3376.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77.

Bazin E, Glemin S, Galtier N. Population size does not influence mitochondrial genetic diversity in animals. Science. 2006;312:570–2.

Belton JM, McCord RP, Gibcus JH, Naumova N, Zhan Y, Dekker J. Hi-C: a comprehensive technique to capture the conformation of genomes. Methods. 2012;58:268–76.

Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B Methodol. 1995;57:289–300.

Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsch G, Putz J, Middendorf M, Stadler PF. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 2013;69:313–9.

Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics. 2011;27:578–9.

Boetzer M, Pirovano W. Toward almost closed genomes with GapFiller. Genome Biol. 2012;13:R56.

Brown AF, Kann LM, Rand DM. Gene flow versus local adaptation in the northern acorn barnacle, Semibalanus balanoides: insights from mitochondrial DNA variation. Evolution. 2001;55:1972–9.

Bushnell B. BBMap short read aligner. Berkeley: University of California; 2016. http://sourceforge.net/projects/bbmap.

Camus MF, Wolff JN, Sgro CM, Dowling DK. Experimental support that natural selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian Drosophila melanogaster. Mol Biol Evol. 2017;34:2600–12.

Chapman JA, Ho I, Sunkara S, Luo S, Schroth GP, Rokhsar DS. Meraculous: de novo genome assembly with short paired-end reads. PLoS One. 2011;6:e23501.

Chevreux B, Wetter T, Suhai S. Genome sequence assembly using trace signals and additional sequence information. Comput Sci Biol. 1999;99:45–56.

Chin CS, Peluso P, Sedlazeck FJ, Nattestad M, Concepcion GT, Clum A, Dunn C, O'Malley R, Figueroa-Balderas R, Morales-Cruz A, Cramer GR, Delledonne M, Luo C, Ecker JR, Cantu D, Rank DR, Schatz MC. Phased diploid genome assembly with single-molecule real-time sequencing. Nat Methods. 2016;13:1050–4.

Crawford DL, Oleksiak MF. Ecological population genomics in the marine environment. Brief Funct Genomics. 2016;15(5):342–51.

Crisp DJ. Racial differences between North American and European forms of Balanus balanoides. J Mar Biol Assoc UK. 1964;44:33.

Crisp DJ. Differences between North American and European Populations of Balanus balanoides revealed by transplantation. J Fish Res Board Can. 1968a;25:2633–41.

Crisp DJ. Distribution of the parasitic isopod Hemioniscus balani with special reference to the east coast of North America. J Fish Res Board Can. 1968b;25:1161–7.

Dudchenko O, Batra SS, Omer AD, Nyquist SK, Hoeger M, Durand NC, Shamim MS, Machol I, Lander ES, Aiden AP, Aiden EL. De novo assembly of the Aedes aegypti genome using Hi-C yields chromosome-length scaffolds. Science. 2017;356:92–5.

Ellegren H. Genome sequencing and population genomics in non-model organisms. Trends Ecol Evol. 2014;29:51–63.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One. 2011;6:e19379.

Elyashiv E, Bullaughey K, Sattath S, Rinott Y, Przeworski M, Sella G. Shifts in the intensity of purifying selection: an analysis of genome-wide polymorphism data from two closely related yeast species. Genome Res. 2010;20:1558–73.

Emerson KJ, Merz CR, Catchen JM, Hohenlohe PA, Cresko WA, Bradshaw WE, Holzapfel CM. Resolving postglacial phylogeography using high-throughput sequencing. Proc Natl Acad Sci U S A. 2010;107:16196–200.

Endler JA. Natural selection in the wild. Princeton: Princeton University Press; 1986.

Eytan RI, Hellberg ME. Nuclear and mitochondrial sequence data reveal and conceal different demographic histories and population genetic processes in Caribbean reef fishes. Evolution. 2010;64:3380–97.

Flight PA, O'Brien MA, Schmidt PS, Rand DM. Genetic structure and the North American postglacial expansion of the barnacle, Semibalanus balanoides. J Hered. 2012;103:153–65.

Flight PA, Rand DM. Genetic variation in the acorn barnacle from allozymes to population genomics. Integr Comp Biol. 2012;52:418–29.

Flight PA, Schoepfer SD, Rand DM. Physiological stress and the fitness effects of Mpi genotypes in the acorn barnacle Semibalanus balanoides. Mar Ecol Prog Ser. 2010;404:139–49.

Fratantoni DM. North Atlantic surface circulation during the 1990's observed with satellite-tracked drifters. J Geophys Res Oceans. 2001;106(C10):22067–93.

Fujimoto M, Bodily PM, Okuda N, Clement MJ, Snell Q. Effects of error-correction of heterozygous next-generation sequencing data. BMC Bioinformatics. 2014;15(Suppl 7):S3.

Gao S, Bertrand D, Chia BK, Nagarajan N. OPERA-LG: efficient and exact scaffolding of large, repeat-rich eukaryotic genomes with performance guarantees. Genome Biol. 2016;17:102.

Hahn C, Bachmann L, Chevreux B. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads – a baiting and iterative mapping approach. Nucleic Acids Res. 2013;41:e129.

Helmuth B, Mieszkowska N, Moore P, Hawkins SJ. Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. Annu Rev Ecol Evol Syst. 2006;37:373–404.

Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 2010;11:94.

Kajitani R, Toshimoto K, Noguchi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H, Kohara Y, Fujiyama A, Hayashi T, Itoh T. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res. 2014;24:1384–95.

Kidd JR, Friedlaender FR, Speed WC, Pakstis AJ, De La Vega FM, Kidd KK. Analyses of a set of 128 ancestry informative single-nucleotide polymorphisms in a global set of 119 population samples. Investigative Genet. 2011;2(1):1.

Kimura M, Ohta T. On the constancy of the evolutionary rate of cistrons. J Mol Evol. 1971;1(1):18–25.

Kofler R, Pandey RV, Schlotterer C. PoPoolation2: identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). Bioinformatics. 2011;27:3435–6.

Lander ES, Waterman MS. Genomic mapping by fingerprinting random clones: a mathematical analysis. Genomics. 1988;2:231–9.

Le SQ, Durbin R. SNP detection and genotyping from low-coverage sequencing data on multiple diploid samples. Genome Res. 2011;21:952–60.

Lê S, Josse J, Husson F. FactoMineR: an R package for multivariate analysis. J Stat Softw. 2008;25:1–18.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Subgroup Genome Project Data Processing. The sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9.

Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM, arXiv Pre-print, version 2. 2013.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. The power and promise of population genomics: from genotyping to genome typing. Nat Genet. 2003;4:981–94.

Luu K, Bazin E, Blum MG. pcadapt: an R package to perform genome scans for selection based on principal component analysis. Mol Ecol Resour. 2017;17:67–77.

Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J, Olsen J, Perez KE, Stam W, Väinölä R, Viard F, Wares J. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology. 2008;89:S108–S22.

Malécot G, Blaringhem L-F. Les mathématiques de l'hérédité. 1948.

Marcais G, Kingsford C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics. 2011;27:764–70.

Marsden CD, Ortega-Del Vecchyo D, O'Brien DP, Taylor JF, Ramirez O, Vila C, Marques-Bonet T, Schnabel RD, Wayne RK, Lohmueller KE. Bottlenecks and selective sweeps during domestication have increased deleterious genetic variation in dogs. Proc Natl Acad Sci U S A. 2016;113:152–7.

Meiklejohn CD, Montooth KL, Rand DM. Positive and negative selection on the mitochondrial genome. Trends Genet. 2007;23:259–63.

Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA. Genotyping-by-sequencing in ecological and conservation genomics. Mol Ecol. 2013;22:2841–7.

Nunez JCB, Oleksiak MF. A cost-effective approach to sequence hundreds of complete mitochondrial genomes. PLoS One. 2016;11:e0160958.

Nunez JCB, Seale TP, Fraser MA, Burton TL, Fortson TN, Hoover D, Travis J, Oleksiak MF, Crawford DL. Population genomics of the euryhaline teleost Poecilia latipinna. PLoS One. 2015;10:e0137077.

Oddes S, Zelig A, Kaplan N. Three invariant Hi-C interaction patterns: applications to genome assembly. Methods. 2018;142:89–99.

Orvik KA, Niiler P. Major pathways of Atlantic water in the northern North Atlantic and Nordic Seas toward Arctic. Geophys Res Lett. 2002;29(19):1896.

Paschou P, Ziv E, Burchard EG, Choudhry S, Rodriguez-Cintron W, Mahoney MW, Drineas P. PCA-correlated SNPs for structure identification in worldwide human populations. PLoS Genet. 2007;3:1672–86.

Patterson N, Price AL, Reich D. Population structure and eigen analysis. PLoS Genet. 2006;2:e190.

Paulino D, Warren RL, Vandervalk BP, Raymond A, Jackman SD, Birol I. Sealer: a scalable gap-closing application for finishing draft genomes. Bmc Bioinformatics. 2015;16:230.

Perez-Losada M, Hoeg JT, Simon-Blecher N, Achituv Y, Jones D, Crandall KA. Molecular phylogeny, systematics and morphological evolution of the acorn barnacles (Thoracica: Sessilia: Balanomorpha). Mol Phylogenet Evol. 2014;81C:147–58.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One. 2012;7:e37135.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006;38:904–9.

Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, Bertoni A, Swerdlow HP, Gu Y. A tale of three next generation sequencing platforms: comparison of Ion torrent, pacific biosciences and illumina MiSeq sequencers. BMC Genomics. 2012;13:341.

Rand DM. Mitigating mutational meltdown in mammalian mitochondria. PLoS Biol. 2008;6:e35.

Rand DM. Thermal habit, metabolic rate and the evolution of mitochondrial DNA. Trends Ecol Evol. 1994;9:125–31.

Rhoads A, Au KF. PacBio sequencing and its applications. Genomics Proteomics Bioinformatics. 2015;13:278–89.

Roman J, Palumbi SR. A global invader at home: population structure of the green crab, Carcinus maenas, in Europe. Mol Ecol. 2004;13:2891–8.

Ruddiman WF, Mcintyre A. The North-Atlantic Ocean during the last deglaciation. Palaeogeogr Palaeoclimatol Palaeoecol. 1981;35:145–214.

Safonova Y, Bankevich A, Pevzner PA. dipSPAdes: assembler for highly polymorphic diploid genomes. J Comput Biol. 2015;22:528–45.

Schlotterer C, Tobler R, Kofler R, Nolte V. Sequencing pools of individuals-mining genome-wide polymorphism data without big funding. Nat Rev Genet. 2014;15:749–63.

Schmidt PS, Bertness MD, Rand DM. Environmental heterogeneity and balancing selection in the acorn barnacle Semibalanus balanoides. Proc Biol Sci. 2000;267:379–84.

Schmidt PS, Rand DM. Intertidal microhabitat and selection at Mpi: interlocus contrasts in the northern acorn barnacle, Semibalanus balanoides. Evolution. 1999;53:135–46.

Schmidt PS, Rand DM. Adaptive maintenance of genetic polymorphism in an intertidal barnacle: habitat- and life-stage-specific survivorship of Mpi genotypes. Evolution. 2001;55:1336–44.

Schmidt PS, Serrão EA, Pearson GA, Riginos C, Rawson PD, Hilbish TJ, Brawley SH, Trussell GC, Carrington E, Wethey DS, Grahame JW, Bonhomme F, Rand DM. Ecological genetics in the North Atlantic: environmental gradients and adaptation at specific loci. Ecology. 2008;89:S91–S107.

Sea Urchin Genome Sequencing Consortium, Sodergren E, Weinstock GM, Davidson EH, Cameron RA, Gibbs RA, Angerer RC, Angerer LM, Arnone MI, Burgess DR, Burke RD, Coffman JA, Dean M, Elphick MR, Ettensohn CA, Foltz KR, Hamdoun A, Hynes RO, Klein WH, Marzluff W, McClay DR, Morris RL, Mushegian A, Rast JP, Smith LC, Thorndyke MC, Vacquier VD, Wessel GM, Wray G, Zhang L, Elsik CG, Ermolaeva O, Hlavina W, Hofmann G, Kitts P, Landrum MJ, Mackey AJ, Maglott D, Panopoulou G, Poustka AJ, Pruitt K, Sapojnikov V, Song X, Souvorov A, Solovyev V, Wei Z, Whittaker CA, Worley K, Durbin KJ, Shen Y, Fedrigo O, Garfield D, Haygood R, Primus A, Satija R, Severson T, Gonzalez-Garay ML, Jackson AR, Milosavljevic A, Tong M, Killian CE, Livingston BT, Wilt FH, Adams N, Belle R, Carbonneau S, Cheung R, Cormier P, Cosson B, Croce J, Fernandez-Guerra A, Geneviere AM, Goel M, Kelkar H, Morales J, Mulner-Lorillon O, Robertson AJ, Goldstone JV, Cole B, Epel D, Gold B, Hahn ME, Howard-Ashby M, Scally M, Stegeman JJ, Allgood EL, Cool J, Judkins KM, McCafferty SS, Musante AM, Obar RA, Rawson AP, Rossetti BJ, Gibbons IR, Hoffman MP, Leone A, Istrail S, Materna SC, Samanta MP, Stolc V, Tongprasit W, Tu Q, Bergeron KF, Brandhorst BP, Whittle J, Berney K, Bottjer DJ, Calestani C, Peterson K, Chow E, Yuan QA, Elhaik E, Graur D, Reese JT, Bosdet I, Heesun S, Marra MA, Schein J, Anderson MK, Brockton V, Buckley KM, Cohen AH, Fugmann SD, Hibino T, Loza-Coll M, Majeske AJ, Messier C, Nair SV, Pancer Z, Terwilliger DP, Agca C, Arboleda E, Chen N, Churcher AM, Hallbook F, Humphrey GW, Idris MM, Kiyama T, Liang S, Mellott D, Mu X, Murray G, Olinski RP, Raible F, Rowe M, Taylor JS, Tessmar-Raible K, Wang D, Wilson KH, Yaguchi S,

Gaasterland T, Galindo BE, Gunaratne HJ, Juliano C, Kinukawa M, Moy GW, Neill AT, Nomura M, Raisch M, Reade A, Roux MM, Song JL, Su YH, Townley IK, Voronina E, Wong JL, Amore G, Branno M, Brown ER, Cavalieri V, Duboc V, Duloquin L, Flytzanis C, Gache C, Lapraz F, Lepage T, Locascio A, Martinez P, Matassi G, Matranga V, Range R, Rizzo F, Rottinger E, Beane W, Bradham C, Byrum C, Glenn T, Hussain S, Manning G, Miranda E, Thomason R, Walton K, Wikramanayke A, Wu SY, Xu R, Brown CT, Chen L, Gray RF, Lee PY, Nam J, Oliveri P, Smith J, Muzny D, Bell S, Chacko J, Cree A, Curry S, Davis C, Dinh H, Dugan-Rocha S, Fowler J, Gill R, Hamilton C, Hernandez J, Hines S, Hume J, Jackson L, Jolivet A, Kovar C, Lee S, Lewis L, Miner G, Morgan M, Nazareth LV, Okwuonu G, Parker D, Pu LL, Thorn R, Wright R. The genome of the sea urchin Strongylocentrotus purpuratus. Science. 2006;314:941–52.

Shen X, Chu KH, Chan BK, Tsang LM. The complete mitochondrial genome of the fire coral-inhabiting barnacle Megabalanus ajax (Sessilia: Balanidae): gene rearrangements and atypical gene content. Mitochondrial DNA. 2014a:1–2.

Shen X, Tsoi KH, Cheang CC. The model barnacle Balanus balanus Linnaeus, 1758 (Crustacea: Maxillopoda: Sessilia) mitochondrial genome and gene rearrangements within the family Balanidae. Mitochondrial DNA. 2014b:1–3.

Silva G, Lima FP, Martel P, Castilho R. Thermal adaptation and clinal mitochondrial DNA variation of European anchovy. Proc Biol Sci. 2014;281.

Simao FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31:3210–2.

Simpson JT, Durbin R. Efficient de novo assembly of large genomes using compressed data structures. Genome Res. 2012;22:549–56.

Slatkin M. A measure of population subdivision based on microsatellite allele frequencies. Genetics. 1995;139:457–62.

Small KS, Brudno M, Hill MM, Sidow A. Extreme genomic variation in a natural population. Proc Natl Acad Sci U S A. 2007;104:5698–703.

Smit AFA, Hubley R, Green P. RepeatMasker Open-4.0. In. 2013–2015.

Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3.

Stanke M, Waack S. Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics. 2003;19:ii215–i25.

Toews DP, Brelsford A. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol. 2012;21:3907–30.

The UniProt Consortium. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2017;45:D158–69.

van Oppen MJH, Draisma SGA, Olsen JL, Stam WT. Multiple trans-Arctic passages in the red alga Phycodrys rubens: evidence from nuclear rDNA ITS sequences. Mar Biol. 1995;123:179–88.

Vermeij GJ. Anatomy of an invasion: the trans-Arctic interchange. Paleobiology. 1991;17:281–307.

Wares JP. Intraspecific variation and geographic isolation in idotea balthica (Isopoda: Valvifera). J Crustac Biol. 2001;21:1007–13.

Wares JP, Cunningham CW. Phylogeography and historical ecology of the North Atlantic intertidal. Evolution. 2001;55:2455–69.

Wright S. Isolation by distance. Genetics. 1943;28:114–38.

Xu H, Luo X, Qian J, Pang X, Song J, Qian G, Chen J, Chen S. FastUniq: a fast de novo duplicates removal tool for paired short reads. PLoS One. 2012;7:e52249.

Ye C, Ma ZS, Cannon CH, Pop M, Yu DW. Exploiting sparseness in de novo genome assembly. Bmc Bioinformatics. 2012;13(Suppl 6):S1.

Ye C, Hill C, Ruan J, Ma Z. DBG2OLC: efficient assembly of large genomes using the compressed overlap graph. arXiv. 2015;1410.2801.

Zimin AV, Marcais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. The MaSuRCA genome assembler. Bioinformatics. 2013;29:2669–77.

Part IV
Seascape Genomics

Seascape Genomics: Contextualizing Adaptive and Neutral Genomic Variation in the Ocean Environment

Libby Liggins, Eric A. Treml, and Cynthia Riginos

Abstract Seventy-one per cent of the earth's surface is covered by ocean which contains almost 80% of the world's phyla – "seascape genomics" is the study of how spatial dependence and environmental features in the ocean influence the geographic structure of genomic patterns in marine organisms. The field extends from seascape genetics where the study of small numbers of neutral loci predominates, to additionally consider larger numbers of loci from throughout the genome that may be of some functional or adaptive significance and are subject to selection. Seascape genomics is conceptually similar to landscape genomics; the disciplines share theoretical underpinnings, and the genetic measures and analytical methods are often the same. However, the spatio-temporal variability of the physical ocean environment and the biological characteristics of marine organisms (e.g. large population sizes and high dispersal ability) present some characteristic challenges and opportunities for spatial population genomics studies. This chapter provides an overview of the field of seascape genomics, outlines concepts and methods to consider when conducting seascape genomics studies, and highlights future research avenues and opportunities for the application of seascape genomics to global issues affecting our marine environment.

Keywords Adaptation · Genetic-environment association · Genotype-by-sequencing · Landscape genomics · Natural selection · Oceanography · Outlier test · Population genomics · Seascape genetics · SNPs

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1 Introduction: What Is Seascape Genomics?

Seascape genomics is a cognate of "landscape genomics" focused on marine habitats and species. These genomics disciplines use landscape (or seascape) composition, configuration, and spatial processes as statistical predictors of population genomic patterns (see Balkenhol et al. 2016a, 2019) and therefore can be considered a part of the broader discipline of population genomics (Dyer 2015; see Luikart et al. 2019 for a recent review of population genomics). As with population genomics, these spatially focused fields have benefitted from advances in genotyping technologies (i.e. next-generation sequencing, NGS; Davey and Blaxter 2010 and associated methods for sampling genomes of non-model organisms: Matz 2018; Andrews et al. 2016; Therkildsen and Palumbi 2017). Because genetic variation can now be evaluated across the entire genome, investigators can ask how different habitat configurations and local and intervening environments influence both adaptive and neutral genomic variation within and among populations. Thus seascape genomics is sometimes operationally split into "neutral" seascape genomics (mainly inferring the influence of gene flow and drift via patterns of neutral genomic variation) and "adaptive" seascape genomics (mainly inferring selection and local adaptation via patterns of adaptive genomic variation) (Manel et al. 2010; Schoville et al. 2012; Balkenhol et al. 2019). However, the greatest insights are to be gained when leveraging genomic data to uncover the underlying processes (e.g. migration, drift, selection) rather than focusing solely on emergent spatial genetic patterns. Examining the role of the seascape in determining the prominence and interrelationships of mutation, drift, migration, and selection enables an investigator to truly ascertain eco-evolutionary processes and to understand which seascape attributes affect the distribution of genomic diversity within and among natural populations.

In this chapter, we provide a general introduction to seascape genomics, building on previous overviews of the discipline and related disciplines (see Sect. 2). We provide a description of the rationale for undertaking population genomics studies in the marine environment and describe the incentives for a seascape genomics approach. We present an overview of how seascape genomics studies have been conducted and some of their insights to date. Finally, we highlight future directions in seascape genomics, especially as it intersects with other lines of inquiry and can contribute insights to anthropogenic challenges facing marine biodiversity.

2 The History and Rise of Seascape Genomics

The question of how space, environment, and habitat features influence microevolutionary processes, and subsequently the geography of genomic variation, has a long history in population genetics (Epling and Dobzhansky 1942; Wright 1943; inspiring "phylogeography" Avise 2000; reviewed in Grummer et al. 2019). The rise of spatially focused microevolutionary research, including seascape genomics, has

been enabled via the maturation of several foundational disciplines and technological advances (reviewed by Epperson 2003; Storfer et al. 2007; Riginos et al. 2016). For instance, landscape and seascape genomics studies are the obvious progression from landscape (Manel et al. 2003; Balkenhol et al. 2016b) and seascape genetics studies (Selkoe et al. 2016a, 2008; Riginos and Liggins 2013) that followed sequencing technology advancements. Furthermore, improved local instrumentation through time and new satellite data provide spatial information at higher spatial and temporal resolution. Concurrently, we now have more advanced statistical methods for handling these spatial and environmental data in the fields of spatial statistics, geoinformatics, physical oceanography, and landscape ecology. There continues to be interdependence and cross-fertilization among all these disciplines, and therefore a researcher interested in seascape genomics should also consult relevant literature in related fields.

The number, and rate, of seascape genomics publications is increasing, yet only 4% of spatial genomics studies are reported to focus on marine species (Grummer et al. 2019). Due to the dominance of terrestrial investigations, the conceptual and methodological developments in spatial genetics have largely been driven by terrestrial studies (see reviews of "landscape genetics/genomics" by Manel et al. 2003; Storfer et al. 2007; Holderegger and Wagner 2008; Balkenhol et al. 2019; and the predominant emphasis in a landmark book: Balkenhol et al. 2016b). Seascape genetics has been reviewed several times over the past decade or so (briefly by Hansen and Hemmer-Hansen 2007; and comprehensively by Selkoe et al. 2016a, 2008; Riginos and Liggins 2013; Liggins et al. 2013), and more recently seascape genomics has been reviewed in isolation (Riginos et al. 2016). Some authors have nested seascape genomics within "waterscape genetics/genomics" (Selkoe et al. 2016b) and "aquatic genetics/genomics" (Grummer et al. 2019) alongside "riverscape genetics/genomics". Although these named subdisciplines refer to different habitats, it is important to note that "landscape genomics-type" questions and approaches may be common across these habitats. Regardless of habitat, it is often the biology, ecology, and life history of study organisms that determine evolutionary processes and hence suitable study approaches.

The predominance of complex life histories in marine species and the spatio-temporal variability of the ocean environment have presented considerable challenges for the ways we quantify the marine environment, measure genetic variation, and relate the environment and genetic patterns (three core challenges identified by Riginos et al. 2016). In view of that, arguably the greatest conceptual and methodological developments that seascape genetics/genomics has contributed to the broader discipline of landscape genetics/genomics have been in regard to species that have high dispersal potential, large effective population sizes, and life histories evolved for spatially and often temporally dynamic habitats. On the other hand, looking to the future, it may be that the specific dynamics and selective pressures in the ocean resulting from global change (e.g. hypoxic conditions, seawater acidity, and sustained harvesting over millennia) inspire questions that are distinct from those asked of other systems. Thus, although landscape genomics is already interdisciplinary – relying on tools from spatial ecology, spatial

statistics, and broader population genomics – seascape genetics has, and seascape genomics likely will, continue to contribute some novel approaches to the panoply of landscape genomics inquiries.

3 Opportunities and Challenges in the Application of Spatial Population Genomics to Marine Organisms

The motives of seascape genetics and seascape genomics are largely the same. Predictably, seascape genomics differs from seascape genetics in the number of loci used across the genome, coincident with the increased possibility that sampled loci have (or are linked to) functional consequences and are thus affected by selection. Most of our knowledge regarding the distribution of genomic variation within and among marine populations is based on decades of studies using low marker densities and predominantly neutral loci (i.e. seascape genetics). While these studies are not directly comparable to what seascape genomics studies have, and will, reveal in years to come, they provide foundational knowledge of the marine system. It is upon this background, and within these identified challenges, that research questions in seascape genomics are motivated. The promise of seascape genomics is that the breadth of questions we are able to answer in the marine environment has increased: in particular, we have much to learn regarding the generation, distribution, and maintenance of adaptive genomic variation of marine populations in regard to the seascape.

3.1 Spatial and Temporal Dynamism of the Seascape and Influences on Genomic Patterns

Marine organisms have a great diversity of life histories (Strathmann 1990). At one extreme, some organisms have direct development and are entirely benthic, or site-attached, throughout their life (including some seahorses, some tunicates, and some echinoderms). In contrast, many marine organisms are entirely planktonic (including diatoms, dinoflagellates, copepods, and krill) or pelagic (including cetaceans and many fishes) and may migrate (e.g. whales and eels). However, most marine species, including most commercially important species, have both a pelagic stage and a benthic stage, and the majority of these are associated with the benthos as adults and are planktonic in their early life (as depicted in Fig. 1). Direct observations of these early life stages are often brief and essentially impossible for many species. For this reason, molecular markers have been essential tools for learning about the population ecology and inferring metapopulation dynamics of marine organisms with bi-partite life cycles (Palumbi 1997; Hellberg 2009). Interestingly, inferences from marine population genetic studies have revealed that despite the long distance

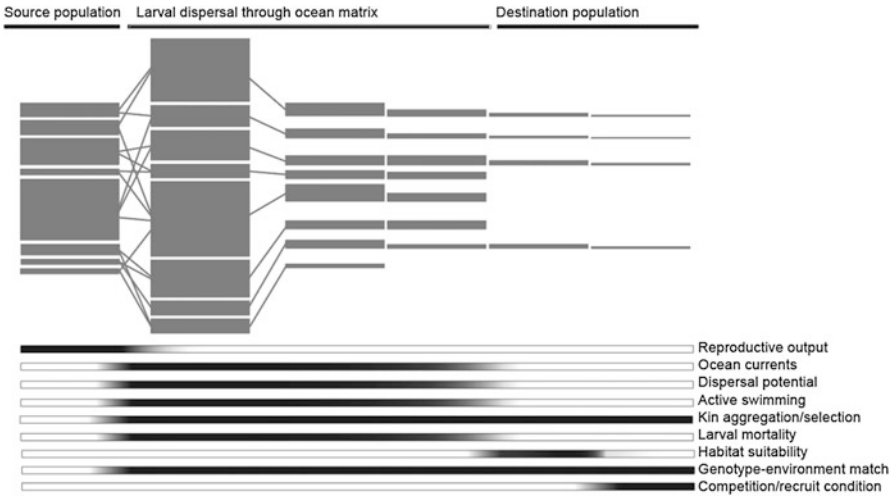

Fig. 1 Some of the biological characteristics of marine species and physical and environmental characteristics of the seascape (bottom) that intersect to influence demographic and selective processes throughout a bi-partite lifecycle and determine the distribution of genomic variation within and among populations. Hypothetical source populations are depicted as independent bands (top left) where bandwidth can be thought of as number of individuals or genomic variation. The bottom panel shows the period over which the biological or seascape characteristics have most relevance. *Reproductive output* of the source population varies as a function of population size and timing of reproduction. During dispersal (from left to right), larvae can be advected by *currents*, mixed (crossed lines) and may become diluted or concentrated represented by bandwidths. Larval *dispersal potential* is related to the early life history traits of a species, such as egg type, pelagic larval duration, and pre-competency period. Larval behaviours such as *kin aggregation* and *active swimming* can augment or counter physical oceanography to concentrate larvae. Bandwidths become smaller depicting larval *mortality* due to the ocean (biotic and abiotic) environment that may be random or determined by a *genetic-environment association* in the matrix (and later in the benthic environment). Successful settlement of larvae into a hypothetical destination population (top right) is dependent on *habitat suitability*. Post-settlement survival in the population is dependent on the *condition* of the recruit, *competition* and high-density blocking by conspecifics, and the presence of kin and may be selective. (This figure and caption combines earlier contributions of Cowen and Sponaugle 2009 and Liggins et al. 2013.)

dispersal potential of these organisms, often the genetic neighbourhood is smaller than expected (Almany et al. 2007; D'Aloia et al. 2015; Oleksiak 2019). In pursuit of understanding the seascape determinants for such non-intuitive metapopulation structures, marine organisms with a bi-partite life history have been the pervasive focus of seascape genetics studies (reviewed in Riginos and Liggins 2013; Selkoe et al. 2016a) and seascape genomics studies to date.

Traits of these early life stages (gametes, eggs, and/or larvae) vary considerably and are thought to affect a species' dispersal potential (Shanks 2009) and influence gene flow (see Fig. 1). Although spatially implicit analyses suggest there is some influence of these traits (i.e. direct versus planktonic development, nutritional provisioning of eggs and larvae, and planktonic larval duration influences the scaling

of genetic differentiation with geographic distance; Selkoe and Toonen 2011; Riginos et al. 2011), there are several other factors that influence the spatially explicit distribution of genetic variation within and among populations (discussed in Davies et al. 2015). For example, the number of reproductive adults in a given population strongly influences gene flow (Whitlock and Macauley 1999; Treml et al. 2012). Furthermore, a species' predicted potential to disperse between two populations versus realized gene flow can be decoupled by the interaction of pelagic life stages with oceanography (as articulated by Galindo et al. 2006; Selkoe et al. 2008) and selection against migrants (Marshall et al. 2010; Waters et al. 2013). Strong ocean currents can connect far-flung populations via dispersive individuals, but can also disconnect populations in non-intuitive ways through the formation of ocean barriers (Treml et al. 2015b). Moreover, whereas ocean currents are a dispersal vector for some passive species (and likely from only some locations and particular seasons), for other species ocean currents may be irrelevant (e.g. benthic-attached or strong swimming juveniles/adults). Thus, ideally we would want to consider the interactions between species' attributes, oceanography, and the geographic structure of the habitat. In pursuit of understanding the relationship between ocean currents and larval dispersal, coupled oceanographic-biological models (hereafter "biophysical" models) have emerged as an important tool in marine ecology and seascape genetics and genomics.

Ocean currents and the ocean environment in general fluctuate through time, affecting food resources, salinity, light, and temperature available within an organism's local environment. Ensuring the survival of the next generation in such a dynamic environment has been touted as one of the reasons the bi-partite lifestyle is prevalent across marine organisms. Other biological characters favoured by spatio-temporally fluctuating environments include high fecundity and varied reproductive success through time ("sweepstakes" reproduction; Hedgecock 1994) – both common among marine organisms. There has been much interest in the consequences of high vagility and these reproductive strategies on neutral genomic patterns for adult marine populations. For the many marine species that disperse widely, population genetics theory predicts high genetic variation and low differentiation over large spatial scales (Waples 1998; Hellberg et al. 2002; Faurby and Barber 2012). Moreover, large effective population sizes (*Ne*) resulting from high vagility and fecundity mean that the relative influence of genetic drift may be low (Wright 1931; Hellberg 2009; Gagnaire et al. 2015). Accordingly, many marine animals appear to be characterized by low neutral population genetic structure (Palumbi 1992; Ward et al. 1994; Waples 1998; Hedrick 1999; Kinlan and Gaines 2003). However, there has been the prevailing speculation that our inferences of population genetic structure to date have been lacking sufficient power (see Sect. 4.5) and that the high-density genome-wide markers used in seascape genomics may provide us new insights into general patterns of neutral population genetic structure in the sea (Oleksiak 2019).

There has been the frequent observation that genetic patterns in the ocean can also vary among populations but without obvious regard to space, in a phenomenon termed "chaotic genetic patchiness" (Johnson and Black 1982; and see Selkoe et al.

2008; Eldon et al. 2016). The characteristic attribute of chaotic genetic patchiness is that spatial genetic patterns of marine animals shift over time, a phenomenon commonly observed when there is temporal replication of sampling (e.g. Johnson and Black 1982; Selkoe et al. 2006; Toonen and Grosberg 2011; Villacorta-Rath et al. 2018; Jackson et al. 2018). A number of explanations proposed to explain temporal shifts and the spatial heterogeneity of genetic patterns include high variance in reproductive success, meaning different parents contributing distinct sets of larvae through time (Hedgecock 1994; Eldon et al. 2016), and temporally variable currents that alter larval sources. However, several other explanations are suggestive of adaptive strategies and a role of selection, including kin aggregation during larval dispersal and recruitment, and selection on larvae during their pelagic stage (Johnson and Black 1984) or at the stage of recruitment to the benthos (Vigliola et al. 2007). Thus, explanations for spatio-temporally dynamic genetic patterns in the ocean include processes that are both neutral and adaptive.

The importance of the local environment for the recruiting individuals and adult populations is well recognized; however, the influence of the large intervening environment (i.e. the matrix) is often overlooked (Riginos et al. 2016; see Fig. 1). Just as spatio-temporal fluctuations in the local marine environment can exert selective pressure on resident benthic populations, the dispersive stages of most marine organisms are likely subjected to strong selection while traversing the dynamic and heterogeneous matrix of the ocean. In fact, the open water environment experienced by larvae can influence their fitness (Shima and Swearer 2009) and their juvenile post-settlement survival (reviewed by Marshall and Morgan 2011), thus likely affecting the genomic patterns emerging across the seascape. The question remains as to whether the dispersal phase is purely a demographic bottleneck or whether temporally variable selection on pelagic larvae regularly affects allele frequencies.

3.2 Adaptive Genomic Patterns in the Seascape Context

Whereas seascapes and the biology of marine organisms present a challenging background within which to understand population demography, the marine environment may offer some advantages for empirical investigations of how selection operates in natural populations (Riginos et al. 2016). There are many strong environmental gradients in the ocean that are frequently replicated, such as intertidal zones, depth, oceanographic fronts, headlands, estuaries, and freshwater outflows (Schmidt et al. 2008; Selkoe et al. 2016a). Such seascape features lend themselves to sampling at paired and environmentally contrasting locations at a fine scale, improving statistical power to detect loci structured by the environment (see Sect. 4.1). In addition, because of their large effective population sizes, marine species may be especially suited to respond to environmental changes via natural selection. The efficacy of selection scales with *Ne* and, therefore, strong selection could be typical in marine populations (Allendorf et al. 2010; Gagnaire et al. 2015; Bierne et al. 2016).

The large effective population sizes of marine organisms coupled with their high vagility provides a unique testing ground to observe how natural selection might proceed in the face of high migration (Hauser and Carvalho 2008). It is likely that adaptations could draw from the large amounts of standing genetic variation found in marine populations (Kelley et al. 2016; as in Colosimo et al. 2005). If this were the case, seascape genomics may help us understand to what extent the same alleles confer some functional benefit across multiple locations with similar environments (Riginos et al. 2016). If environment-associated alleles can be identified and shown to have different origins, such as by new mutations, then this is evidence for independent adaptive evolution (a phenomenon found in many fishes, Bernatchez 2016; Le Moan et al. 2016). In contrast, environment-associated alleles that are identical or related indicate that there is a shared historical origin to the adaptation (i.e. parallel adaptive evolution from standing genetic variation). In the marine environment, it has been suggested that parallel adaptive evolution may be more prevalent than in terrestrial or freshwater systems, as extensive gene flow enables the reuse of the same alleles from standing genetic variation. In practice, however, competing scenarios can be difficult to distinguish (Bierne et al. 2013).

In summary, the marine realm has immense spatial and temporal variability, over a vast range of scales. Marine organisms have evolved a diverse array of life histories to succeed in this spatio-temporally dynamic environment. Most life histories are complex (i.e. bi-partite), which is presumably a strategy to maintain viable metapopulation structures. Based on spatial genetic studies, these metapopulation structures and their causes are often non-intuitive and may be spatio-temporally dynamic themselves. With the onset of seascape genomics, it is opportune to understand the consequences of these life histories and metapopulation structures for how marine species have adapted to the marine environment. Understanding patterns of neutral and adaptive genomic variation of marine populations in relation to contemporary seascapes will help us understand how marine species may be equipped to cope with directional shifts in their environment resulting from global changes. The challenges of overfishing, habitat degradation, and climate changes are already influencing our marine communities and populations and presumably are driving adaptive responses in these populations that we now have the tools to assess.

4 Considerations and Approaches in Seascape Genomics

Seascape genomics can now take advantage of sequencing technologies providing us with more varied and potentially precise genomic information combined with higher resolution spatial and oceanographic information. However, to make effective use of these data-rich resources, the importance of taking an informed and experimental approach to a seascape genomics study cannot be underestimated (a sentiment also shared in Storfer et al. 2010; Liggins et al. 2013; Riginos et al. 2016; Balkenhol and Fortin 2016; Balkenhol et al. 2019). For instance, formulating a seascape genomics research question or hypothesis requires a robust understanding

of the focal organism's biology and how it is likely to interact with the seascape of interest (Fig. 1). The subsequent study design, sampling extent and approach, choice of predictors (i.e. seascape), responses (i.e. genomic measures), and analytical steps are then crucial in determining whether a researcher might be able to sufficiently address their question or hypothesis. All of these steps are co-dependent (Riginos et al. 2016; Balkenhol et al. 2019) and should be considered early in the research project. (See Storfer et al. 2007, Balkenhol et al. 2016a, and Balkenhol and Fortin 2016 for an extended discussion of study approaches in landscape genetics and genomics; and see Liggins et al. 2013 and Riginos et al. 2016 for a summary of issues specifically relevant to seascape genetics and genomics studies.)

Here, we outline some important considerations for seascape genomics studies and the tools and approaches that seascape genomics studies have taken to date. Within these steps, we highlight some areas of innovation in seascape genetics/genomics, specifically in taking strategic sampling approaches (Box 1), the use of biophysical models (Box 2), and the incorporation of asymmetric processes in seascape genetics/genomics (Box 3).

4.1 Study Design

It is widely appreciated that the spatial sampling strategy will affect one's ability to address a seascape genomics research question or hypothesis; however, the importance of the sampling in time is less acknowledged. Seascape factors that are likely to influence the genomic structure of marine species differ widely in their spatial and temporal *grain* size and also in their permanence (referred to as their *stationarity*, see Riginos et al. 2016 for elaboration on these definitions; and see Riginos and Liggins 2013 for some example features). Moreover, the occurrence of temporal shifts in genomic variation further suggests that consideration of sample timing is important (Johnson and Black 1984; Hedgecock 1994; Eldon et al. 2016; discussed in Sect. 3.1). Depending on the study motives, operationally this may mean: sampling each population several times, so that the temporal fluctuations in allele frequencies are also quantified when describing spatial genetic patterns; keeping sample timing consistent (i.e. same age group and timing across locations); or sampling in step with the temporal feature of interest (see Liggins et al. 2013 for more discussion). For example, in a recent example, Henriques et al. (2016) sampled shelf-associated hake (*Merluccius capensis*) over three successive years, including a year of increased upwelling. The authors showed that the hypoxic conditions caused by the upwelling drove gene flow from the south to north, across an otherwise stable genetic boundary. Thus, the resolution or grain of the spatio-temporal sampling of genomic variation should be guided by how the seascape variables might influence the neutral or adaptive processes of interest.

The grain and *extent*, representing the size of the sampled area or length of sampled time, jointly determine the *scale* of a sampling design (Riginos et al. 2016). Ideally, a genomics sampling design should be informed by species biology

(including demography) and the grain size of the seascape features or data of interest. Accordingly, such a sampling design should therefore consider *autocorrelation* (Tobler 1970; Getis and Ord 2010), or the spatio-temporal correlation due to proximity, of the seascape feature that will be used as a predictor. Autocorrelation between values for seascape or environmental features of sampled locations means the data are not independent (Dale and Fortin 2014), thus not meeting the assumptions of classic parametric statistics.

Another case of correlation relevant to studies investigating multiple seascape features is *collinearity*. In marine systems, common examples of collinearity among seascape features include mean annual temperature with latitude and depth with photosynthetically active radiation. Collinearity may lead to problems in parameter estimation in parametric regression-based analyses (Dormann et al. 2013; Prunier et al. 2015). Therefore, if seascape features (i.e. predictors) are highly correlated, this collinearity will need to be accommodated with a modified analysis (e.g. eigenanalysis; principal component analysis, PCA) or one of the predictors removed from the study (Wagner and Fortin 2016). For these reasons, for a study to distinguish the influence of competing seascape features, sampling design should seek to minimize the collinearity among them. (See Riginos et al. 2016 for an overview of several concepts relevant to the sampling of spatio-temporal features, their properties, and influence on analysis.)

The ability of different sampling designs to discriminate among hypotheses can be statistically evaluated based on the planned sampling of the seascape features alone, ahead of any genomic inquiries. Such evaluations can proceed regardless of whether the seascape features of interest are continuous, categorical, or ordinal. Riginos et al. (2016) provide an example where sampling locations are selected based on maximizing the distance in the continuous environmental principal component (PC) space and minimizing the geographic distance between them. Such a spatial sampling design would help a researcher distinguish between patterns of genomic variation driven by geographic distance among sampling sites versus the environmental distance among sites (as recommended by Selkoe et al. 2008 and Liggins et al. 2013). In contrast, to address an a priori hypothesis regarding a specific seascape feature, sampling proximate or paired locations that differ only by the factor of interest has been suggested as a powerful approach (Rellstab et al. 2015; Lotterhos and Whitlock 2015), where presumably the investigator checks for collinearity as part of the experimental design. Alternatively, we present a hypothetical example for the implementation of a stratified-random sampling scheme developed for a seascape genomics study in Box 1. Such an approach has not yet been implemented as far as we are aware, but might be useful when trying to understand how multiple, but a priori defined, seascape variables may interact to determine patterns of genomic variation.

Where the study has clearly articulated hypotheses as to how the seascape feature will influence migration, drift, and/or selection, then population genetic (or genomic) simulations (Hoban et al. 2012) can provide valuable assessments of the sampling design (see Landguth et al. 2016 for examples). Such an approach has the advantage of evaluating the sampling design from the perspective of the genetic/genomic measures that the researcher will use to confirm or refute a hypothesis. Thus, such

simulations will additionally provide information regarding the sensitivity of the genetic measures to the seascape-genomic interaction of interest (as well as enabling power tests to help decisions discussed in Sect. 4.4 regarding the number of loci, individuals, and populations required; Parobek et al. 2017).

Several seascape genomics studies have planned their sampling design with regard to correlations among spatial variables or purposely taken replicate samples across environmental contrasts (e.g. Limborg et al. 2012; Therkildsen et al. 2013; Bongaerts et al. 2017). For instance, some studies have employed a replicated paired sampling design to evaluate the role of parallel adaptive evolution. For example, both Westram et al. (2014) and Ravinet et al. (2016) evaluate the formation of ecomorphs in the littorine snail (*Littorina saxatilis*) using paired sampling across two habitat types: "wave" and "crab". Le Moan et al. (2016) similarly took replicate samples of anchovies (*Engraulis encrasicolus*) across paired "open water" and "coastal" locations. In all cases, a few shared outlier loci were found among multiple habitat contrasts. This suggests that at least some shared variation may be used to enable parallel adaptation across multiple habitats, for both a direct developer with no pelagic larval phase (i.e. the littorine snail) and a mobile fish with planktonic larvae (i.e. the anchovy).

Box 1 Developing a Stratified-Random Sampling Scheme in Seascape Genomics

Seascape genomics studies can greatly increase their inferential power by quantifying the seascape features of interest ahead of time and using this to guide their field sampling (see Sect. 4.1 and Riginos et al. 2016 for more discussion). One approach that is often advocated is a "stratified-random" sampling design. Such a sampling design can be hard to conceptualize when interested in seascape features or environmental variables that are continuous and/or vary through time.

Here, we conceptually step through the process of planning a stratified-random sampling design for a hypothetical seascape genomics study of an urchin in New Zealand. (For similar examples interested in maximizing contrasts among competing variables, or identifying steep environmental gradients, see Riginos et al. 2016.) In our example, we are interested in how the genomic variation within and among urchin populations (either neutral or adaptive) is associated with directional changes in the local seascape environment and how the demographic setting of a population may influence this. We build our parameter space (Fig. 2) using a biophysical urchin dispersal model to inform axes representing local retention (I), immigration (II), and environmental change based on sea surface temperature (SST, III). We choose these three variables because we are interested in the relative importance of immigration versus local retention in determining the species' adaptive response to environmental change (e.g. change in SST). Nonetheless, any hypothesized drivers could be used in their place, and the subsequent steps would be similar.

(continued)

Box 1 (continued)

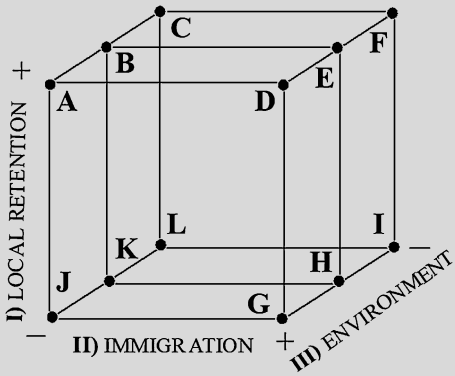

Fig. 2 Demographic-environment multivariate parameter space. A to L are our "strata" and represent strategic contrasts in the parameter values for the drivers we are interested in: i.e. (I) local retention, (II) immigration, and (III) environmental change

The demographic axes are estimated using a biophysical larval dispersal modelling framework (see Box 2). The dispersal model includes high-resolution urchin habitat data and traits, such as reproductive output, spawning phenology, larval type, and competency characteristics. This mechanistic dispersal model tracks a "cloud" of virtual urchin larvae, effectively quantifying the entire dispersal kernel as it moves through the seascape. Modelled output data will include the dispersal probability matrix, the direction, strength, and distance of migration between all pairs of urchin habitat patches. These data are summarized for all habitat patches, to provide values of local retention (I) and immigration (II, Fig. 3). Environmental change (III) is defined as the recent change in SST (i.e. running weekly mean, 1958–2018) relative to the thermal optimum for the urchin's early life development stages. Mortality is greatest during the early life stages of most marine reef species, and during this stage, urchin developmental success and survival is greatly affected by temperature.

All habitat patches are projected in multivariate space according to their local retention (I), immigration (II), and environmental change (III) values and classified into the 12 extreme demographic-environmental contrasts (Fig. 2, A to L). These are our strata. We might anticipate that not all contrasts will have representative habitat patches, but where possible, replicate habitat patches from each strata would be selected at random for sampling in the field. Alternatively, these demographic-environment contrasts (i.e. strata) could be used as scenarios for population genetics/genomics simulations to infer likely outcomes and/or assess the sensitivity of genetic measures to the underlying causes of the spatial genomic patterns.

(continued)

Box 1 (continued)

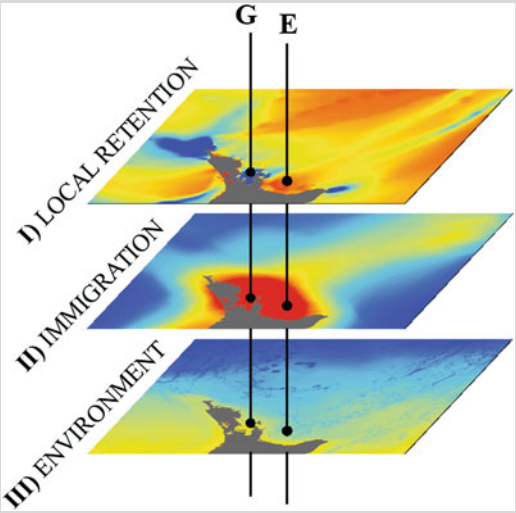

Fig. 3 Hypothetical samples of our strata (i.e. demographic-environment contrasts). With reference to Fig. 2, G represents a patch where local retention is low (I), immigration is high (II), and environmental change has been high (III). In contrast, E represents a patch where both local retention and immigration are high, and environmental change has been moderate. Replicate samples would be taken for each stratum (A to L)

4.2 Quantifying Seascape Features and Processes

Seascape genomics studies are interested in the association between genomic variation and local seascape or environmental features, as well as the environment, or matrix, between those features (e.g. expanses of open ocean, habitat patches, and hydrodynamic barriers). The resolution, extent, and frequency of seascape and environmental data have increased substantially in the last decade, as well as the accessibility of these remote-sensed and in situ oceanographic data (for some example spatial and environmental parameters used in seascape genomics studies and sources, see Riginos et al. 2016 and Grummer et al. 2019). Nonetheless, the challenges described in Sect. 4.1 (e.g. autocorrelation, collinearity) apply to the quantification or characterization of seascape features to be used as predictors. The ocean environment and ecological processes (such as dispersal) are rarely stationary, often varying in space and time (*nonstationarity*). Features of the seascape are also often autocorrelated, and the structure of this nonindependence may vary in direction (*isotropic*), resulting in directionality in the patterns. For example, asymmetrical patterns and relationships, such as caused by ocean currents, are *anisotropic* (see Box 2 on biophysical models and Box 3 focused on characterizing asymmetry). The challenge is quantifying these seascape and environmental features

of interest in a way that is appropriate for their hypothesized relationship with genomic variation and in a form that enables the relationship to be statistically evaluated (see Sect. 4.6).

One of the most fundamental hypotheses underlying seascape genomics studies is that there should be some association between genetic (or genomic) patterns and space. The isolation-by-distance hypothesis (IBD, Wright 1943; Slatkin 1993; Rousset 2000) describes the autocorrelation between geographic distance and genetic differentiation for continuously distributed populations (or individuals) that approximate an equilibrium between migration and genetic drift. IBD analyses traditionally use straight-line (Euclidean) geographic distances; however, with our improved ability to resolve the seascape, we can now use ecologically relevant distances. The simplest of these is overwater distance (a type of least-cost-path, Spear et al. 2010), constraining gene flow to occur through non-land paths and assuming that all intervening parts of the seascape are equally traversable and isotropic. Overwater distance measures have been used extensively as a predictor in seascape genomics studies to date as they are more realistic than Euclidean-based assumptions. Even so, Bonanomi et al. (2015) contrasted the performance of such overwater distances with a least cost path around the coast between adult cod (*Gadus morhua*) populations and spawning grounds based on habitat suitability modelling. The study found that the distances based on habitat suitability predicted individual genotypes better than overwater distance alone.

Other isolation-based approaches include isolation-by-environment where the degree of genomic differentiation among sampling units is expected to increase with increasing environmental dissimilarity (Wang and Bradburd 2014); isolation-by-resistance (McRae 2006) where ecological distances are modelled by weighting the cost (resistance) of traversing various seascape features; and circuit theory (McRae et al. 2008) which builds on this approach to take every possible path among populations into consideration simultaneously. Often these approaches used frequently in terrestrial landscape settings are inadequate in the marine system where inferred distances may be dynamic in time, and connections may have directionality. In such cases, asymmetric oceanographic distance (derived from an ocean circulation model) or dispersal distances derived from biophysical models of larval dispersal, parameterized with biological attributes of the organism, may better capture the physical and/or biological processes affecting gene flow (see Box 2).

The most extreme case of resistance, or limitation to connection among locations via dispersal and gene flow, is by a barrier or boundary. Obviously landmasses form boundaries and may be complete barriers to movement. More complex cases are permeable barriers that reduce but might not preclude gene exchange entirely (e.g. Treml et al. 2015b). Boundaries that have been investigated in seascape genomics studies include those caused by upwelling and ocean currents (e.g. Saenz-Agudelo et al. 2015; Xuereb et al. 2018a).

The majority of seascape genomics studies interested in detecting patterns in adaptive genomic variation, or associating outliers or allele frequencies of putative candidate loci, with the seascape, have focused on seascape features local to sampled populations. In these cases, summary values have been used to describe the

environment at static locations, such as mean or maximum sea surface temperature, or sea surface salinity, and rarely consider the range or variability through time (but see Sylvester et al. 2018).

Box 2 Biophysical Modelling in Seascape Genomics

Biophysical models can help quantify the connectivity, dynamics, and asymmetric nature of the ocean environment and are particularly useful for understanding the movement of pelagic gametes or larvae over large distances (Leis et al. 2011). These models range from using physical ocean circulation as a proxy for dispersal (e.g. White et al. 2010) to coupled biological-physical models (hereafter biophysical models) that also include virtual larvae with individual biological attributes and behaviour (e.g. Paris et al. 2007; Treml et al. 2015a). Seascape genetics and genomics studies have benefited from considering genetic data alongside both simple and complex models. Nonetheless, our knowledge regarding the important role that life history features and behaviours of marine organisms play in determining dispersal (Strathmann 1990; Gerlach et al. 2007; Kingsford et al. 2002) suggests that biologically informed models may provide a more realistic proxy of the expected dispersal process (see Fig. 1). Thus, biophysical models have emerged as a way to generate seascape genetics hypotheses (e.g. Wood et al. 2014; Treml et al. 2015b), produce biophysical data to include in the analysis of genetic data, and ultimately examine the dispersal mechanisms underlying genetic patterns.

Seascape genomics questions can be informed by a variety of products derived from biophysical models such as the dispersal pathway of an individual, a population's dispersal kernel describing the probability of successful dispersal with distance (both useful in parentage and assignment tests, e.g. Le Port et al. 2017; Bode et al. 2019), indices reflecting the amount of immigration versus local retention (e.g. Wood et al. 2014; see Box 1), and various connectivity matrices describing dispersal among locations. Which model output is of most interest depends on the research question, the genetic data, and study design. In fact, all considerations relevant to study design apply here (see Sects. 4.1 and 4.2): to the choice of biological parameters, the spatio-temporal resolution (or grain size of the physical model and habitat patches), and the derivation of the model outputs (for a more in-depth discussion, see Liggins et al. 2013 and Treml et al. 2015a). For instance, connectivity matrices from biophysical models are pairwise and directional and thus provide an appropriate match for migration among populations (also directional; see Crandall et al. 2012; Davies et al. 2015); however, most population genetics statistics are either symmetric (e.g. pairwise F_{ST}) or relate to a specific population (e.g. HWE; allele frequency spectra). As a consequence, biophysically derived data will often be converted into symmetric measures

(continued)

Box 2 (continued)
of connectivity or distance (average strength) or used as a localized estimator of connectivity (e.g. Paterno et al. 2017), capturing site-specific connectivity qualities (e.g. maximum flow, betweenness centrality, source strength). While these derived indices allow the association of the biophysical model with genomic data to be statistically evaluated using standard methods (see Sect. 4.6), the data will lose some of its information and realism (Vuilleumier and Possingham 2006; Kool et al. 2013). Nonetheless, there are some innovative ways in which researchers have incorporated the inherent asymmetry of the ocean, including for biophysical models and genetic data, into their analyses (see Box 3 on characterizing asymmetry in seascape genomics).

Despite the wide use of biophysical models to characterize migration processes in the ocean, several studies have evidenced the value in using such migration matrices alongside other relevant predictors of selection, drift, and mutation. In reality, the distribution of genomic variation, and particularly adaptive variation, also depends on many other environmental and biotic factors (Fig. 1). Thus, unexplained variance in the association of biophysical model outputs with patterns in genomic data can help highlight where other seascape features or processes may be important. For example, in a study of American lobster (*Homarus americanus*) by Benestan et al. (2016), although their model of larval dispersal explained the majority of the variance found in the patterns of genomic variation, residual variance for putatively adaptive variation was found to be significantly associated with minimum annual sea surface temperature. In another study, Barth et al. (2017) were able to suggest a role of local adaptation to fjord habitats in maintaining population genomic structure in cod (*Gadus morhua*), despite high connectivity with offshore populations based on a biophysical model. With the same goal in mind – to understand the relative influence of the larval dispersal versus local, post-settlement processes in shaping the distribution of genetic variation – Ewers-Saucedo et al. (2016) instead used a coupled "biophysical larval dispersal and fitness model" to study the co-existence and segregation of two barnacle (*Notochthamalus scabrosus*) lineages. The authors found that by incorporating population census, fecundity, larval dispersal, and a spatially explicit model of post-settlement competition, they were able to demonstrate that the distribution of the genetic types (lineages) were likely maintained through their distinct environmental/fitness optima.

In summary, as we become immersed in the genomics era and are interested in adaptive genomic variation alongside neutral genomic variation, biophysical models will continue to serve as a valuable method in the toolkit of seascape genomics. Furthermore, as our understanding of the factors shaping the distribution of neutral and adaptive genetic variation is enhanced through multidisciplinary studies, biophysical modelling frameworks may be well suited to further parametrization relevant to these eco-evolutionary settings.

4.3 Genomic Markers

Genomics methods and NGS are facilitating population genomics, and therefore seascape genomics studies, of non-model organisms (Luikart et al. 2003; Hohenlohe et al. 2010; Holliday et al. 2019). These methods offer greater precision to quantify neutral genomic variation through the promise of larger numbers of loci and higher levels of polymorphism (i.e. how the processes of migration, mutation, and drift are influenced by the seascape) and the possibility to infer patterns of adaptive variation via loci that are of some functional significance (i.e. how the process of selection is influenced by the seascape). There are a growing number of ways by which a researcher may access this genomic information, and although we still have much to learn about genomic architecture and genomic processes, it is clear that the approach used influences not only the number of loci (from hundreds to all variable loci across genomes) but also the nature of the markers and the subsequent analyses that can be used (see Davey and Blaxter 2010; Andrews et al. 2016).

Genomics methods can be largely divided into targeted and non-targeted approaches. Targeted approaches include arrays and sequence capture for predetermined single nucleotide polymorphisms, SNPs (e.g. SNP arrays, Seeb et al. 2011; genotyping-in-thousands by sequencing, Campbell et al. 2014), exons (e.g. exon bait capture, Bi et al. 2012; expressed exome capture, Puritz and Lotterhos 2018), transcripts (e.g. targeted RNA-seq, Mercer et al. 2012), or informative sequence loci flanking ultra-conserved elements (UCEs, Faircloth et al. 2012). The advantages of these approaches are that they are less likely to return contaminant loci, allow for much greater control of the number of loci (therefore also ensuring optimal coverage), and usually work with varying degrees of DNA quality and quantity (not the case if the focus is RNA, however). But, these approaches require initial development, and often optimization, which is costly and time-consuming. Nonetheless, many of the early seascape genomics studies conducted focused on commercially fished species (e.g. cod, herring, hake, sole) for which there are substantial genomic resources available. The most commonly used markers for these species were SNPs designed from transcriptomic data targeting gene coding regions of known functional significance (Riginos et al. 2016).

Over recent years, most seascape genomics studies have used non-targeted approaches that provide an appropriate and informative method in the absence of pre-existing genetic resources. Non-targeted approaches range from whole-genome shotgun resequencing (WGS; Therkildsen and Palumbi 2017) and transcriptome sequencing (RNA-seq, de Wit et al. 2012) to reduced representation methods (reviewed by Andrews et al. 2016) targeting sequence regions adjacent to restriction sites (e.g. double digest restriction site-associated DNA, ddRAD; restriction site-associated DNA sequencing, RADseq; genotype-by-sequencing, GBS; and complexity reduction of polymorphic sequences, CRoPS) or other repetitive sequences (e.g. Nextera-tagmented reductively amplified DNA, nextRAD). These methods do not require any prior development and aim to provide a panel of loci randomly distributed across the genome (or transcriptome). Studies motivated to answer

questions at the population-level (see Sect. 4.4) may also use a pooled sequencing approach, where libraries are constructed for many individuals rather than each individual separately (Schlötterer et al. 2014, as in Westram et al. 2014 and Guo et al. 2015). Although methods for estimating population statistics for these pooled sequencing approaches are developed (e.g. Kofler et al. 2011), the covariance among loci (including linkage disequilibria) within the population cannot be estimated, and, therefore, individual-based analyses, such as admixture or parentage detection, cannot be used. Furthermore, a disadvantage common to all of these non-targeted approaches is that researchers are often required to have a large quantity of high-quality starting DNA (or RNA in the case of RNA-seq) for every individual.

The bioinformatics steps of variant calling from the derived genomic data and obtaining sufficient locus coverage can be challenging for marine species. As a consequence of having large population sizes, the high levels of polymorphism in marine populations can cause problems for the identification of SNPs (discussed in Lowry et al. 2017). Polymorphism within the sequences can drive some bioinformatics pipelines to erroneously split reads from the same locus into multiple clusters (Puritz et al. 2014; Mastretta-Yanes et al. 2015). Also, when using a higher clustering threshold to avoid this issue, some alignment-based clustering approaches (e.g. Eaton 2014) can falsely join paralogs as orthologs. In addition, polymorphism at restriction sites are known to lead to a large number of null alleles when using restriction enzyme approaches (as in Ravinet et al. 2016) or targeted PCR-based methods on marine species. (See Willette et al. 2014 for more discussion on the use of NGS methods in marine systems.)

Having access to annotated reference genomes and/or transcriptomes can support the calling of variants and help avoid many of the above issues. Moreover, such resources would enable targeted approaches across a wider diversity of marine species. However, these genetic resources are few for marine species; only 18 genomes have been assembled for marine species, as opposed to around 70 for terrestrial species (as of 2015, Kelley et al. 2016). This divide is likely partially driven by levels of interest in terrestrial species versus marine species, but the high levels of heterozygosity in marine species also causes issues in de novo genome assembly (Kelley et al. 2016).

4.4 Locus, Individuals, or Populations?

A fundamental consideration in planning a study is whether individuals or populations will be the sampling unit (Anderson et al. 2010). Where the seascape is the focus, it may be deemed more cost-effective (from a genotyping point of view) to sample individuals at more locations over a broader spatial extent than populations at fewer locations and reduce spatial resolution or study extent (Riginos et al. 2016). Alternatively, a pooled (population) sequencing approach might be considered (see Sect. 4.3). However, population-level sampling dominates in seascape genomics (Riginos et al. 2016). In part, this may reflect the habit of the focal

species studied so far – that is, species with patchy habitat and schooling marine fishes with large geographic ranges. In seascape genetics, studies over large spatial extents were also noted to primarily use population-level sampling, whereas studies interested in smaller spatial extents and temporally variable parameters used individual-level sampling (see Sect. 4.1; Riginos and Liggins 2013; Liggins et al. 2013). For a population-based approach, although simulations suggest that only 3–6 individuals per location might be sufficient to detect genetic differentiation (Prunier et al. 2013) and to discern outlier loci for biallelic markers such as SNPs (Lotterhos and Whitlock 2015), it is unlikely that such small sample sizes would provide a reliable estimate of allele frequencies. Certainly, for multiallelic loci such as DNA sequences, larger population sample sizes are advisable (Riginos et al. 2016) and would support demographic modelling (see Sect. 4.6 and Box 3).

Taking a population-based sampling approach does not preclude the use of individual-based analyses or locus-based analyses for that matter. In the case of the majority of seascape genomics studies conducted to date, a combination of population-, individual-, and locus/loci-specific analyses has been typical. When using neutral genomic variation to infer population demography, population-level analyses typically rely on allele frequencies (such as F_{ST}) that can be analysed under the assumption that populations are in Hardy-Weinberg equilibrium (HWE). Genetic differences between pairs of individuals based on multilocus genotypes (e.g. biallelic SNPs) can be used similarly in conventional population-based statistics (Rousset 2000). Individual-based analyses are most distinctly useful in inferring parentage, relatedness, and to assign individuals to populations (as in Saenz-Agudelo et al. 2009) or clusters (as in Pritchard et al. 2000). Lastly, when the analyses endeavour to detect loci under selection, population-based analyses are most common; however, outlier detection based on genotyped individuals is possible (e.g. using the methods: LFMM, Frichot et al. 2013; and PCAdapt, Duforet-Frebourg et al. 2014).

4.5 Quantifying Genomic Patterns and Processes

Response variables used in seascape genomics studies include those that provide a measure within a single spatial unit (an individual, population, or geographic region; i.e. node-based, Wagner and Fortin 2016) and those that reflect a difference or connection between spatial units, based on either individual genotypes, population summaries, or sets of putatively neutral or adaptive loci (i.e. link-based). For the most part, summary statistics from population genetics and genomics are used as these responses. For instance, indices of genetic diversity, such as allelic diversity, allelic richness, heterozygosity, and percentage of polymorphic loci, are used as measures aligned to spatial units. To measure between two or more spatial units, genetic distances and indices of genetic differentiation are often used (e.g. F_{ST}, Wright 1943; Nei 1972). However, classic population genetics models that inform

many of these indices have underlying assumptions that may lead to erroneous interpretations of genetic data for marine organisms that have large ranges, large population sizes, and high levels of dispersal among (sub)populations (Selkoe et al. 2008; Karl et al. 2012).

Riginos et al. (2016) discussed four theoretical problems underpinning the inference of neutral genomic population structure for marine organisms (also discussed in Whitlock and McCauley 1999; Waples and Gaggiotti 2006; Faurby and Barber 2012; Palumbi and Pinsky 2013; Gagnaire et al. 2015; Gagnaire and Gaggiotti 2016). First, differentiation is typically low between populations because of high gene flow and, therefore, is difficult to statistically distinguish from zero (Waples 1998). Second, the high reproductive output, and ranges of marine species, means that global effective population sizes are large and sustain high global genetic diversities (i.e. weak genetic drift), constraining the maximum differentiation value for markers with more than two alleles (e.g. multiallelic SNPs, reviewed in Meirmans and Hedrick 2011). Third, due to the combination of weak drift, high migration, and often asymmetric (source-sink) migration, populations are rarely at equilibrium, meaning it is difficult to distinguish between historical and contemporary gene flow (Slatkin 1985; Marko and Hart 2011). Fourth, as previously discussed, spatially and temporally inconsistent patterns of genetic structure (i.e. chaotic genetic patchiness) may be indicative of variance in reproductive success or patterns of immigration over time, violating the HWE assumptions of large and constant population sizes. As a consequence, characterizing metapopulation structure in the marine system continues to be challenging (Gagnaire et al. 2015; Gaggiotti 2017).

Seascape genomics studies often use averaged F_{ST} between pairs of populations (or individuals); however, other methods and metrics can be used to understand spatial patterns of neutral genomic structure and the underlying processes. For instance, parentage analysis, clustering methods (including assignment tests; François and Waits 2015), population graphs (including the estimation of conditional genetic distance, cGD; Dyer and Nason 2004; Dyer et al. 2010), and ordinations based on genotypes alone (such as PCA or discriminant analysis of principal components, DAPC) without interim summaries of the genetic data are useful for describing relationships among individuals and for discovering emergent spatial genetic structuring. Gagnaire et al. (2015) also suggest some metrics that may be better suited to detecting weak genetic differentiation typical for marine populations, including haplotype sharing, focusing on rare alleles, or "migrant tracts" of DNA segments that resist recombination after admixture.

In study species where gene flow should be substantial and/or past changes in population sizes are likely, coalescent methods or demographic modelling approaches based on allele frequency spectra may be more appropriate estimators of genetic connections than those based on genetic distances or differentiation indices (e.g. Le Moan et al. 2016; see more discussion in Marko and Hart 2011 and Box 3). Several of these methods have the advantage of providing information regarding the directionality of dispersal or gene flow and therefore may be more useful for evaluating asymmetric processes, such as transport by currents and source-sink dynamics (see Box 3). It should be noted, however, that these alternative methods

and metrics for evaluating neutral genomic structure may have properties that preclude statistical evaluation of their values alongside predictors derived from the seascape, or at least the analyses to do so require careful consideration (see Sect. 4.6).

The loci retrieved for any seascape genomics analysis will comprise loci that have experienced a range of selection coefficients from negligible to substantive. Most population genomics studies try to uncover loci that are candidates for carrying a strong imprint of selection (as opposed to more subtle polygenic selection, see Gagnaire and Gaggiotti 2016). Depending on the study's objectives, these candidate loci may then be removed from subsequent analyses aimed at inferring the seascape features affecting neutral population genomic structure and gene flow (e.g. Dalongeville et al. 2018a; Xuereb et al. 2018a), or outlier loci may be analysed in isolation to uncover and describe adaptive genomic structure and causes of environmentally mediated selection (e.g. Dalongeville et al. 2018b; Xuereb et al. 2018b). Often the putatively neutral and adaptive loci will be analysed independently, but using the same methods (see Sect. 4.6), to demonstrate the differences in their spatial structuring across the seascape (e.g. Sandoval-Castillo et al. 2018), with an implicit assumption that loci are not physically linked (see Sect. 6.2).

The inference of putatively adaptive (outlier) loci can be via: the evaluation of locus-specific pairwise F_{ST} values, where extreme values indicate that differentiation may be driven by adaptive rather than neutral processes (F_{ST}-outlier approach); unconstrained ordination of loci where various measures of multivariate distance may be used to identify outliers (e.g. PCAdapt, Duforet-Frebourg et al. 2014; multilocus population graphs and cGD; Dyer and Nason 2004; Dyer et al. 2010); or where allele frequencies for a locus are directly associated with the environmental parameter(s) of interest (genetic-environment association analysis, covered in Sect. 4.6). Most studies typically use at least two methods from alternative strategies, taking advantage of their complementary strengths, and their different inherent biases towards false positives and false negatives (see reviews by Lotterhos and Whitlock 2014, 2015; de Villemereuil et al. 2014; Rellstab et al. 2015; François et al. 2016; Gagnaire and Gaggiotti 2016).

As neutral genomic structure underlies the evaluation of loci putatively under selection, the problems in understanding neutral genomic structure may influence our inference of adaptive genomic structure for marine populations (Kelley et al. 2016). In particular, F_{ST}-outlier based methods may be affected by the performance of F_{ST} in marine metapopulations, leading to false positives and/or negatives in outlier loci. Across all approaches however, it is recognized that genetic signatures of selection may be confounded with signatures of neutral processes (i.e. historical or demographic; Excoffier et al. 2009). The sensitivities of several methods to spatial genetic correlation structures formed through IBD, range expansions, and hierarchical population structure have been described and evaluated (Lotterhos and Whitlock 2014; de Villemereuil et al. 2014; de Villemereuil and Gaggiotti 2015; Whitlock and Lotterhos 2015; Frichot et al. 2015; Gagnaire and Gaggiotti 2016). On this basis, seascape genomics studies typically proceed in the detection of selection with the inclusion of some control for population structure (Excoffier et al. 2009; de Villemereuil et al. 2014) but often also conduct an analysis without such controls,

so as to avoid being too conservative (Forester et al. 2018). Based on uncertainty in our inference of metapopulation structures and the modes by which selection operates in marine environments, using a suite of approaches to detect putatively adaptive loci is recommended (e.g. including F_{ST}-outlier and environmental association analyses, both with and without corrections for neutral population genomic structure; see Dalongeville et al. 2018b for an empirical test of several approaches and methods relevant to seascape genomics studies). Typically, subsequent analyses, focused on putatively adaptive loci or identifying candidate genes under selection, will proceed by using the loci within the intersection of all methods or some subset of these.

4.6 Making "Seascape Genomics" Inferences

Seascape genomics inferences rely upon analyses that can evaluate the association of several, competing seascape features, including environmental gradients, habitat configurations, and matrix characteristics, on spatial genomic patterns. Therefore, these analyses routinely encompass multiple predictors (i.e. seascape variables) and multiple response variables (e.g. allele frequencies for several loci). Predictor variables are likely to be collinear (or correlated, as described in Sect. 4.2), and loci are also often not independent due to linkage and shared historical relationships (Stone et al. 2011). Thus, while there are many ways to qualitatively evaluate the correspondence between the seascape and spatial genomic patterns, here we focus on analyses that *statistically evaluate* the association between seascape predictors and neutral and/or adaptive genomic variation. Moreover, we focus on the most recently used methods in seascape genomics that attempt to handle the challenging properties of genome-wide data and environmental data derived from the seascape. In the general field of landscape genetics/genomics, it is encouraged to undertake several different analytical approaches to inform study results, taking advantage of the strengths and limitations of the available methods (Rellstab et al. 2015; for a detailed review of methods see Wagner and Fortin 2016; some appropriate methods and relevant software are provided in Grummer et al. 2019).

To allow straightforward approaches to the analysis of seascape genomic data, often the dimensionality of predictor, or response variables, is reduced. For example, summary statistics such as mean F_{ST} or cGD can be used to represent multiple loci (whether they be genome-wide, or putatively neutral, or adaptive; see Guillot et al. 2009 and Wagner and Fortin 2016). Subsequently, Mantel tests (Mantel 1967), partial Mantel tests (Smouse et al. 1986), or multiple regression of distance matrices (Legendre et al. 1994) can be used to test for a significant association between seascape predictor(s) (such as a distance measure, see Sect. 4.2) and genetic summary statistics. However, Mantel tests (and derivatives) are prone to false positives and are not appropriate when there is high spatial autocorrelation among sampled locations (discussed by Guillot and Rousset 2013; Legendre et al. 2015). Moreover, in these distance-based approaches, allele frequencies from each sampled location

contribute to multiple pairwise observations, and therefore the observations are not independent. In contrast, for genetic response variables that are tied to a specific location (e.g. per cent polymorphic loci), although spatial autocorrelation may exist among the sampled sites, robust inferences based on multiple regression techniques can be used (Legendre et al. 2015). For example, generalized linear mixed models (e.g. gINLAnd, Guillot et al. 2014) are popularly used for inferring associations between the environment and allele frequencies and can account for spatial autocorrelation using geographic coordinates or coordinates derived from multidimensional scaling of other distance matrices, such as overwater distance (see Sandoval-Castillo et al. 2018 for an example).

Several different methods come under the umbrella of genetic-environment association analyses (GEAs). An implicit advantage of GEAs is that they use allele frequencies directly as the genetic response variables (rather than a genetic summary statistic) to investigate associations with environmental variables. They are predominantly used in detecting loci putatively under selection, while also charactering the environmental conditions contributing to adaptive genetic variation (Joost et al. 2007; Schoville et al. 2012; Sork et al. 2013; Rellstab et al. 2015). Several GEA methods are limited to the evaluation of a single environmental variable at a time (i.e. BayEnv: Günther and Coop 2013; LEA: Frichot et al. 2013; BayeScEnv: de Villemereuil and Gaggiotti 2015), which can lead to spurious correlations when there is collinearity among the predictor variables derived from the seascape (Riginos et al. 2016). In an attempt to incorporate multiple predictor variables into the one analysis, the common features of the predictor variables are sometimes summarized as orthogonal PCs, to be used as a single predictor variable in the analysis (de Villemereuil and Gaggiotti 2015; see Riginos et al. 2016 for a hypothetical example).

Multivariate statistics have many useful properties in the context of landscape genomics and seascape genomics (Riginos et al. 2016; Rajora et al. 2016). These methods are flexible in the types of input data, they manage collinearity through ordination, have modifications to measure and address autocorrelation and asymmetry, and by definition multivariate methods can have multiple response variables (see Borcard et al. 1992; Jombart et al. 2009; Manel et al. 2010; Wagner and Fortin 2016 for detailed discussions of multivariate statistics in landscape genetics). For both the inference of neutral genomic patterns and their association with seascape predictors, and for GEAs that help to define candidate loci and causes of adaptive genetic structure, redundancy analysis (RDA) – one type of multivariate analysis – has been used frequently in recent seascape genomics studies. RDA is an extension of linear regression that summarizes suites of associations between multiple predictors and response variables, using ordination to reduce dimensionality and handle collinearity. Possible response variables include those that are site-associated, such as allele frequencies for all loci of sampled populations, or principal coordinates analysis (PCoA) can be used to decompose distance matrices (e.g. F_{ST}, cGD) into orthogonal eigenvectors for use in distance-based RDA (dbRDA, Legendre and Anderson 1999). Furthermore, within the RDA framework (and other multivariate methods), spatial eigenfunction analysis can be used to account for spatial correlation

structures, including neutral population structure (Dray et al. 2006; see Forester et al. 2018; e.g. Xuereb et al. 2018b) and asymmetric predictors such as ocean currents (see Box 3).

Multivariate methods of GEAs, such as RDA, have been demonstrated to have higher power to detect locally adapted loci and maintain a balance of low false-positive and high true-positive rates under a variety of demographic histories, sampling designs, habitat configurations, dispersal, and levels of selection (Forester et al. 2016, 2018; Capblancq et al. 2018). Furthermore, while many outlier detection methods overlook selected loci that have a small effect (i.e. polygenic loci), these multivariate methods are well suited to detect weak multilocus responses as they implicitly model the covariance of loci in response to the environment (Bourret et al. 2013; Rellstab et al. 2015; Wellenreuther and Hansson 2016). (For more discussion regarding polygenic selection in marine populations, see Gagnaire and Gaggiotti 2016.)

For those not familiar with multivariate statistics, one drawback of these methods is that they lack intuitive visualizations and statistics for the association between loci and the environment. For this reason, several studies have adopted post hoc analyses subsequent to the multivariate method. For instance, to further investigate the spatial distribution of putatively adaptive alleles, a linear regression (or generalized additive model, GAM) between the minor allele frequencies (MAFs) of candidate loci and the best-associated environmental variables is often conducted, to ensure significant locus–environment associations (see Hoey and Pinsky 2018 and Xuereb et al. 2018b for examples). In the case of suspected polygenic selection, polygenic score analysis evaluates the additive effect of all candidate loci within a given individual in response to the environment (Gagnaire and Gaggiotti 2016). Candidate loci are identified as the loci for which alleles have a positive correlation with the focal environmental variable. A polygenic score based on these loci is then calculated for each individual by summing the number of favourable alleles within a particular environment across all candidate loci. Finally, these polygenic scores are used to evaluate the relationship an individual has with the focal environment (see Xuereb et al. 2018b for an example).

The landscape (and seascape) genomics community is increasingly proactive in the uptake of new analytical techniques as they appear in the fields of statistics and landscape ecology. Undoubtedly, there will be new methods developed in coming years that will be of particular use in spatial population genomics settings. For instance, machine-learning approaches such as Gradient Forest (see Bay et al. 2018; Fitzpatrick and Keller 2015 for examples) and Random Forest regression (see Sylvester et al. 2018 for a recent implementation in a seascape context) have not yet been used widely and may be of great relevance to studies with a large number of predictor variables (because of uncertainty regarding the important features of the seascape) and where there is some interest in understanding the robustness of the inference. One issue that has not been coherently addressed by the seascape genomics community is an overreliance on summary metrics based on observed genetic data, such as F_{ST} and expected heterozygosity, as estimators of gene flow or drift with the implicit assumption that populations are in migration-drift and mutation-

drift equilibria. By contrast, demographic modelling (such as Gutenkunst et al. 2009; Roux et al. 2013) offers approaches for estimating the actual evolutionary parameters of interest, although this modelling cannot be readily scaled up to landscape scale investigations (see Box 3). There certainly is not one superior approach to seascape genomics analysis, so the appropriate approach should be carefully considered in the case of each study.

Box 3 Characterizing Asymmetry in Seascape Genomics

Asymmetry (a special case of anisotropy) is an expected characteristic of marine environments especially with respect to larval dispersal given the prevalence of directional currents. There is no single methodology that can provide a rigorous evaluation of observed spatial genomic patterns against expectations arising from asymmetric processes (such as dispersal derived from biophysical models, Box 2). The common approach of converting asymmetric predictors to symmetric matrices is unsatisfying as it loses key information regarding those predictions (for more discussion see Kool et al. 2013; Riginos et al. 2016). Similarly, when symmetric metrics of genetic patterns (such as F_{ST}) are applied to situations with asymmetric gene flow, estimates of gene flow can be highly inaccurate (Wilkinson-Herbots and Ettridge 2004; Marko and Hart 2011). To maintain predicted asymmetries using currently available methods, however, involves a fundamental trade-off of (A) being able to consider many spatial samples (populations or individuals) in a single analysis with the implicit assumption that genomic differentiation scales solely with contemporary gene flow versus (B) using computationally intensive demographic modelling to jointly estimate gene flow, drift, and divergence times for a limited number of populations over evolutionary time scales.

One way to simultaneously consider many spatial samples (strategy A) is by using regression of distance matrices (predictors such as dispersal probability against pairwise genetic distances of samples). For example, forward matrix projections using gene flow values derived from biophysical models were well correlated to empirical genetic results for a Caribbean coral (*Montastraea annularis*, Foster et al. 2012). Alternatively, several recent studies have turned to an ordination method (asymmetric eigenvector modelling, AEM; Blanchet et al. 2011) to decompose the (asymmetric) predictor matrix into orthogonal spatial autocorrelation structures and evaluate these vectors against observed allele frequencies via multivariate regression (i.e. RDA). Using this method, spatial genetic structure for American lobster (*Homarus americanus*, Benestan et al. 2016), Californian sea cucumbers (*Parastichopus californicus*, Xuereb et al. 2018a), Mediterranean striped red mullet (*Mullus surmuletus*, Dalongeville et al. 2018a), and Great Barrier Reef corals (*Acropora tenuis* and *Acropora millepora*, Riginos et al. 2019) was well predicted by spatial autocorrelation structures derived from biophysical models. This approach is attractive because the computation is relatively

(continued)

Box 3 (continued)
straightforward and it is also possible to examine the correlations between predictors and subsets of loci providing another approach to identifying candidate loci for selection (Capblancq et al. 2018; e.g. Dalongeville et al. 2018a). However, while this approach can directly compare observed allele frequencies against asymmetric predictors such as biophysical models, it ignores other possible influences on spatial patterns of allele frequencies such as differential rates of genetic drift arising from variation in population sizes among locations, divergence time, and endogenous influences on gene flow (see Bierne et al. 2013; and empirical examples from sea bass, *Dicentrarchus labrax*, Duranton et al. 2018; and sea horses, *Hippocampus guttulatus*, Riquet et al. 2019).

Demographic modelling (strategy B) attempts to uncover probable combinations of demographic (and, to a limited extent, selective) parameter values that are consistent with observed spatial genetic patterns (e.g. Gutenkunst et al. 2009; Roux et al. 2013; see Riginos et al. 2016 for more in-depth discussion). This approach therefore can decouple gene flow estimates from population size and divergence. An example of using such migration estimates derived from demographic modelling (based on allele frequency spectra of SNPs) was undertaken in corals, where there was a positive correlation between pairwise combinations for five populations for both biophysical and genetic-derived migration estimates with greater southward dispersal (Matz et al. 2018). Although demographic modelling can estimate the process of interest such as gene flow or migration, scaling up such analyses to infer seascape-wide dynamics is not feasible. With an increasing number of populations, there is an exponential increase in the number of potential free parameters; to estimate migration rates among n populations would yield $n!/(n-2)!$ parameters for directional migration, for instance. Another issue with using demographic modelling-derived migration estimates is that these values represent averages over time since divergence between populations and are therefore not commensurate with the timeframe of biophysical models. Even when divergence is recent, the uncertainty surrounding parameter estimation for recent events is high (Robinson et al. 2014).

There is complementarity among these approaches however (strategy A and B). For instance, Riginos et al. (2019) used demographic modelling for a subset of populations to demonstrate that gene flow among coral populations conformed to an IBD model (and not a vicariance scenario) and then undertook AEM analyses for all available populations to demonstrate correlations between dispersal predicted by a biophysical model and spatial genetic structure. Overall, such approaches that can detect or represent asymmetric migration should outperform genetic response variables that provide a single bidirectional value between populations. Nonetheless, there is much scope for further theory and methods to help uncover and evaluate the influence of

(continued)

Box 3 (continued)
asymmetric processes on spatial genetic variation. Undoubtedly the on-going theoretical and analytical advances in population genomics, including methods that relax assumptions regarding gene flow-drift equilibrium and symmetrical gene flow, will deliver more useful approaches for seascape genomics.

5 Key Insights Provided by Seascape Genomics

Despite the few strictly seascape genomics studies published to date, the rigour with which these studies have associated the seascape and patterns of genomic variation has ensured that they provide new understanding of marine metapopulation dynamics (Sect. 5.1), the process of adaptive divergence in the ocean (Sect. 5.2), and the causes of adaptive divergence in the marine environment (Sect. 5.3). Below we summarize the insights provided by seascape genomics studies so far.

5.1 Patterns and Scales of Spatial Genomic Structuring in the Ocean

With the advent of genome-wide markers, there was an anticipation of increased power and resolution to distil previously intractable patterns of population genetic differentiation for marine populations previously observed using mitochondrial DNA markers and microsatellites. Population genomics studies of marine organisms have indeed identified structuring between subpopulations that other markers have failed to detect (reviewed in Kelley et al. 2016). Nonetheless, where the low marker- and genome-wide marker datasets are comparable, the patterns of structure are consistent, although SNP datasets have greater power to resolve patterns of genomic structure (e.g. Sylvester et al. 2018 used both microsatellites and SNPs in their seascape genomics analysis of Atlantic salmon, *Salmo salar*). Thus, we anticipate that the major conclusions from previous studies based on classical markers will be sound even when re-examined using genome-wide markers.

Accompanying the use of genome-wide markers, inferences from seascape genomics studies have benefitted from advances in our parameterization of ocean-mediated dispersal (see Box 2) and analytical methods to incorporate these data in our analysis (Box 3). For instance, Xuereb et al. (2018a) determined that oceanography was a better predictor of neutral genomic structure than geographic distance for the giant California sea cucumber (*Parastichopus californicus*) over some spatial scales. Moreover, Benestan et al. (2016) used a biophysical model to determine that larval connectivity was the most important factor influencing the neutral population genomic structure of the American lobster (*Homarus americanus*) in Eastern

Canada. In both studies, the authors were able to retain the inherent asymmetry of the dispersal probabilities in the analysis and could evaluate them alongside other seascape predictors, through the use of multivariate and eigenfunction analyses.

The increased uptake of robust analytical methods has helped us resolve not only which predictors are important but the scales over which different seascape predictors matter. Building on the studies of Schunter et al. (2011), Munguia-Vega et al. (2014), and Young et al. (2015) that demonstrated that biophysical dispersal distance is a better predictor of genetic variation than geographic distance at spatial scales of 250–4,000 km; studies by Dalongeville et al. (2018a) and Xuereb et al. (2018a) revealed the scale over which larval dispersal shapes spatial genomic patterns for the Mediterranean striped red mullet (*Mullus surmuletus*) and giant Californian sea cucumber, respectively. In both studies, the extent at which spatial genomic patterns transitioned from being mediated by larval dispersal to multigenerational stepping stone processes (i.e. geographic distance) was well described using eigenfunction analyses in RDA.

Theoretically, we anticipate that there will be instances where metapopulation structures also influence patterns of adaptive genomic variation, and conversely that environmental gradients will influence the patterns of neutral genomic variation. For instance, if the movement of individuals is limited by strong selection against immigrants (Nosil et al. 2009), populations would diverge at both adaptive and neutral loci (Schoville et al. 2012), and neutral loci may appear to have environmental associations purely through their physical linkage on a chromosome with selected loci (reviewed by Gagnaire et al. 2015). Similarly, in low gene flow situations, both selected loci and linked neutral loci can get "trapped" geographically (Barton 1979; Gagnaire et al. 2015). Several seascape genomics studies have provided evidence for these phenomena. Spatial clustering of groups based on putatively adaptive loci and then neutral loci for both herring (*Clupea harengus*, Limborg et al. 2012) and sole (*Solea solea*, Diopere et al. 2017) recovered the same clusters, but with more pronounced spatial structure for the adaptive loci.

Accounting for the potential autocorrelation among spatial patterns of neutral and adaptive genomic variation in seascape genomics analyses has helped us disentangle the processes responsible for their conflated patterns. For instance, after multivariate analyses incorporating spatial variables, Diopere et al. (2017) demonstrated that despite the similar patterns of spatial structure for putatively neutral and adaptive variation in sole, environmental variables explained a significant amount of variability among the outlier loci, but not among non-outlier loci. Moreover, although sea surface temperature, salinity, and bottom shear stress explained significant genotypic variation for both outlier and non-outlier loci across populations of turbot (*Scophthalmus maximus*), there were additional environmental factors that were significant for outlier genotypes, but not neutral loci (Vandamme et al. 2014). These studies address the differences in patterns and scales of structuring we may expect for neutral and adaptive genomic variation over space, however, patterns and scales over time are yet to be addressed (see Sect. 6.2).

5.2 Origins of Adaptive Genomic Variants in Marine Populations

It has been suggested that the high dispersal of many marine organisms may reduce the potential for local selection (Slatkin 1985; Lenormand 2002) because locally adapted individuals may be swamped by immigration and/or are unlikely to migrate to an environment they are adapted to. However, theoretical models and empirical evidence, including several recent seascape genomics studies, now suggest that local adaptations can be maintained despite high gene flow (reviewed in Tigano and Friesen 2016). In fact, seascape studies suggest that gene flow, and metapopulation structures, do not have a strong bearing on adaptive outcomes necessarily. For example, spatially structured and environmentally associated adaptive genomic variation has been proposed for panmictic metapopulations (e.g. flounder, *Paralichthys dentatus*, Hoey and Pinsky 2018; goby, *Psammogobius knysnaensis*, Teske et al. 2019), stepping stone metapopulations (e.g. abalone, *Haliotis laevigata*, Sandoval-Castillo et al. 2018), populations connected via contemporary larval dispersal (e.g. sea cucumber, Xuereb et al. 2018a, b), and peripherally isolated and small populations of Atlantic Salmon (Sylvester et al. 2018). Thus, seascape genomics studies increasingly suggest selection as the major driver of spatial genomic structuring in the ocean, irrespective of migration and drift.

Knowledge about metapopulation structure and gene flow may help us understand the potential sources of adaptive variation (e.g. standing genetic variation, new mutations, or adaptive introgression; Tigano and Friesen 2016) and therefore the mechanisms by which adaptive divergence occurs. For instance, in their study of a greenlip abalone metapopulation maintained by stepping stone migration, Sandoval-Castillo et al. (2018) hypothesized that the main source of adaptive variation was standing variation from which local genetic variants were segregating. At the other end of the spectrum, even in the presence of strong drift and low gene flow, Sylvester et al. (2018) suggest that adaptation of Atlantic salmon to their peripheral environment was possible due to sufficient standing variation being present in the population. Further suggestion for the importance of standing genetic variation and parallel adaptation in the marine environment is provided by the paired contrast studies of Ravinet et al. (2016) and Westram et al. (2014) for adjacent ecomorphs in the littorine snail and for open water and coastal anchovies (Le Moan et al. 2016). In both study systems, outlier loci had consistent habitat associations, suggesting that despite the differences in the study species dispersal potential (littorine snail are direct developers and anchovy are a mobile fish with planktonic larvae), shared variation may often be used in parallel across habitats. Based on these studies, it seems likely that the large effective population sizes, and therefore high levels of genetic diversity maintained by marine organisms, equips them with sufficient standing genetic variation to adapt to heterogeneous environments, and even spatially segregated, but similar environments. However, we still have much to learn about the process of adaptation in the ocean, and spurious genotype by environment associations arising from undescribed linkage cannot be discounted (see Bierne et al. 2013 and Sect. 6.2 for an extended discussion).

Many studies have now identified loci putatively showing adaptive divergence with environment, despite the maintenance of high gene flow and consequently low neutral genomic structure (e.g. Schmidt and Rand 1999; Gagnaire et al. 2012; De Wit and Palumbi 2013; Pespeni and Palumbi 2013; Therkildsen et al. 2013; Benestan et al. 2016; Gleason and Burton 2016; Hoey and Pinsky 2018; Sandoval-Castillo et al. 2018; Dalongeville et al. 2018b; Xuereb et al. 2018b). For such disparate patterns of neutral and adaptive genetic structure to be maintained, spatial balancing selection must be occurring within in each generation. For instance, Benestan et al. (2016) suggest that selection in American lobster populations is mainly driven by minimum temperatures encountered by larval or benthic stages. In the case of summer flounder, Hoey and Pinsky (2018) suggest that selection following dispersal in each generation appears to be the most likely process maintaining genetic differentiation with relation to bottom temperature. The authors recommend that evidence for this selective bottleneck within each generation could be provided by comparing allele frequencies of larval summer flounder to those in surviving adults, where the larval metapopulations would be expected to be genetically homogenous, whereas the adult population would differentiate according to the previously described genetic-environment associations. If verified across several studies, this high gene flow maintained by many marine organisms, coupled with the efficacy of spatially varying selection drawing upon large amounts of standing genetic variation, and the potential prevalence of polygenic adaptation enabling individuals to attain fitness through several genes of small effect (rather than having one allele or gene of consequence; e.g. Xuereb et al. 2018b; but see Yeaman 2015) would contribute to marine populations maintaining adaptive polymorphisms and having great adaptive potential across varying environments.

5.3 Understanding the Environmental Sources of Adaptive Divergence in Marine Populations

Seascape genomics studies have identified potential environmental predictors of adaptive genetic variation including sea surface temperature (e.g. acorn barnacle, *Semibalanus balanoides*, Véliz et al. 2004; American lobster, Benestan et al. 2016; red abalone, *Haliotis rufescens*, de Wit and Palumbi 2013; greenlip abalone, Sandoval-Castillo et al. 2018; Atlantic salmon, Sylvester et al. 2018 and references therein), sea bottom temperature (e.g. summer flounder, Hoey and Pinsky 2018), salinity (e.g. Atlantic salmon, *Salmo salar*, Bourret et al. 2013; European sea bass, *Dicentrarchus labrax*, Tine et al. 2014; Mediterranean striped red mullet, Dalongeville et al. 2018b), bathymetry (fish tusk, *Brosme brosme*, Knutsen et al. 2009), depth (cod, *Gadus morhua*, Case et al. 2005), and oxygen concentration (e.g. greenlip abalone, Sandoval-Castillo et al. 2018), to name a few. A recent study has further suggested that the same species across different seascapes may have different determinants of spatial selection. Xuereb et al. (2018b) determined that

over the broadest spatial extent adaptive genomic variation for the Californian sea cucumber was associated with mean sea bottom temperature; whereas within a subregion of the study extent, adaptive genomic variation was further structured by surface salinity and current velocity. Thus, these data suggest that despite evidence and potential for parallel adaptive evolution, associations between environmental predictors and an adaptive response in one region may not be transferable across a species full range.

Nonetheless, for the scales and species studied to date, the most investigated and potentially important predictor of spatial patterns in adaptive genomic variation is ocean temperature (as described in Oleksiak 2019). Water temperature is renowned for being an important factor in determining a species' niche (Angilletta and Angilletta 2009). Ambient temperature determines the aerobic scope (Pörtner and Knust 2007), metabolism (Johnston and Dunn 1987), development (O'Connor et al. 2007), and other physiological processes for many marine organisms; therefore, it is not surprising that it may be one of the most important determinants of environmentally mediated selection in the ocean. Ocean temperatures are also one of the most monitored features of the seascape, as future temperature changes are expected under climate change. Consequently, understanding the potential impacts of changing ocean temperatures, and the potential, and genetic basis for local adaptation to a changed temperature regime has been a popular research pursuit (Savolainen et al. 2013). Seascape genomics studies have found different aspects of local temperature regimes to be important. Although mean sea surface temperature is the most investigated, minimum sea surface temperature (e.g. abalone, de Wit and Palumbi 2013) and mean bottom temperature (e.g. sea cucumber, Xuereb et al. 2018b) also have relevance for some organisms, and temperature ranges including warm extremes were important for a peripheral population of Atlantic salmon (Sylvester et al. 2018).

For both temperature and salinity (the second most investigated environmental variable in seascape genomics studies), there are intuitive biological reasons for why they exert selective pressure on marine organisms. Nonetheless, identifying an association between allele frequencies and values of a seascape variable does not confirm a causal relationship. Our inferences are biased by, and restricted to, the environmental variables that are quantified from the seascape and will not include all potentially correlated variables. The matching of putatively adaptive loci to genomic regions and specific genes on an annotated reference can provide some support (Manel et al. 2016). Several studies have matched outlier loci to genes involved in osmoregulation (reviewed for teleosts in Dennenmoser et al. 2017; e.g. Dalongeville et al. 2018b) and thermal adaptation (e.g. Benestan et al. 2016) for example. However, a convincing case for causation requires the demonstration of a mechanistic link between the environmental variable and locus/loci (either neutral or selective) via functional genomics, reciprocal transplantation, or common garden experiments (Feder and Mitchell-Olds 2003; Lowry and Willis 2010). For example, in mussels (*Mytilus edulis*), there is a clear functional link between the *Lap* locus, fitness of individual mussels according to their salinity environment, and resulting spatial genetic patterns (from the work of Koehn et al. 1980; Koehn and Siebenaller

1981). Lastly, endogenous reproductive incompatibilities are common in the sea and can maintain genetic divergence for a given locus or linkage group. Such signatures of reproductive isolation may be difficult to distinguish from environmentally mediated selection and often segregate with environmental features (Bierne et al. 2011). Until we understand linkage across the genome better (see Sect. 6.2), our inferences of local adaptation and spatial balancing selection in the ocean (see Sect. 5.2) and their underlying causes will need to remain cautious.

6 Conclusions and Future Perspectives

Seascape genomics is a rapidly developing field of inference leveraging technological and analytical advances of several related disciplines. Early seascape genomics studies have affirmed our intuition that using more numerous and genome-wide markers provide researchers with higher resolution to detect genomic structuring across space and in relation to the environment. These studies have mostly corroborated the findings regarding metapopulation structures of marine organisms based on earlier seascape genetic studies that have used lower numbers of markers. However, seascape genomics studies have additionally revealed patterns of adaptive divergence that are often over much smaller spatial scales than neutral genomic divergence. Our early interpretations of seascape genomic patterns suggest selection may be a major driver of spatial genomic structuring in the marine environment – indicating that we should potentially expect a reshuffling of the genomic composition of populations in response to new environmental regimes over very short periods of time.

Our understanding of the genomic architectures of marine organisms, our ability to statistically evaluate patterns, and therefore our inference of adaptive processes within marine populations will improve in years to come especially as more complete genomes become resolved. To corroborate our early seascape genomics findings and enhance the purview and relevance of seascape genomics, below we highlight some avenues for future seascape genomics research, both in the eco-evolutionary sciences and research agendas relevant to global issues affecting marine biodiversity.

6.1 Mapping Adaptive Genomic Variation

A potential issue that has not been widely acknowledged in landscape genomics is how linkage blocks, including chromosomal inversions, can affect interpretations regarding selection. Because of the rarity of well-assembled genomes and genetic maps for marine species, seascape genomics studies to date have largely made the simplifying assumption that surveyed loci are unlinked. Yet, it is increasingly appreciated that islands of differentiation (Wolf and Ellegren 2017) and

chromosomal inversions may be common and potentially play a key role in adaptive evolution (Wellenreuther and Bernatchez 2018). For example, Bradbury et al. (2013) showed that inferred outlier loci for Atlantic cod (*Gadus morhua*) were highly clustered within three chromosomes. The repression of recombination in these inverted chromosomal regions meant that oceanic and coastal cod populations were able to adapt to local oxygen, temperature, and salinity regimes despite high levels of gene flow (Sodeland et al. 2016). When loci are unmapped, such linkage relationships among loci are unlikely to be recognized. Instead, many loci, incorrectly assumed to be unlinked, may appear to be under selection, especially showing genetic-environment associations (Bierne et al. 2013, as observed with sea horses, *Hippocampus guttulatus*: Riquet et al. 2019) or caught up with weak barriers (Gagnaire et al. 2015, as in sea bass, *Dicentrarchus labrax*: Duranton et al. 2018). Although creating genetic maps via multigenerational breeding experiments is very difficult, some clever alternatives to uncover linkage relationships are now being explored, for example, using trios of offspring and parents (Duranton et al. 2018) or using alignment to scaffolds of closely related species (Riquet et al. 2019). Future investigations that comprehensively combine knowledge of genome structures with seascape features are sure to yield nuanced insights regarding the maintenance of adaptive polymorphisms in spite of high gene flow and how different genomic regions respond to selective pressures and dispersal barriers.

6.2 Resolving the Importance and Scale of Chaotic Genetic Patchiness

Given the common observation of chaotic genetic patchiness among putatively neutral loci (such as microsatellites), it is clear that many marine populations are unlikely to approximate a migration-drift equilibrium (see Sect. 3.1). This observation suggests that single time-point sampling (as is typical for seascape genomics) may yield anomalous or non-replicable results such as suggesting dispersal barriers or populations with atypical allele frequencies, erroneously suggestive of adaptive processes (Lotterhos and Whitlock 2014; Gaggiotti 2017). However, seascape thinking can contribute to investigations of chaotic genetic patchiness. For example, if sampled genetic cohorts could be associated with the relevant oceanographic conditions during which dispersal occurred, it might be possible to make (and test) predictions arising directly from these oceanographic conditions, where replication across time (different cohorts each with time-of-dispersal appropriate predictions) would strengthen these inferences. Even if no convincing associations between oceanography and population genetic structure are uncovered, simply determining the scales over which chaotic genetic patchiness occurs (and for which species) and the potential implications of this phenomenon for our inference of outlier loci would be a beneficial contribution to our seascape genomics understanding.

It is also possible that selective pressures themselves may be highly variable in time, and, therefore, adaptive advantages of specific alleles could vary with time too, leading to a perceived pattern of spatio-temporal chaotic genetic patchiness in some situations. Seascape genomics studies have already evidenced their potential to uncover genetic-environment associations over much smaller spatial scales than previously anticipated, or expected, in high gene flow species. Now, the question is – at what spatio-temporal scale does chaos turn into order? It might be possible to link sources of selection to locus-specific responses by considering environmental variables at the spatio-temporal resolution of the perceived chaos. We only know of a few studies that have repeated outlier analyses for different cohorts (cardinalfish, *Siphamia tubifer*, Gould and Dunlap 2017; lobster, *Jasus edwardsii*, Villacorta-Rath et al. 2018; gobies, *Bathygobius cocosensis*, Thia et al. pers. comm) with all finding that most outlier loci differ by timepoint. Resolving whether temporally variable outliers are simply artefacts of dispersal and drift or whether selective pressures differ by time and place will require a carefully conceived spatio-temporal sampling approach. Nonetheless, understanding *how* nonstationary is normal for chaotic genetic patchiness will be critical for decisively inferring a change in the neutral-adaptive seascape.

6.3 More Seascapes and More Species

The field of seascape genomics, by definition, is inclusive of all seascapes and constituent species. The majority of population genomics studies in the marine environment still focus on the nearshore and near-surface periphery of the oceans (Riginos et al. 2016). In part, this is due to our dependency on having seascape data derived by local and accessible static or shipboard instrumentation or from remote-sensing products and oceanographic models. However, whereas there was similarly a bias towards species of fisheries and commercial interest in the early stages of the genomics era (possibly due to the availability of genetic and funding resources), there are now more diverse taxonomic groups being studied (e.g. gobies, Teske et al. 2019; Thia et al. pers. comm; cardinalfish, Gould and Dunlap 2017). Nevertheless, "seascape genomics" – to study how spatial dependence and environmental features in the ocean influence the geographic structure of genomic patterns in marine organisms – could be literally taken to mean replicate sampling across the seascape at the level of *genomes*. Although such multispecies approaches have been very few (but see Bongaerts et al. 2017), this approach has been described as "Landscape Community Genomics" (LCG, Hand et al. 2015). LCG is interested in assessing how seascape features may influence species similarly, or differently, including their interactions and, therefore, how neutral and adaptive processes within species may influence community composition and ultimately ecosystem functioning.

Multispecies comparisons can uncover spatial factors that have broad scale effects across ecological communities, in that shared patterns across species could filter out short-term chaotic variability. Multispecies seascape genetics studies have

suggested that incongruences in neutral genetic patterns are common with the likely reasons suggested to be differences in species biology and demography (Ayre et al. 2009; Dawson et al. 2014; Bongaerts et al. 2017); however, consistencies are also observed particularly over large geographic scales (Pelc et al. 2009; Toonen et al. 2011; Gaither and Rocha 2013; Liggins et al. 2016). Considering patterns that may be scalable across the biological hierarchy (e.g. DeBoer et al. 2014; Crandall et al. 2019) and including adaptive variation is a natural extension of earlier multispecies studies. For instance, in a recent seascape genomics study of a South African goby, despite homogeneity across the study extent based on putatively neutral loci, the outlier loci showed regional divergence aligned with known biogeographic disjunctions and breaks for intraspecific lineages (Teske et al. 2019). The outlier loci sat in temperature-related gene regions, conforming with conventional wisdom that the biogeographic disjunction was related to temperature. Whether such scaling in divergence patterns across the biological hierarchy is common, and whether the putatively adaptive loci for other co-distributed species reveal some commonalities in terms, spatial divergence or related gene function remains to be investigated.

Arguably, "seascape genomics" could also be as much about the *seascape* as it is the genomics. In the most obvious sense, this could be thought of as studying spatially explicit seascape features that are replicated, either through space or time, such as spatial environmental gradients or upwelling events through time. Such seascape genomics studies are already underway for individual species, but multispecies studies where seascape features are replicated have been underutilized (but see Bongaerts et al. 2017). Coordinated global research in future may enable truly replicated studies where large-scale gradients are the replicates, as well as species. Replicate sampling of the seascape could also involve co-sampling of species in parameter space rather than geographic space (a spatially implicit approach, e.g. Riginos et al. 2011). This parameter space could be defined individually for each species and may represent a species' environmental niche space where, for example, core populations, trailing edge populations, and leading edge populations could be classified according to the species environmental envelope, but without regard to geographic space. Alternatively, a demographic-environmental space as described in Box 1 could be defined for multiple species, whereby two species sampled from the same geographic location may sit at different positions in the parameter space depending on the inferred immigration and retention values for each species at that location. Such spatially implicit approaches may help moderate or appropriately incorporate (depending on the study objectives) the differences in species-specific ecologies (for niche-based approaches) and demographies (for the coupled demographic-environment approach) providing more power to address the focal seascape features.

6.4 Seascape Genomics as an Integrated and Applied Discipline

Seascape genomics studies have most often been motivated by the evolutionary and ecological sciences, in our pursuit of questions not otherwise answerable for certain taxa. Looking forward, as our ability to measure genomic variation and to quantify and model the seascape improves, seascape genomics will likely become a more diverse and broadly used discipline. In particular, we anticipate greater application in the conservation and applied sciences such as fisheries (Bernatchez et al. 2017) and in addressing issues of global change. Marine organisms that we rely upon, and that make up our environment, are threatened by overfishing, pollution, and climate change (to name a few; McCauley et al. 2015). In theory, seascape genomics can help us both characterize the responses of marine organisms to these threats and infer their resiliency or adaptive potential (Kelley et al. 2016). Based on our intuition from seascape genomics studies conducted to date, marine species may be well equipped for responding to altered selective regimes, including those arising from humans (Allendorf et al. 2010), and adaptive potential may be high (Hoey and Pinsky 2018). The real application of seascape genomics to these important threats will likely be the ultimate test of this intuition.

Evidence that we can detect the impacts of fishing on marine populations (Allendorf et al. 2008; Pinsky and Palumbi 2014; reviewed in Bernatchez et al. 2017) provides a proof of concept that seascape genomics may be a means to detect and monitor impacts of fishing and other threats to the persistence of natural populations. Seascape genomics studies conducted to date have provided information relevant to the (re)definition of fishery stocks (e.g. greenlip abalone, Sandoval-Castillo et al. 2018; North Atlantic saithe, *Pollachius virens*, Saha et al. 2015) and to inform aquaculture and restocking of wild populations (e.g. for the greenlip abalone). In particular, a coupled biophysical model-population genomics approach may be valuable in defining stocks, their metapopulation (stock) structures, and therefore their resilience and short-term adaptive potential (Baltazar-Soares et al. 2018). Furthermore, continued monitoring of such populations following harvesting, farming, and restocking would provide a valuable time series, from which impacts of environmental changes to the seascape and/or management practices could be measured. Such an approach would both help seascape genomics become more prospective as a field and would help our fisheries and aquaculture industries actively manage genetic diversity and evolutionary incentives alongside other industry priorities.

If we can detect selection occurring within generations, we should be able to detect shifting and detrimental selective regimes related to the threats facing marine organisms over short timescales. This would require a focus on seascape variables that are relevant to the stressors and acquiring relevant spatio-temporal data. For example, in Box 1, we derive the demographic-environmental seascape parameter space for a potentially range-extending species, designed to inform population sampling looking for a genomic response – neutral or adaptive – to a

contemporaneous change in the seascape (i.e. sea surface temperature). Knowledge regarding genomic responses to shifts in our environment, and the underlying processes, may enable predictions regarding the distribution and abundance of species in future climate scenarios (Hansen et al. 2012; Sylvester et al. 2018). It has been suggested that current genetic-environmental associations could be used to inform translocations, captive breeding programmes, and the design of pre-adapted populations for future climate scenarios (Manel et al. 2018; Ralls et al. 2018). Similarly, taking adaptive genomic variation and its association with the seascape into conservation decision-making surrounding species management (Flanagan et al. 2018; Hoffmann et al. 2015) and marine protected area networks has been widely discussed (von der Heyden 2017).

Unquestionably the more applied and integrative that seascape genomics becomes – receiving input from disparate disciplines – the more useful and diverse the field will be. A seascape genomics approach would certainly add value as part of the toolkit to help manage our global challenges in biodiversity, food security, and environmental sustainability. However, for seascape genomics to keep step with these possibilities, it requires robust study design, transparent filtering, analyses, and interpretation, thus enabling findings to be reputable and defensible as we would expect for any other data-rich and crucially important scientific discipline. To realize this goal, there will need to be a focus on defining applicable deliverables and communicating these effectively to a broad variety of specialists in other fields and occupations (Shafer et al. 2015).

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References

Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N. Genetic effects of harvest on wild animal populations. Trends Ecol Evol. 2008;23:327–37.

Allendorf FW, Hohenlohe PA, Luikart G. Genomics and the future of conservation genetics. Nat Rev Genet. 2010;11:697.

Almany GR, Berumen ML, Thorrold SR, Planes S, Jones GP. Local replenishment of coral reef fish populations in a marine reserve. Science. 2007;316:742–4.

Anderson CD, Epperson BK, Fortin MJ, Holderegger R, James PM, Rosenberg MS, Scribner KT, et al. Considering spatial and temporal scale in landscape-genetic studies of gene flow. Mol Ecol. 2010;19:3565–75.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. Harnessing the power of RADseq for ecological and evolutionary genomics. Nat Rev Genet. 2016;17:81–92.

Angilletta MJ Jr, Angilletta MJ. Thermal adaptation: a theoretical and empirical synthesis. Oxford: Oxford University Press; 2009.

Avise JC. Phylogeography: the history and formation of species. Cambridge: Harvard University Press; 2000.

Ayre DJ, Minchinton TE, Perrin C. Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? Mol Ecol. 2009;18:1887–903.

Balkenhol N, Fortin M-J. Basics of study design: sampling landscape heterogeneity and genetic variation for landscape genetic studies. In: Balkenhol N, Cushman S, Storfer A, Waits L, editors. Landscape genetics: concepts, methods, applications. West Sussex: Wiley; 2016. p. 58–75.

Balkenhol N, Cushman S, Storfer A, Waits LP. Introduction to landscape genetics: defining, learning and applying an interdisciplinary field. In: Balkenhol N, Cushman S, Storfer A, Waits L, editors. Landscape genetics: concepts, methods, applications. West Sussex: Wiley; 2016a. p. 1–17.

Balkenhol N, Cushman S, Storfer A, Waits LP. Landscape genetics: concepts, methods, applications. West Sussex: Wiley; 2016b.

Balkenhol N, Dudaniec RY, Krutovsky KV, Johnson JS, Cairns DM, Segelbacher G, Selkoe KA, et al. Landscape genomics: understanding relationships between environmental heterogeneity and genomic characteristics of populations. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 261–322.

Baltazar-Soares M, Hinrichsen HH, Eizaguirre C, Handling editor: Mikko Heino. Integrating population genomics and biophysical models towards evolutionary-based fisheries management. ICES J Mar Sci. 2018;75:1245–57.

Barth JM, Berg PR, Jonsson PR, Bonanomi S, Corell H, Hemmer-Hansen J, Jakobsen KS, Johannesson K, Jorde PE, Knutsen H, Moksnes PO. Genome architecture enables local adaptation of Atlantic cod despite high connectivity. Mol Ecol. 2017;26:4452–66.

Barton NH. Gene flow past a cline. Heredity. 1979;43:333.

Bay RA, Harrigan RJ, Le Underwood V, Gibbs HL, Smith TB, Ruegg K. Genomic signals of selection predict climate-driven population declines in a migratory bird. Science. 2018;359:83–6.

Benestan L, Quinn BK, Maaroufi H, Laporte M, Clark FK, Greenwood SJ, Rochette R, et al. Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (*Homarus americanus*). Mol Ecol. 2016;25:5073–92.

Bernatchez L. On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. J Fish Biol. 2016;89:2519–56.

Bernatchez L, Wellenreuther M, Araneda C, Ashton DT, Barth JM, Beacham TD, Maes GE, et al. Harnessing the power of genomics to secure the future of seafood. Trends Ecol Evol. 2017;32:665–80.

Bi K, Vanderpool D, Singhal S, Linderoth T, Moritz C, Good JM. Transcriptome-based exon capture enables highly cost-effective comparative genomic data collection at moderate evolutionary scales. BMC Genomics. 2012;13:403.

Bierne N, Welch J, Loire E, Bonhomme F, David P. The coupling hypothesis: why genome scans may fail to map local adaptation genes. Mol Ecol. 2011;20:2044–72.

Bierne N, Gagnaire PA, David P. The geography of introgression in a patchy environment and the thorn in the side of ecological speciation. Curr Zool. 2013;59:72–86.

Bierne N, Bonhomme F, Arnaud-Haond S. Editorial dedicated population genomics for the silent world: the specific questions of marine population genetics. Curr Zool. 2016;62:545–50.

Blanchet FG, Legendre P, Maranger R, Monti D, Pepin P. Modelling the effect of directional spatial ecological processes at different scales. Oecologia. 2011;166:357–68.

Bode M, Leis JM, Mason LB, Williamson DH, Harrison HB, Choukroun S, Jones GP. Successful validation of a larval dispersal model using genetic parentage data. PLoS Biol. 2019;17: e3000380.

Bonanomi S, Pellissier L, Therkildsen NO, Hedeholm RB, Retzel A, Meldrup D, Olsen SM, et al. Archived DNA reveals fisheries and climate induced collapse of a major fishery. Sci Rep. 2015;5:15395.

Bongaerts P, Riginos C, Brunner R, Englebert N, Smith SR, Hoegh-Guldberg O. Deep reefs are not universal refuges: reseeding potential varies among coral species. Sci Adv. 2017;3:e1602373.

Borcard D, Legendre P, Drapeau P. Partialling out the spatial component of ecological variation. Ecology. 1992;73:1045–55.

Bourret V, Kent MP, Primmer CR, Vasemägi A, Karlsson S, Hindar K, et al. SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). Mol Ecol. 2013;22:532–51.

Bradbury IR, Hubert S, Higgins B, Bowman S, Borza T, Paterson IG, et al. Genomic islands of divergence and their consequences for the resolution of spatial structure in an exploited marine fish. Evol Appl. 2013;6:450–61.

Campbell NR, Harmon SA, Narum SR. Genotyping-in-thousands by sequencing (GT-seq): a cost effective SNP genotyping method based on custom amplicon sequencing. Mol Ecol Resour. 2014;15(4):855–67.

Capblancq T, Luu K, Blum MG, Bazin E. Evaluation of redundancy analysis to identify signatures of local adaptation. Mol Ecol Resour. 2018;18:1223–33.

Case RA, Hutchinson WF, Hauser L, Van Oosterhout C, Carvalho GR. Macro-and micro-geographic variation in pantophysin (PanI) allele frequencies in NE Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser. 2005;301:267–78.

Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G, Dickson M, Grimwood J, Schmutz J, et al. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science. 2005;307:1928–33.

Cowen RK, Sponaugle S. Larval dispersal and marine population connectivity. Ann Rev Mar Sci. 2009;1:443–66.

Crandall ED, Treml EA, Barber PH. Coalescent and biophysical models of stepping-stone gene flow in neritid snails. Mol Ecol. 2012;21:5579–98.

Crandall ED, Riginos C, Bird CE, Liggins L, Treml E, Beger M, Barber PH, Connolly SR, Cowman PF, DiBattista JD, Eble JA. The molecular biogeography of the Indo-Pacific: testing hypotheses with multispecies genetic patterns. Glob Ecol Biogeogr. 2019;28:943–60.

D'Aloia CC, Bogdanowicz SM, Francis RK, Majoris JE, Harrison RG, Buston PM. Patterns, causes, and consequences of marine larval dispersal. Proc Natl Acad Sci U S A. 2015;112:13940–5.

Dale MR, Fortin MJ. Spatial analysis: a guide for ecologists: Cambridge University Press; 2014.

Dalongeville A, Andrello M, Mouillot D, Lobreaux S, Fortin MJ, Lasram F, Belmaker J, Rocklin D, Manel S. Geographic isolation and larval dispersal shape seascape genetic patterns differently according to spatial scale. Evol Appl. 2018a;11:1437–47.

Dalongeville A, Benestan L, Mouillot D, Lobreaux S, Manel S. Combining six genome scan methods to detect candidate genes to salinity in the Mediterranean striped red mullet (*Mullus surmuletus*). BMC Genomics. 2018b;19:217.

Davey JW, Blaxter ML. RADSeq: next-generation population genetics. Brief Funct Genomics. 2010;9:416–23.

Davies SW, Treml EA, Kenkel CD, Matz MV. Exploring the role of Micronesian islands in the maintenance of coral genetic diversity in the Pacific Ocean. Mol Ecol. 2015;24:70–82.

Dawson MN, Hays CG, Grosberg RK, Raimondi PT. Dispersal potential and population genetic structure in the marine intertidal of the eastern North Pacific. Ecol Monogr. 2014;84:435–56.

de Villemereuil P, Gaggiotti OE. A new FST-based method to uncover local adaptation using environmental variables. Methods Ecol Evol. 2015;6:1248–58.

de Villemereuil P, Frichot É, Bazin É, François O, Gaggiotti OE. Genome scan methods against more complex models: when and how much should we trust them? Mol Ecol. 2014;23:2006–19.

de Wit P, Palumbi SR. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Mol Ecol. 2013;22:2884–97.

de Wit P, Pespeni MH, Ladner JT, Barshis DJ, Seneca F, Jaris H, Therkildsen NO, Morikawa M, Palumbi SR. The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. Mol Ecol Resour. 2012;12:1058–67.

DeBoer TS, Naguit MR, Erdmann MV, Ablan-Lagman MC, Carpenter KE, Toha AH, Barber PH. Concordance between phylogeographic and biogeographic boundaries in the Coral Triangle: conservation implications based on comparative analyses of multiple giant clam species. Bull Mar Sci. 2014;90:277–300.

Dennenmoser S, Vamosi SM, Nolte AW, Rogers SM. Adaptive genomic divergence under high gene flow between freshwater and brackish-water ecotypes of prickly sculpin (*Cottus asper*) revealed by Pool-Seq. Mol Ecol. 2017;26:25–42.

Diopere E, Vandamme SG, Hablützel PI, Cariani A, Van Houdt J, Rijnsdorp A, Tinti F, FishPopTrace Consortium, Volckaert FA, Maes GE. Seascape genetics of a flatfish reveals local selection under high levels of gene flow. ICES J Mar Sci. 2017;75:675–89.

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JR, et al. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography. 2013;36:27–46.

Dray S, Legendre P, Peres-Neto PR. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol Model. 2006;196:483–93.

Duforet-Frebourg N, Bazin E, Blum MG. Genome scans for detecting footprints of local adaptation using a Bayesian factor model. Mol Biol Evol. 2014;31:2483–95.

Duranton M, Allal F, Fraïsse C, Bierne N, Bonhomme F, Gagnaire PA. The origin and remolding of genomic islands of differentiation in the European sea bass. Nat Commun. 2018;9:2518.

Dyer RJ. Is there such a thing as landscape genetics? Mol Ecol. 2015;24:3518–28.

Dyer RJ, Nason JD. Population graphs: the graph theoretic shape of genetic structure. Mol Ecol. 2004;13:1713–27.

Dyer RJ, Nason JD, Garrick RC. Landscape modelling of gene flow: improved power using conditional genetic distance derived from the topology of population networks. Mol Ecol. 2010;19:3746–59.

Eaton DA. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics. 2014;30:1844–9.

Eldon B, Riquet F, Yearsley J, Jollivet D, Broquet T. Current hypotheses to explain genetic chaos under the sea. Curr Zoo. 2016;62:551–66.

Epling C, Dobzhansky T. Genetics of natural populations. VI. Microgeographic races in *Linanthus parryae*. Genetics. 1942;27:317.

Epperson BK. Geographical genetics (MPB-38). Princeton: Princeton University Press; 2003.

Ewers-Saucedo C, Pringle JM, Sepúlveda HH, Byers JE, Navarrete SA, Wares JP. The oceanic concordance of phylogeography and biogeography: a case study in *Notochthamalus*. Ecol Evol. 2016;6:4403–20.

Excoffier L, Hofer T, Foll M. Detecting loci under selection in a hierarchically structured population. Heredity. 2009;103:285.

Faircloth BC, McCormack JE, Crawford NG, Harvey MG, Brumfield RT, Glenn TC. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Syst Biol. 2012;61:717–26.

Faurby S, Barber PH. Theoretical limits to the correlation between pelagic larval duration and population genetic structure. Mol Ecol. 2012;21:3419–32.

Feder ME, Mitchell-Olds T. Evolutionary and ecological functional genomics. Nat Rev Genet. 2003;4:649.

Fitzpatrick MC, Keller SR. Ecological genomics meets community-level modelling of biodiversity: mapping the genomic landscape of current and future environmental adaptation. Ecol Lett. 2015;18:1–6.

Flanagan SP, Forester BR, Latch EK, Aitken SN, Hoban S. Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. Evol Appl. 2018;11:1035–52.

Forester BR, Jones MR, Joost S, Landguth EL, Lasky JR. Detecting spatial genetic signatures of local adaptation in heterogeneous landscapes. Mol Ecol. 2016;25:104–20.

Forester BR, Lasky JR, Wagner HH, Urban DL. Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. Mol Ecol. 2018;27:2215–33.

Foster NL, Paris CB, Kool JT, Baums IB, Stevens JR, Sanchez JA, Bastidas C, et al. Connectivity of Caribbean coral populations: complementary insights from empirical and modelled gene flow. Mol Ecol. 2012;21:1143–57.

François O, Waits LP. Clustering and assignment methods in landscape genetics. In: Balkenhol N, Storfer A, Cushman SA, Waits LP, editors. Landscape genetics: concepts, methods, applications. Wiley: Chichester; 2015. p. 114–28.

François O, Martins H, Caye K, Schoville SD. Controlling false discoveries in genome scans for selection. Mol Ecol. 2016;25:454–69.

Frichot E, Schoville SD, Bouchard G, François O. Testing for associations between loci and environmental gradients using latent factor mixed models. Mol Biol Evol. 2013;30:1687–99.

Frichot E, Schoville SD, de Villemereuil P, Gaggiotti OE, François O. Detecting adaptive evolution based on association with ecological gradients: orientation matters! Heredity. 2015;115:22.

Gaggiotti OE. Metapopulations of marine species with larval dispersal: a counterpoint to Ilkka's Glanville fritillary metapopulations. Ann Zool Fenn. 2017;54(1–4):97–113. Finnish Zoological and Botanical Publishing Board

Gagnaire PA, Gaggiotti OE. Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. Curr Zoo. 2016;62:603–16.

Gagnaire PA, Normandeau E, Côté C, Hansen MM, Bernatchez L. The genetic consequences of spatially varying selection in the panmictic American eel (*Anguilla rostrata*). Genetics. 2012;190:725–36.

Gagnaire PA, Broquet T, Aurelle D, Viard F, Souissi A, Bonhomme F, Arnaud-Haond S, et al. Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. Evol Appl. 2015;8:769–86.

Gaither MR, Rocha LA. Origins of species richness in the Indo-Malay-Philippine biodiversity hotspot: evidence for the centre of overlap hypothesis. J Biogeogr. 2013;40:1638–48.

Galindo HM, Olson DB, Palumbi SR. Seascape genetics: a coupled oceanographic-genetic model predicts population structure of Caribbean corals. Curr Biol. 2006;16:1622–6.

Gerlach G, Atema J, Kingsford MJ, Black KP, Miller-Sims V. Smelling home can prevent dispersal of reef fish larvae. Proc Natl Acad Sci U S A. 2007;104:858–63.

Getis A, Ord JK. The analysis of spatial association by use of distance statistics. In: Perspectives on spatial data analysis. Berlin: Springer; 2010. p. 127–45.

Gleason LU, Burton RS. Genomic evidence for ecological divergence against a background of population homogeneity in the marine snail *Chlorostoma funebralis*. Mol Ecol. 2016;25:3557–73.

Gould AL, Dunlap PV. Genomic analysis of a cardinalfish with larval homing potential reveals genetic admixture in the Okinawa Islands. Mol Ecol. 2017;26:3870–82.

Grummer JA, Beheregaray LB, Bernatchez L, Hand BK, Luikart G, Narum SR, et al. Aquatic landscape genomics and environmental effects on genetic variation. Trends Ecol Evol. 2019;34 (7):641–54.

Guillot G, Rousset F. Dismantling the Mantel tests. Methods Ecol Evol. 2013;4:336–44.

Guillot G, Leblois R, Coulon A, Frantz AC. Statistical methods in spatial genetics. Mol Ecol. 2009;18:4734–56.

Guillot G, Vitalis R, le Rouzic A, Gautier M. Detecting correlation between allele frequencies and environmental variables as a signature of selection. A fast computational approach for genome-wide studies. Spat Stat. 2014;8:145–55.

Günther T, Coop G. Robust identification of local adaptation from allele frequencies. Genetics. 2013;195:205–20.

Guo B, DeFaveri J, Sotelo G, Nair A, Merilä J. Population genomic evidence for adaptive differentiation in Baltic Sea three-spined sticklebacks. BMC Biol. 2015;13:19.

Gutenkunst RN, Hernandez RD, Williamson SH, Bustamante CD. Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. PLoS Genet. 2009;5:e1000695.

Hand BK, Lowe WH, Kovach RP, Muhlfeld CC, Luikart G. Landscape community genomics: understanding eco-evolutionary processes in complex environments. Trends Ecol Evol. 2015;30:161–8.

Hansen MM, Hemmer-Hansen J. Landscape genetics goes to sea. J Biol. 2007;6:6.
Hansen MM, Olivieri I, Waller DM, Nielsen EE, GeM Working Group. Monitoring adaptive genetic responses to environmental change. Mol Ecol. 2012;21:1311–29.
Hauser L, Carvalho GR. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 2008;9:333–62.
Hedgecock D. Does variance in reproductive success limit effective population sizes of marine organisms. Gen Evol Aqua Org. 1994;122:122–34.
Hedrick PW. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution. 1999;53:313–8.
Hellberg ME. Gene flow and isolation among populations of marine animals. Annu Rev Ecol Evol Syst. 2009;40:291–310.
Hellberg ME, Burton RS, Neigel JE, Palumbi SR. Genetic assessment of connectivity among marine populations. Bull Mar Sci. 2002;70:273–90.
Henriques R, von der Heyden S, Lipinski MR, du Toit N, Kainge P, Bloomer P, Matthee CA. Spatio-temporal genetic structure and the effects of long-term fishing in two partially sympatric offshore demersal fishes. Mol Ecol. 2016;25:5843–61.
Hoban S, Bertorelle G, Gaggiotti OE. Computer simulations: tools for population and evolutionary genetics. Nat Rev Genet. 2012;13:110.
Hoey JA, Pinsky ML. Genomic signatures of environmental selection despite near-panmixia in summer flounder. Evol App. 2018;11:1732–47.
Hoffmann A, Griffin P, Dillon S, Catullo R, Rane R, Byrne M, Jordan R, Oakeshott J, Weeks A, Joseph L, Lockhart P. A framework for incorporating evolutionary genomics into biodiversity conservation and management. Clim Change Resp. 2015;2:1.
Hohenlohe PA, Phillips PC, Cresko WA. Using population genomics to detect selection in natural populations: key concepts and methodological considerations. Int J Plant Sci. 2010;171:1059–71.
Holderegger R, Wagner HH. Landscape genetics. Bioscience. 2008;58:199–207.
Holliday JA, Hallerman EM, Haak DC. Genotyping and sequencing technologies in population genetics and genomics. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 83–126.
Jackson TM, Roegner GC, O'Malley KG. Evidence for interannual variation in genetic structure of Dungeness crab (*Cancer magister*) along the California Current System. Mol Ecol. 2018;27:352–68.
Johnson MS, Black R. Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar Biol. 1982;70:157–64.
Johnson MS, Black R. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution. 1984;38:1371–83.
Johnston IA, Dunn JE. Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. Symp Soc Exp Biol. 1987;41:67–93.
Jombart T, Pontier D, Dufour AB. Genetic markers in the playground of multivariate analysis. Heredity. 2009;102:330.
Joost S, Bonin A, Bruford MW, Després L, Conord C, Erhardt G, Taberlet P. A spatial analysis method (SAM) to detect candidate loci for selection: towards a landscape genomics approach to adaptation. Mol Ecol. 2007;16:3955–69.
Karl SA, Toonen RJ, Grant WS, Bowen BW. Common misconceptions in molecular ecology: echoes of the modern synthesis. Mol Ecol. 2012;21:4171–89.
Kelley JL, Brown AP, Therkildsen NO, Foote AD. The life aquatic: advances in marine vertebrate genomics. Nat Rev Genet. 2016;17:523–34.
Kingsford MJ, Leis JM, Shanks A, Lindeman KC, Morgan SG, Pineda J. Sensory environments, larval abilities and local self-recruitment. Bull Mar Sci. 2002;70:309–40.
Kinlan BP, Gaines SD. Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology. 2003;84:2007–20.
Knutsen H, Jorde PE, Sannæs H, Rus Hoelzel A, Bergstad OA, Stefanni S, et al. Bathymetric barriers promoting genetic structure in the deepwater demersal fish tusk (*Brosme brosme*). Mol Ecol. 2009;18:3151–62.

Koehn RK, Siebenaller JF. Biochemical studies of aminopeptidase polymorphism in *Mytilus edulis*. II. Dependence of reaction rate on physical factors and enzyme concentration. Biochem Genet. 1981;19:1143–62.

Koehn RK, Newell RI, Immermann F. Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc Natl Acad Sci U S A. 1980;77:5385–9.

Kofler R, Orozco-terWengel P, De Maio N, Pandey RV, Nolte V, Futschik A, Kosiol C, et al. PoPoolation: a toolbox for population genetic analysis of next generation sequencing data from pooled individuals. PLoS One. 2011;6:e15925.

Kool JT, Moilanen A, Treml EA. Population connectivity: recent advances and new perspectives. Landsc Ecol. 2013;28:165–85.

Landguth E, Cushman SA, Balkenhol N. Simulation modeling in landscape genetics. In: Balkenhol N, Cushman S, Storfer A, Waits L, editors. Landscape genetics: concepts, methods, applications. West Sussex: Wiley; 2016. p. 99–113.

Le Moan A, Gagnaire PA, Bonhomme F. Parallel genetic divergence among coastal–marine ecotype pairs of European anchovy explained by differential introgression after secondary contact. Mol Ecol. 2016;25:3187–202.

Le Port A, Montgomery JC, Smith AN, Croucher AE, McLeod IM, Lavery SD. Temperate marine protected area provides recruitment subsidies to local fisheries. Proc R Soc Lond B Biol Sci. 2017;284:20171300.

Legendre P, Anderson MJ. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol Monogr. 1999;69:1–24.

Legendre P, Lapointe FJ, Casgrain P. Modeling brain evolution from behavior: a permutational regression approach. Evolution. 1994;48:1487–99.

Legendre P, Fortin MJ, Borcard D. Should the Mantel test be used in spatial analysis? Methods Ecol Evol. 2015;6:1239–47.

Leis JM, van Herwerden L, Patterson HM. Estimating connectivity in marine fish populations: what works best? Oceanogr Mar Biol. 2011;49:193–234.

Lenormand T. Gene flow and the limits to natural selection. Trends Ecol Evol. 2002;17:183–9.

Liggins L, Treml EA, Riginos C. Taking the plunge: an introduction to undertaking seascape genetic studies and using biophysical models. Geogr Compass. 2013;7:173–96.

Liggins L, Treml EA, Possingham HP, Riginos C. Seascape features, rather than dispersal traits, predict spatial genetic patterns in co-distributed reef fishes. J Biogeogr. 2016;43:256–67.

Limborg MT, Helyar SJ, De Bruyn M, Taylor MI, Nielsen EE, Ogden RO, Carvalho GR, FPT Consortium, Bekkevold D. Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). Mol Ecol. 2012;21:3686–703.

Lotterhos KE, Whitlock MC. Evaluation of demographic history and neutral parameterization on the performance of FST outlier tests. Mol Ecol. 2014;23:2178–92.

Lotterhos KE, Whitlock MC. The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. Mol Ecol. 2015;24:1031–46.

Lowry DB, Willis JH. A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. PLoS Biol. 2010;8: e1000500.

Lowry DB, Hoban S, Kelley JL, Lotterhos KE, Reed LK, Antolin MF, Storfer A. Responsible RAD: striving for best practices in population genomic studies of adaptation. Mol Ecol Resour. 2017;17:366–9.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet. 2003;4:981–94.

Luikart G, Kardos M, Hand BK, Rajora OP, Aitken SN, Hohenlohe PA. Population genomics: advancing understanding of nature. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 3–79.

Manel S, Schwartz MK, Luikart G, Taberlet P. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol. 2003;18:189–97.

Manel S, Joost S, Epperson BK, Holderegger R, Storfer A, Rosenberg MS, Scribner KT, Bonin A, Fortin MJ. Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. Mol Ecol. 2010;19:3760–72.

Manel S, Perrier C, Pratlong M, Abi-Rached L, Paganini J, Pontarotti P, Aurelle D. Genomic resources and their influence on the detection of the signal of positive selection in genome scans. Mol Ecol. 2016;25:170–84.

Manel S, Andrello M, Henry K, Verdelet D, Darracq A, Guerin PE, Desprez B, Devaux P. Predicting genotype environmental range from genome–environment associations. Mol Ecol. 2018;27:2823–33.

Mantel N. The detection of disease clustering and a generalized regression approach. Cancer Res. 1967;27:209–20.

Marko PB, Hart MW. The complex analytical landscape of gene flow inference. Trends Ecol Evol. 2011;26:448–56.

Marshall DJ, Morgan SG. Ecological and evolutionary consequences of linked life-history stages in the sea. Curr Biol. 2011;21:R718–25.

Marshall DJ, Monro K, Bode M, Keough MJ, Swearer S. Phenotype–environment mismatches reduce connectivity in the sea. Ecol Lett. 2010;13:128–40.

Mastretta-Yanes A, Arrigo N, Alvarez N, Jorgensen TH, Piñero D, Emerson BC. Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. Mol Ecol Resour. 2015;15:28–41.

Matz MV. Fantastic beasts and how to sequence them: ecological genomics for obscure model organisms. Trends Genet. 2018;34:121–32.

Matz MV, Treml EA, Aglyamova GV, Bay LK. Potential and limits for rapid genetic adaptation to warming in a Great Barrier Reef coral. PLoS Genet. 2018;14:e1007220.

McCauley DJ, Pinsky ML, Palumbi SR, Estes JA, Joyce FH, Warner RR. Marine defaunation: animal loss in the global ocean. Science. 2015;347:1255641.

McRae BH. Isolation by resistance. Evolution. 2006;60:1551–61.

McRae BH, Dickson BG, Keitt TH, Shah VB. Using circuit theory to model connectivity in ecology and conservation. Ecology. 2008;89(10):2712–24.

Meirmans PG, Hedrick PW. Assessing population structure: FST and related measures. Mol Ecol Resour. 2011;11:5–18.

Mercer TR, Gerhardt DJ, Dinger ME, Crawford J, Trapnell C, Jeddeloh JA, Mattick JS, et al. Targeted RNA sequencing reveals the deep complexity of the human transcriptome. Nat Biotechnol. 2012;30:99.

Munguia-Vega A, Jackson A, Marinone SG, Erisman B, Moreno-Baez M, Girón-Nava A, Pfister T, Aburto-Oropeza O, Torre J. Asymmetric connectivity of spawning aggregations of a commercially important marine fish using a multidisciplinary approach. PeerJ. 2014;2:e511.

Nei M. Genetic distance between populations. Am Nat. 1972;106:283–92.

Nosil P, Funk DJ, Ortiz-Barrientos DA. Divergent selection and heterogeneous genomic divergence. Mol Ecol. 2009;18:375–402.

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM. Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. Proc Natl Acad Sci. 2007;104:1266–71.

Oleksiak MF. Adaptation without boundaries: population genomics in marine systems. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer Nature Switzerland AG; 2019. p. 587–612.

Palumbi SR. Marine speciation on a small planet. Trends Ecol Evol. 1992;7:114–8.

Palumbi SR. Molecular biogeography of the Pacific. Coral Reefs. 1997;16:S47–52.

Palumbi SR, Pinsky ML. Marine dispersal, ecology and conservation. In: Bertness MD, Bruno JF, Silliman B, Stachowicz JJ, editors. Marine community ecology. 2nd ed. Sunderland: Sinauer; 2013. p. 57–83.

Paris CB, Chérubin LM, Cowen RK. Surfing, spinning, or diving from reef to reef: effects on population connectivity. Mar Ecol Prog Ser. 2007;347:285–300.

Parobek CM, Archer FI, DePrenger-Levin ME, Hoban SM, Liggins L, Strand AE. Skelesim: an extensible, general framework for population genetic simulation in R. Mol Ecol Resour. 2017;17:101–9.

Paterno M, Schiavina M, Aglieri G, Ben Souissi J, Boscari E, Casagrandi R, Chassanite A, Chiantore M, Congiu L, Guarnieri G, Kruschel C. Population genomics meet Lagrangian simulations: oceanographic patterns and long larval duration ensure connectivity among *Paracentrotus lividus* populations in the Adriatic and Ionian seas. Ecol Evol. 2017;7:2463–79.

Pelc RA, Warner RR, Gaines SD. Geographical patterns of genetic structure in marine species with contrasting life histories. J Biogeogr. 2009;36:1881–90.

Pespeni MH, Palumbi SR. Signals of selection in outlier loci in a widely dispersing species across an environmental mosaic. Mol Ecol. 2013;22:3580–97.

Pinsky ML, Palumbi SR. Meta-analysis reveals lower genetic diversity in overfished populations. Mol Ecol. 2014;23:29–39.

Pörtner HO, Knust R. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science. 2007;315:95–7.

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945–59.

Prunier JG, Kaufmann B, Fenet S, Picard D, Pompanon F, Joly P, Lena JP. Optimizing the trade-off between spatial and genetic sampling efforts in patchy populations: towards a better assessment of functional connectivity using an individual-based sampling scheme. Mol Ecol. 2013;22:5516–30.

Prunier JG, Colyn M, Legendre X, Nimon KF, Flamand MC. Multicollinearity in spatial genetics: separating the wheat from the chaff using commonality analyses. Mol Ecol. 2015;24:263–83.

Puritz JB, Lotterhos KE. Expressed exome capture sequencing: a method for cost-effective exome sequencing for all organisms. Mol Ecol Resour. 2018;18:1209–22.

Puritz JB, Hollenbeck CM, Gold JR. dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ. 2014;2:e431.

Rajora OP, Eckert AJ, Zinck JWR. Single-locus versus multilocus patterns of local adaptation to climate in eastern white pine (*Pinus strobus*, Pinaceae). PLoS One. 2016;11:e0158691. https://doi.org/10.1371/journal.pone.0158691.

Ralls K, Ballou JD, Dudash MR, Eldridge MD, Fenster CB, Lacy RC, et al. Call for a paradigm shift in the genetic management of fragmented populations. Conserv Lett. 2018;11:e12412.

Ravinet M, Westram A, Johannesson K, Butlin R, André C, Panova M. Shared and nonshared genomic divergence in parallel ecotypes of *Littorina saxatilis* at a local scale. Mol Ecol. 2016;25:287–305.

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. A practical guide to environmental association analysis in landscape genomics. Mol Ecol. 2015;24:4348–70.

Riginos C, Liggins L. Seascape genetics: populations, individuals, and genes marooned and adrift. Geogr Compass. 2013;7:197–216.

Riginos C, Douglas KE, Jin Y, Shanahan DF, Treml EA. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography. 2011;34:566–75.

Riginos C, Crandall ED, Liggins L, Bongaerts P, Treml E. Navigating the currents of seascape genomics: how spatial analyses can augment population genomic studies. Curr Zoo. 2016;62:581–601.

Riginos C, Hock K, Matias AM, Mumby PJ, van Oppen MJH, Lukoschek V. Asymmetric dispersal is a critical element of concordance between biophysical dispersal models and spatial genetic structure in Great Barrier Reef corals. Div Dist. 2019; https://doi.org/10.1111/ddi.12969.

Riquet F, Liautard-Haag C, Woodall L, Bouza C, Louisy P, Hamer B, et al. Parallel pattern of differentiation at a genomic island shared between clinal and mosaic hybrid zones in a complex of cryptic seahorse lineages. Evolution. 2019;73:817–35.

Robinson JD, Coffman AJ, Hickerson MJ, Gutenkunst RN. Sampling strategies for frequency spectrum-based population genomic inference. BMC Evol Biol. 2014;14:254.

Rousset F. Genetic differentiation between individuals. J Evol Biol. 2000;13:58–62.

Roux C, Tsagkogeorga G, Bierne N, Galtier N. Crossing the species barrier: genomic hotspots of introgression between two highly divergent *Ciona intestinalis* species. Mol Biol Evol. 2013;30:1574–87.

Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S. Estimating connectivity in marine populations: an empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. Mol Ecol. 2009;18:1765–76.

Saenz-Agudelo P, DiBattista JD, Piatek MJ, Gaither MR, Harrison HB, Nanninga GB, et al. Seascape genetics along environmental gradients in the Arabian Peninsula: insights from ddRAD sequencing of anemonefishes. Mol Ecol. 2015;24:6241–55.

Saha A, Hauser L, Kent M, Planque B, Neat F, Kirubakaran TG, Huse I, Homrum EÍ, Fevolden SE, Lien S, Johansen T. Seascape genetics of saithe (*Pollachius virens*) across the North Atlantic using single nucleotide polymorphisms. ICES J Mar Sci. 2015;72:2732–41.

Sandoval-Castillo J, Robinson NA, Hart AM, Strain LW, Beheregaray LB. Seascape genomics reveals adaptive divergence in a connected and commercially important mollusc, the greenlip abalone (*Haliotis laevigata*), along a longitudinal environmental gradient. Mol Ecol. 2018;27:1603–20.

Savolainen O, Lascoux M, Merilä J. Ecological genomics of local adaptation. Nat Rev Genet. 2013;14:807.

Schlötterer C, Tobler R, Kofler R, Nolte V. Sequencing pools of individuals – mining genome-wide polymorphism data without big funding. Nat Rev Genet. 2014;15:749.

Schmidt PS, Rand DM. Intertidal microhabitat and selection at MPI: interlocus contrasts in the northern acorn barnacle, *Semibalanus balanoides*. Evolution. 1999;53:135–46.

Schmidt PS, Serrao EA, Pearson GA, Riginos C, Rawson PD, Hilbish TJ, et al. Ecological genetics in the North Atlantic: environmental gradients and adaptation at specific loci. Ecology. 2008;89: S91–107.

Schoville SD, Bonin A, François O, Lobreaux S, Melodelima C, Manel S. Adaptive genetic variation on the landscape: methods and cases. Annu Rev Ecol Evol Syst. 2012;43:23–43.

Schunter C, Carreras-Carbonell J, Macpherson E, Tintoré J, Vidal-Vijande E, Pascual A, Guidetti P, et al. Matching genetics with oceanography: directional gene flow in a Mediterranean fish species. Mol Ecol. 2011;20:5167–81.

Seeb JE, Carvalho G, Hauser L, Naish K, Roberts S, Seeb LW. Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. Mol Ecol Resour. 2011;11:1–8.

Selkoe KA, Toonen RJ. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. Mar Ecol Prog Ser. 2011;436:291–305.

Selkoe KA, Gaines SD, Caselle JE, Warner RR. Current shifts and kin aggregation explain genetic patchiness in fish recruits. Ecology. 2006;87:3082–94.

Selkoe KA, Henzler CM, Gaines SD. Seascape genetics and the spatial ecology of marine populations. Fish Fish. 2008;9:363–77.

Selkoe KA, D'Aloia CC, Crandall ED, Iacchei M, Liggins L, Puritz JB, et al. A decade of seascape genetics: contributions to basic and applied marine connectivity. Mar Ecol Prog Ser. 2016a;554:1–19.

Selkoe KA, Scribner KT, Galindo HM. Waterscape genetics – applications of landscape genetics to rivers, lakes, and seas. In: Balkenhol N, Cushman S, Storfer A, Waits L, editors. Landscape genetics: concepts, methods, applications. West Sussex: Wiley; 2016b. p. 220–46.

Shafer AB, Wolf JB, Alves PC, Bergström L, Bruford MW, Brännström I, Colling G, Dalén L, De Meester L, Ekblom R, Fawcett KD. Genomics and the challenging translation into conservation practice. Trends Ecol Evol. 2015;30:78–87.

Shanks AL. Pelagic larval duration and dispersal distance revisited. Biol Bull. 2009;216:373–85.

Shima JS, Swearer SE. Larval quality is shaped by matrix effects: implications for connectivity in a marine metapopulation. Ecology. 2009;90:1255–67.

Slatkin M. Rare alleles as indicators of gene flow. Evolution. 1985;39:53–65.

Slatkin M. Isolation by distance in equilibrium and non-equilibrium populations. Evolution. 1993;47:264–79.

Smouse PE, Long JC, Sokal RR. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst Zool. 1986;35:627–32.

Sodeland M, Jorde PE, Lien S, Jentoft S, Berg PR, Grove H, et al. Islands of divergence' in the Atlantic cod represent polymorphic chromosomal rearrangements. Genome Biol Evol. 2016;8:1012–22.

Sork VL, Aitken SN, Dyer RJ, Eckert AJ, Legendre P, Neale DB. Putting the landscape into the genomics of trees: approaches for understanding local adaptation and population responses to changing climate. Trends Gen Genom. 2013;9:901–11.

Spear SF, Balkenhol N, Fortin MJ, McRae BH, Scribner KI. Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. Mol Ecol. 2010;19:3576–91.

Stone GN, Nee S, Felsenstein J. Controlling for non-independence in comparative analysis of patterns across populations within species. Phil Trans Roy Soc B: Biol Sci. 2011;366:1410–24.

Storfer A, Murphy MA, Evans JS, Goldberg CS, Robinson S, Spear SF, et al. Putting the 'landscape' in landscape genetics. Heredity. 2007;98:128.

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP. Landscape genetics: where are we now? Mol Ecol. 2010;19:3496–514.

Strathmann RR. Why life histories evolve differently in the sea. Am Zool. 1990;30:197–207.

Sylvester EV, Beiko RG, Bentzen P, Paterson I, Horne JB, Watson B, Lehnert S, et al. Environmental extremes drive population structure at the northern range limit of Atlantic salmon in North America. Mol Ecol. 2018;27:4026–40.

Teske PR, Sandoval-Castillo J, Golla TR, Emami-Khoyi A, Tine M, von der Heyden S, Beheregaray LB. Thermal selection as a driver of marine ecological speciation. Proc R Soc B. 2019;286:20182023.

Therkildsen NO, Palumbi SR. Practical low-coverage genome-wide sequencing of hundreds of individually barcoded samples for population and evolutionary genomics in nonmodel species. Mol Ecol Resour. 2017;17:194–208.

Therkildsen NO, Hemmer-Hansen J, Hedeholm RB, Wisz MS, Pampoulie C, Meldrup D, et al. Spatiotemporal SNP analysis reveals pronounced biocomplexity at the northern range margin of Atlantic cod *Gadus morhua*. Evol Appl. 2013;6:690–705.

Tigano A, Friesen VL. Genomics of local adaptation with gene flow. Mol Ecol. 2016;25:2144–64.

Tine M, Kuhl H, Gagnaire P-A, Louro B, Desmarais E, Martins RST, et al. European sea bass genome and its variation provide insights into adaptation to euryhalinity and speciation. Nat Commun. 2014;5:5770.

Tobler WR. A computer movie simulating urban growth in the Detroit region. Econ Geogr. 1970;46 (sup1):234–40.

Toonen RJ, Grosberg RK. Causes of chaos: spatial and temporal genetic heterogeneity in the intertidal anomuran crab *Petrolisthes cinctipes*. Phylogeogr Popul Genet Crustacea. 2011;2011:75–107.

Toonen RJ, Andrews KR, Baums IB, Bird CE, Concepcion GT, Daly-Engel TS, et al. Defining boundaries for ecosystem-based management: a multispecies case study of marine connectivity across the Hawaiian Archipelago. J Marine Biol. 2011;2011. pii: 460173

Treml EA, Roberts JJ, Chao Y, Halpin PN, Possingham HP, Riginos C. Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations. Integr Compar Biol. 2012;52:525–37.

Treml EA, Ford JR, Black KP, Swearer SE. Identifying the key biophysical drivers, connectivity outcomes, and metapopulation consequences of larval dispersal in the sea. Mov Ecol. 2015a;3:17.

Treml EA, Roberts J, Halpin PN, Possingham HP, Riginos C. The emergent geography of biophysical dispersal barriers across the Indo-West Pacific. Div Dist. 2015b;21:465–76.

Vandamme SG, Maes GE, Raeymaekers JA, Cottenie K, Imsland AK, Hellemans B, et al. Regional environmental pressure influences population differentiation in turbot (*S cophthalmus maximus*). Mol Ecol. 2014;23:618–36.

Véliz D, Bourget E, Bernatchez L. Regional variation in the spatial scale of selection at MPI∗ and GPI∗ in the acorn barnacle *Semibalanus balanoides* (Crustacea). J Evol Biol. 2004;17:953–66.

Vigliola L, Doherty PJ, Meekan MG, Drown DM, Jones ME, Barber PH. Genetic identity determines risk of post-settlement mortality of a marine fish. Ecology. 2007;88:1263–77.

Villacorta-Rath C, Souza CA, Murphy NP, Green BS, Gardner C, Strugnell JM. Temporal genetic patterns of diversity and structure evidence chaotic genetic patchiness in a spiny lobster. Mol Ecol. 2018;27:54–65.

von der Heyden S. Making evolutionary history count: biodiversity planning for coral reef fishes and the conservation of evolutionary processes. Coral Reefs. 2017;36:183–94.

Vuilleumier S, Possingham HP. Does colonization asymmetry matter in metapopulations? Proc R Soc Lond B Biol Sci. 2006;273:1637–42.

Wagner H, Fortin M-J. Basics of spatial data analysis: linking landscape and genetic data for landscape genetic studies. In: Balkenhol N, Cushman S, Storfer A, Waits L, editors. Landscape genetics: concepts, methods, applications. West Sussex: Wiley; 2016. p. 77–98.

Wang IJ, Bradburd GS. Isolation by environment. Mol Ecol. 2014;23:5649–62.

Waples RS. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J Hered. 1998;89:438–50.

Waples RS, Gaggiotti O. Invited review: what is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol. 2006;15:1419–39.

Ward RD, Woodwark M, Skibinski DO. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J Fish Biol. 1994;44:213–32.

Waters JM, Fraser CI, Hewitt GM. Founder takes all: density-dependent processes structure biodiversity. Trends Ecol Evol. 2013;28:78–85.

Wellenreuther M, Bernatchez L. Eco-evolutionary genomics of chromosomal inversions. Trends Ecol Evol. 2018;33:427–40.

Wellenreuther M, Hansson B. Detecting polygenic evolution: problems, pitfalls, and promises. Trends Genet. 2016;32:155–64.

Westram AM, Galindo J, Alm Rosenblad M, Grahame JW, Panova M, Butlin RK. Do the same genes underlie parallel phenotypic divergence in different *Littorina saxatilis* populations? Mol Ecol. 2014;23:4603–16.

White C, Selkoe KA, Watson J, Siegel DA, Zacherl DC, Toonen RJ. Ocean currents help explain population genetic structure. Proc R Soc Lond B Biol Sci. 2010;277:1685–94.

Whitlock MC, Lotterhos KE. Reliable detection of loci responsible for local adaptation: inference of a null model through trimming the distribution of FST. Am Nat. 2015;186:S24–36.

Whitlock MC, Mccauley DE. Indirect measures of gene flow and migration: FST$\neq$ 1/(4Nm+ 1). Heredity. 1999;82:117–25.

Wilkinson-Herbots HM, Ettridge R. The effect of unequal migration rates on. FST Theor Popul Biol. 2004;66:185–97.

Willette DA, Allendorf FW, Barber PH, Barshis DJ, Carpenter KE, Crandall ED, et al. So, you want to use next-generation sequencing in marine systems? Insight from the Pan-Pacific Advanced Studies Institute. Bull Mar Sci. 2014;90:79–122.

Wolf JB, Ellegren H. Making sense of genomic islands of differentiation in light of speciation. Nat Rev Genet. 2017;18:87.

Wood S, Paris CB, Ridgwell A, Hendy EJ. Modelling dispersal and connectivity of broadcast spawning corals at the global scale. Global Ecol Biog. 2014;23(1):1.

Wright S. Evolution in Mendelian populations. Genetics. 1931;16:97.

Wright S. Isolation by distance. Genetics. 1943;28:114–38.

Xuereb A, Benestan L, Normandeau É, Daigle RM, Curtis JM, Bernatchez L, Fortin MJ. Asymmetric oceanographic processes mediate connectivity and population genetic structure, as revealed by RAD seq, in a highly dispersive marine invertebrate (*Parastichopus californicus*). Mol Ecol. 2018a;27:2347–64.

Xuereb A, Kimber CM, Curtis JM, Bernatchez L, Fortin MJ. Putatively adaptive genetic variation in the giant California sea cucumber (*Parastichopus californicus*) as revealed by environmental association analysis of restriction-site associated DNA sequencing data. Mol Ecol. 2018b;27:5035–48.

Yeaman S. Local adaptation by alleles of small effect. Am Nat. 2015;186:S74–89.

Part V
Adaptation, Acclimation, and Speciation

Clinal Adaptation in the Marine Environment

David I. Dayan

Abstract Biologists hoping to understand the population genetics and evolution of marine organisms face a common challenge. Clear boundaries that define populations, shape gene flow, and drive natural selection are not apparent when looking across a featureless seascape. Instead, many marine species are broadly and continuously distributed across gradients in environmental variables such as pH, temperature, and salinity. Clinal adaptation to these environmental gradients is rampant among marine species and occurs across a broad range of demographic contexts. This chapter describes how the recent application of population genomics tools is beginning to reveal the genetic basis of clinal adaptation to environmental gradients in the sea. First, the chapter outlines the demographic and alternative selective scenarios that produce clinal variation in allele frequency and may result in spurious identification of adaptive genetic variants. Once these pitfalls are considered, the chapter briefly overviews population genomic techniques for identifying adaptive variants. Then, relevant and recent empirical studies are reviewed to draw generalizations about the genetic basis of clinal adaptation in the marine environment. Finally, future directions for the field are outlined, emphasizing an increased integration of the phenotype and genetic architecture in analyses of clinal adaptation and highlighting the potential of new tools such as machine learning and polygenic analysis.

Keywords Clinal adaptation · Clines · Environmental association analysis · Environmental gradients · Genome-wide scans for selection · Local adaptation · Outlier analysis · Population genomics

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1 Overview and Historical Perspective

The questions that drive population genetic studies of marine organisms have historically been framed by what makes the marine environment unique. Limited apparent barriers to dispersal across the seascape and large population sizes lead to an expectation that marine populations should be largely undifferentiated; the effect of drift is limited by large effective population size and local adaptation is opposed by high migration rate. However, as observed by Hauser and Carvalho (2008) over a decade ago, the field of marine population genetics has undergone a paradigm shift. The view of the ocean as a genetically homogenous landscape was challenged by the dual observations of significant genetic differentiation among populations within many species and evidence of local adaptation across both large spatial scales (e.g., ocean basins) and small ones (e.g., within single estuaries) (Sanford and Kelly 2011). Since then, improved oceanographic modeling and understanding of the ecology marine species (McManus and Woodson 2012; Prairie et al. 2012) have allowed investigation into how patchiness and discontinuity in larval dispersal across a range of spatial and temporal scales leads to more strongly reduced migration and smaller effective population size than initially expected (Eldon et al. 2016; Selkoe et al. 2006, 2010). This new understanding has led some authors to question whether such broad generalization in the distinction between marine and terrestrial species in population genetics is meaningful (Bierne et al. 2016). In place of a field focused on the apparent paradox of local adaptation and genetic differentiation in spite of homogenizing gene flow, population genetic studies in marine populations now provide broad and general insights into the ecology and evolution of natural populations that demonstrate a wide range of demographic structures, from genetically isolated demes within a metapopulation to isolation by distance across large spatial scales and even complete panmixia within species.

Within this broad field, this chapter focuses on clinal adaptation to environmental gradients. I discuss current techniques in population genomics to identify the molecular basis of clinal adaptation from genomic data, with an emphasis on the demographic and alternative selective scenarios that produce clinal variation in allele frequency and may result in spurious identification of adaptive variants. I also briefly review relevant and recent empirical studies to draw generalizations about clinal adaptation in the marine environment and provide insights into the genetic basis of adaptation in general. Finally, I outline some future directions for the field, emphasizing an increased integration of the phenotype and genetic architecture in analyses of clinal adaptation and highlighting the potential of new tools such as machine learning and polygenic analysis.

2 Clinal Adaptation

Clinal adaptation is a particular form of local adaptation, where the adaptive phenotype varies continuously across a spatial or ecological gradient (Endler 1977). Many marine species have broad ranges and as a consequence are often distributed across large environmental gradients (Schmidt et al. 2008). Clinal variation in ecologically relevant traits that correlates with these environmental gradients is suggestive of local adaptation. There is abundant evidence of such clinal phenotype-environment associations in marine species (for reviews, see Fraser et al. (2011), salmonids; Sanford and Kelly (2011), marine invertebrates; and Conover et al. (2006), marine fishes). For example, in the Atlantic silverside, *Menidia menidia* growth rate, temperature of sex determination, and vertebral number gradually vary and strongly correlate with a gradient in temperature along their distribution (Hice et al. 2012). Yet, assessing local adaptation in marine organisms using more rigorous criteria (e.g., local vs. foreign and home vs. away (Kawecki and Ebert 2004)) is often difficult, because many marine species are not readily subjected to reciprocal transplant and common garden studies (Sotka 2012). An exception comes from marine intertidal invertebrates, where extensive local adaptation is observed at both broad, species-wide scales and at smaller scales using reciprocal transplants and common gardens (Burford et al. 2014; Kosloski et al. 2017; Leong et al. 2017; Sanford and Kelly 2011). For more difficult to study species, evidence of local adaptation to environmental gradients stems from comparisons between the extent of trait variation and neutral genetic differentiation (P_{ST}–F_{ST} comparisons) (Leinonen et al. 2006). While these results are best interpreted with caution because the genetic architecture of traits varies across populations and environments (Brommer 2011; Parsons et al. 2016; Pujol et al. 2008), many marine species demonstrate variation in quantitative traits that exceed expectations given genetic drift alone. These putatively non-neutral patterns often correlate with environmental gradients, suggesting clinal adaptation (Defaveri and Merila 2013; Flanagan et al. 2016; Ledoux et al. 2015; Mariani et al. 2012). For example, lateral plate number variation in threespine stickleback along a salinity and temperature gradient across the Baltic Sea greatly exceeds the neutral divergence observed genome-wide in the same region (Defaveri and Merila 2013).

A central goal in marine population genomics is identifying the genetic variants that underlie these clinal adaptations. Most studies in marine organisms conduct genome-wide scans for selection, using patterns in genomic data to identify the molecular basis of adaptation (Table 1). While these studies typically focus on the role that spatially varying selection plays in shaping genetic variation across the seascape and among populations, similar patterns are also produced by many neutral demographic (Endler 1977) and alternative selective scenarios (Barton and Hewitt 1989; Bierne 2010; Bierne et al. 2011). Therefore, methodological approaches to identify adaptive genetic variation face a central challenge: establishing a biologically accurate model of allele frequency change that can serve as a null hypothesis.

Table 1 Sample of empirical studies using environmental association analysis (EAA) and/or outlier analysis to identify genetic variants that may contribute to local adaption to marine environmental gradients

Author, year	Species	EAA method	Outlier method
Drury et al. (2016)	*Acropora cervicornis*		Fdist2
Bay and Palumbi (2014)	*Acropora hyacinthus*	ANOVA	Bootstrap F_{ST}
Benestan et al. (2016)	American lobster	RDA, Pearson correlation test, BayeScEnv, LFMM	BayeScan, ARLEQUIN and OUTFLANK
Pujolar et al. (2014)	*Anguilla anguilla*	BayEnv	BayeScan
Gagnaire et al. (2012)	*Anguilla rostrata*	Logistic regression, sPCA	Arlequin
Bradbury et al. (2010)	Atlantic cod	Multiple regression, mantel test	BayeScan, ArlEQUIN
Therkildsen et al. (2013a)	Atlantic cod	Pearson correlation	BayeScan, Ftemp, fdist2
Therkildsen et al. (2013b)	Atlantic cod	BayEnv	Ftemp
Corander et al. (2013)	Atlantic herring		BAPS
Gaggiotti et al. (2009)	Atlantic herring	Geste	BayeScan
Limborg et al. (2012)	Atlantic herring	BayEnv	Arlequin, BayeScan
Lamichhaney et al. (2012)	Atlantic herring		Simulated F_{ST}
Lamichhaney et al. (2017)	Atlantic herring		Chi-squared test, simulated F_{ST}
Martinez Barrio et al. (2016)	Atlantic herring		Chi-squared test
Bourret et al. (2013)	Atlantic salmon		Arlequin, Fdist2, sliding window Fdist2
Jeffery et al. (2017)	Atlantic salmon	RDA, sPCA	BayeScan, ArlEQUIN
Le Moan et al. (2016)	European anchovy		Fdist2
Milano et al. (2014)	European hake	BayEnv	Arlequin, fdist2
Tepolt and Palumbi (2015)	Green crab		Fdist2
Hess et al. (2013)	Pacific lamprey	MATSAM	Fdist2
Pespeni et al. (2017)	Purple Sea urchin	Partial mantel test	

(continued)

Table 1 (continued)

Author, year	Species	EAA method	Outlier method
De Wit and Palumbi (2013)	Red abalone		Fdist2
Van Wyngaarden et al. (2017)	Sea scallop		BayeScan, Arlequin
Guo et al. (2015)	Threespine stickleback	BayEnv	BayeScan, sliding window F_{ST}
Barth et al. (2017)	Atlantic cod		
Barney et al. (2017)	Atlantic cod		LD outlier
Berg et al. (2015)	Atlantic cod	BayEnv	BayeScan, fdist2
Hecht et al. (2015)	Chinook salmon	RDA	Fdist2

Approaches can be broadly divided into two categories. Approaches in the first category, known as outlier analyses, are based on the assumption that neutral demographic processes such as gene flow, genetic drift, and changes in population size have genome-wide impacts and affect all loci approximately equally, while selection acts only on the subset of loci that underlie traits under selection and linked neutral variants. Outlier analyses use empirical data to simulate null distributions of population genetic parameters under varying demographic scenarios, with candidate loci identified as those that appear in the extreme tails of the distribution (Hoban et al. 2016). The second category of approaches, known collectively as environmental association analysis, identifies allele frequency variation that is highly correlated with environmental variables assumed to drive spatially varying selection. These approaches address the central challenge of establishing an accurate null hypothesis by the inclusion of neutral population structure or spatial relationships among sampling sites that recapitulate neutral structure as covariates in the full model.

2.1 Neutral Clines

Marine species often demonstrate complex demographies owing to spatially restricted gene flow over their large ranges and shared histories of expansion from refugia or secondary contact since the last deglaciation. These neutral demographic scenarios can produce allele frequency patterns among populations that resemble those produces by clinal adaptation and can fool genome-wide scans for selection.

The most intuitive neutral process that produces clinal variation in allele frequency is isolation by distance (IBD) (Wright 1943). Limited dispersal imposed by physical limitations placed on organisms results in a correlation between the relatedness of individuals and the spatial distance between them (Aguillon et al. 2017). At the level of populations, this spatial autocorrelation of genetic variation breaks a key assumption of the island model that is the basis of many genome-wide

scans for selection; migration and gene flow are inversely correlated with the geographic distance between populations due to limited dispersal. When coupled with the effects of mutation and genetic drift, IBD produces genetic clines that resemble the allele frequency variation shaped by clinal adaptation (Meirmans 2012; Vasemagi 2006) but have genome-wide impacts. Spatially restricted gene flow resulting in stable patterns of IBD produces clinal variation in many marine species (see Table 1). The extent to which IBD clines and adaptive genetic clines can be parsed depends on the relative spatial scales of dispersal and the environmental gradient driving selection (Orsini et al. 2013).

In addition to IBD, expansion from refugia and secondary contact of isolated populations can produce neutral genetic clines (Endler 1977; Klopfstein et al. 2006; Slatkin 1973). As non-equilibrium processes, range expansions and secondary contact produce clines that are eventually eroded by gene flow but can persist for many generations. Many marine species share a recent natural history of secondary contact or expansion from refugia following the most recent Pleistocene deglaciation (Ludt et al. 2015; Maggs et al. 2008). In some, there is evidence that spatially restricted gene flow has maintained these neutral non-equilibrium genetic clines, e.g., Assis et al. (2014), Marko (2004), and Smith et al. (2001), while other marine species do not demonstrate contemporary signatures of their postglacial range shifts, e.g., Bradbury et al. (2010). For studies attempting to identify clines shaped natural selection, neutral clines generated by secondary contact or expansion from refugia are particularly confounding, because the axis of the range shift is frequently aligned with the major axis of contemporary environmental variation. For example, the estuarine fish *Fundulus heteroclitus* is distributed along the Atlantic coast of North America such that the species experiences a steep thermal gradient across its range. Spatially varying selection along this gradient has led to the adaptive fixation of alternative lactose dehydrogenase (LDH) allozymes at either end of the species range (Crawford and Powers 1989) with clinal variation in LDH allele frequency contributing adaptive clinal variation in ecologically relevant traits (DiMichele and Powers 1982), demonstrating that selection has shaped allele frequencies at some loci in this species. Yet, coincident with this adaptive cline are neutral clines owing to secondary recontact of two distinct clades of *F. heteroclitus* that diverged by allopatry during the last glacial maximum (Adams et al. 2006; McKenzie et al. 2016). A simulation study of secondary contact in *F. heteroclitus* across a wide range of dispersal distances suggests that secondary contact alone can produce the extent of genome-wide clinal variation observed in this species (Strand et al. 2012).

2.2 Alternative Selective Processes

Spatially varying selection is not the only selective regime that can produce genetic clines. Endogenous and background selection can also produce clinal variation and are generally underappreciated or unexamined among empirical studies of clinal

adaptation (Bierne 2010; Bierne et al. 2011). Endogenous selection occurs when gene flow among two previously isolated groups is limited by negative genomic interactions between alleles from alternative genomic backgrounds, i.e., negative epistasis. These endogenous barriers to gene flow produce tension zones, where equilibrium between dispersal and selection against hybridization produces stable genetic clines centered at the point of contact between the groups. While these endogenous clines and clines maintained by exogenous selection, e.g., spatially varying selection, produce different patterns of allele frequency across space (Kruuk et al. 1999; Vasemagi 2006), where tension zones occur across environmental gradients, they can be conflated for exogenous genetic clines. An even more confounding scenario stems from the observation that tension zones are not spatially restricted and can move owing to drift (Barton and Hewitt 1989). As they move tension zones are attracted to natural features that restrict gene flow, such as fronts for oceanic species or rivers for coastal species. Similarly, they can also become coupled with exogenous selection. In this scenario, termed the coupling hypothesis, the spatial position of clines is determined by exogenous selection, but at many genetic loci, endogenous selection is responsible for the clines (Bierne et al. 2011).

Endogenous selection can also lead to genetic clines outside of the tension zone and produce spatial structure within each of the hybridizing populations. Differential introgression due to partial linkage among neutral loci and loci underlying barriers to gene flow produces clines at linked neutral loci known as introgression tails that extend far from the point of contact between the differentiated populations (Payseur 2010). Introgression tails can be conflated for within-population clines due to spatially varying selection if this scenario is not explicitly examined (Gagnaire et al. 2011; Gosset and Bierne 2013).

Finally, the effect of selection on linked variation is reduced by recombination as a globally advantageous allele sweeps through a subdivided population. Individuals close to the geographic origin of the beneficial allele demonstrate reduced genetic diversity in the chromosomal neighborhood of the advantageous allele, while more distantly located individuals do not demonstrate a loss of diversity (Slatkin and Wiehe 1998). This global hitchhiking in a subdivided population can produce clines at linked loci that are suggestive of spatially varying selection but are in fact due to the spread of a globally advantageous allele (Bierne 2010). Similar patterns are predicted for disadvantageous alleles subject to purifying selection, such that the effect of background selection (Charlesworth et al. 1997) on linked loci varies across subdivided populations and results in clines that resemble those resulting from spatially varying selection (Hu and He 2005).

3 Approaches

3.1 Outlier Analysis

Outlier analyses rely on the assumption that population genetic parameters describing the site frequency spectrum (SFS), linkage disequilibrium (LD), and population differentiation (F_{ST}) demonstrate locus-specific effects under selection, but neutral processes have genome-wide impacts. For example, an adaptive sweep will produce an excess of rare variants and a localized reduction in genetic diversity in the vicinity of the adaptive locus, skewing descriptors of SFS such as Tajima's *D* at the adaptive locus and linked neutral variants relative to the distribution of these statistics at neutral loci (Hoban et al. 2016). The haplotype bearing the adaptive allele will spread rapidly in the population, producing extended regions of high LD in the vicinity of adaptive locus (Smith and Haigh 1974). The extent of these blocks of high LD along the genome are measured using a suite of statistics such as the integrated haplotype score (iHS) or extended haplotype homozygosity (ehh) (Vitti et al. 2013). This divergent selection across populations will also produce locus specific genetic differentiation (F_{ST}) that stands out from genome-wide averages (Beaumont and Nichols 1996).

In all three major classes of outlier analyses (SFS, LD, and F_{ST}), adaptive loci are identified as those in the tails of the distribution of the statistic. However, complex demographic scenarios inflate the variance of the null distribution of the parameters used for outlier detection, leading to an increase in false-positive rates. Approaches that rely on signals within a single population, such as those based on LD, are relatively robust to these demographic effects (Pavlidis et al. 2010) but require high-density, phased genomic data that are often outside the reach of studies using marine organisms. The most commonly used parameter in outlier scans of marine species, population differentiation, measured with F_{ST} or related measures of genetic distance, is perhaps the most negatively affected. This problem is especially relevant for studies attempting to identify loci responsible for clinal adaptation in marine organisms, where IBD, range expansion, and secondary contact are common.

To ameliorate the false-positive problem imposed by demography, some F_{ST} outlier analysis methods explicitly model the distribution of the test statistic under the assumed demography of the populations in question and then compare empirical values of the statistic to these null models. However, some methods are limited in the types of demography that can be modeled (e.g., the Fdist2 family of algorithms is limited to island and hierarchical island models) (Excoffier et al. 2009), and even in the case of highly flexible methods (e.g., BayeScan (Foll and Gaggiotti 2008) and BayScEnv (de Villemereuil et al. 2015), achieving a low false-positive rate critically depends on modeling the correct demographic scenario. For example, simulation studies demonstrate that Fdist2 has a higher false-positive rate under IBD or range expansion when the simulated null dataset attempts to account for neutral structure than when the algorithm is used without neutral parameterization at all (Lotterhos and Whitlock 2014). Given the complex demographies of clinally adapted marine species, the scenarios where these outlier approaches are effective are limited.

A second class of F_{ST} outlier methods avoids the challenge of correctly modeling demographic effects entirely by instead relying on measure of genetic relatedness or covariance among individuals to control for coancestry among samples (e.g., BayEnv2 (Gunther and Coop 2013), SOM/HMM (Jones et al. 2012), FLK (Bonhomme et al. 2010), PCAdapt (Duforet-Frebourg et al. 2014)). The greater flexibility afforded by this approach to control for the effect of demography on population differentiation should improve the performance of these methods in detecting the genetic variants responsible for clinal adaptation in the marine environment. Indeed, simulations suggest that these methods perform better than their counterparts under IBD, range expansions, and secondary contact but still exhibit increased false positives relative to the island model and when non-neutral loci are used during neutral parameterization (Lotterhos and Whitlock 2014).

Perhaps the greatest challenge in applying outlier analyses to clinal adaptation in the marine environment stems from the division of continuously distributed individuals along an environmental, genetic, or phenotypic cline into discrete populations (Jones et al. 2013; Joost et al. 2013). In order to measure population differentiation, one must first define populations, and this task is not clear for marine species with no clear barriers to dispersal among individuals across the species range. This challenge is often addressed in marine population genomics study by grouping individuals into synthetic populations using clustering algorithms such as STRUCTURE (Falush et al. 2003). Alternatively, an analysis of molecular variance (AMOVA) (Excoffier et al. 2009) using a priori predictions about hierarchical structure among sampling locations is often used to test the significance of grouping sampling locations into clusters. In either case, these synthetic groupings of individuals along a cline (clusters) are subsequently used as "populations" in outlier analysis. However, these algorithms will produce genetic clusters even under pure IBD scenarios (Bradburd et al. 2017; Frantz et al. 2009; Serre and Pääbo 2004), and the resultant groupings of individuals as populations in outlier analyses may produce spurious results (Jensen et al. 2005). Additionally, the low genetic differentiation, high gene flow, and large effective population sizes characteristic of many marine species make population subdivision difficult, even when such structure exists (Waples et al. 2008).

Taken together, the challenges associated with correctly controlling for demography in outlier analysis of clinally adapted marine species place serious constraints on the number of study systems where they are appropriate. Because of this limitation, outlier analysis is rarely used as the sole evidence in support of candidate loci (Table 1), especially with increasing recognition of these shortcomings in recent years.

3.2 Environmental Association Analysis

Environmental association analysis (EAA) rests on the assumption that spatially varying selection results in a correlation between allele frequencies and environmental variables. EAA is the theoretical basis of landscape genetics and is also

referred to as gene-environment association (Manel and Holderegger 2013). EAA has several distinct advantages over outlier analysis for identifying the genetic variants underlying clinal adaptation, although the two techniques are often used in concert. Most significantly, EAA is spatially explicit, allowing researchers to appropriately model spatial autocorrelation that arises as a consequence of IBD or range expansion. Also, many EAA methods do not require continuously distributed species to be clustered into synthetic populations, and in addition to revealing the genetic variants that may drive selection, EAA methods can identify the environmental variables that most strongly correlate with adaptive genetic patterns.

EAA includes a diverse array of statistical approaches for discovering gene-environment associations. EAA approaches control for neutral genetic structure through incorporation of genetic covariance among samples and populations or spatial relationships among sampling locations as factors or covariates in the model. Methods based on the former are not well suited to clinal adaptation. Relatedness and kinship approaches, such as those intended for mixed linear modeling of genome-wide association studies (e.g., EMMA/TASSEL), are challenging to use in wild, non-model organisms because most require very dense genotyping and exhibit high variance except among closely related individuals (Kumar et al. 2016). The use of model-based coancestry estimation and clustering algorithms such as latent factors (LFMM (Frichot et al. 2013) and PCAdapt (Duforet-Frebourg et al. 2014)) or Q-matrices from STRUCTURE (EMMA/TASSEL) require subdivision of continuously distributed individuals into discrete groups, before neutral structure can be incorporated into the model. Finally, approaches that rely on the identification of a set of putatively neutral markers for neutral parameterization (e.g., BayEnv2) present different challenges because the neutral dataset has to be carefully matched to the test dataset with respect to genomic position and minor allele frequency (Berg and Coop 2014), and inclusion of non-neutral loci can bias the test statistic (Lotterhos and Whitlock 2014).

In contrast to methods that directly control for neutral genetic structure, a second category of EAA methods takes advantage of the spatial nature of local adaptation and demographic processes such as IBD and range expansion by taking a spatially explicit approach (Forester et al. 2016). Spatial methods do not depend on specific population genetic assumptions and therefore can be applied to the marine environment where population genetic characteristics of species are often well outside normal parameter spaces, e.g., low differentiation coupled with extreme effective population size (Gagnaire et al. 2015) and sweepstakes reproduction with collective dispersal (Eldon et al. 2016). At their simplest, these methods incorporate Euclidean geographic distance as a predictor of genetic distance among samples in the EAA model. More powerful approaches such as Moran's eigenvector maps (Dray et al. 2006) and their extensions (e.g., asymmetric eigenvector maps) (Blanchet et al. 2008), however, can simultaneously account for spatial effects at a range of spatial scales and are used in conjunction with multivariate constrained ordination methods developed among ecologists such as canonical correspondence analysis (CCA) and redundancy analysis (RDA) (Legendre and Legendre 2012). The goal of constrained

ordination in EAA is to generate orthogonal linear combinations of multiple multivariate explanatory variables (spatial relationships and environmental variables) that explain linear combinations of the response data (allele frequency). Several aspects of ordination techniques make them particularly effective for EAA: individual level analysis is possible, the ordination process reduces the dimensionality of large genetic datasets into a small set of uncorrelated genetic axes increasing power to identify polygenic selection, ordination can address collinearity among explanatory variables, and there is a wealth of downstream statistical analyses that provide unique insights into how environmental factors and demography interact to produce spatial genetic patterns. For example, partial RDA allows redundancy analysis to be conducted on the residuals of the response variable after modeling the effect of one or more of the explanatory variables using RDA, allowing researchers to identify the relative contribution of neutral (spatial) vs. putatively adaptive (environmental) processes on genetic variation through variance partitioning. The ability to build models at varying levels of complexity, coupled with significance testing through permutation, also permits identification of the most important environmental variables that drive putatively adaptive genetic divergence.

The flexibility of spatial multivariate ordination techniques offers distinct advantages for marine clinal adaptation. For example, while Moran's eigenvector maps can account for spatial autocorrelation in genetic data across multiple scales, spatial autocorrelation in the marine environment is rarely symmetrical. Instead, asymmetries in larval dispersal due to prevailing currents and flow in the marine environment produce anisotropy in the degree of neutral coancestry among individuals across the seascape (Riginos et al. 2016), e.g., source-sink dynamics. Coupled biophysical modeling offers a solution (Galindo et al. 2010). Constrained ordination techniques can be extended by incorporating estimates of larval dispersal between habitats driven by currents and larval behavior, permitting more accurate null models of gene flow along the cline. Benestan et al. (2016) utilize a biophysical model of American lobster larval dispersal to generate asymmetric eigenvector maps as explanatory variables in an RDA of lobster genetic variation across the species range. Spatial variables that incorporate estimates of larval dispersal with ocean currents (asymmetric eigenvector maps) explained three times the genetic variation than explained by spatial distribution alone (distance-based eigenvector maps).

4 Review

While the extent of limitations for genome scan methods may seem daunting, there is a diverse, recent literature using both outlier and environmental association methods to uncover the molecular basis of clinal adaptation in the marine environment (see Table 1 for a subset of this work). Key to the interpretation of these studies is careful consideration of the potential pitfalls of the methods (de Villemereuil et al. 2014; Forester et al. 2016; Lotterhos and Whitlock 2014, 2015), the use of multiple complimentary methods, and the understanding that their results are only the first

step in a long process validating the causal roles of candidate loci. Keeping the limitations of outlier analysis and EAA in mind, these studies provide several insights into marine clinal adaptation: clinal adaptation can occur in the ocean across a wide range of gene flow regimes, clinal adaptation is driven by both subtle allele frequency variation across environmental gradients and large allele frequency variation at groups of loci in tight linkage, and evidence of hard sweeps from new mutations are not observed.

4.1 Evidence of Adaptation Despite Gene Flow

The generalization that marine species have such high rates of gene flow as to be essentially panmictic, making any evidence of local adaptation a paradox, is no longer the prevailing view (Hauser and Carvalho 2008). However, many marine species exist as large, continuously distributed populations characterized by low differentiation, high gene flow, and subtle patterns of IBD. In these species, the extent of local adaptation is determined by a balance between gene flow and natural selection (Lenormand 2002; Slatkin 1973). These species potentially experience high enough gene flow to significantly curtail local adaptation. However, the population genetic characteristics of this stereotypical marine species also suggest that clinal adaptation should be common, because large effective population size leads to efficient selection and reduced impact of genetic drift.

Marine population genomic studies consistently identify candidate loci for adaptation to environmental gradients in spite of high gene flow. Atlantic herring (*Clupea harengus*) closely fit the population genetic stereotype for marine species. High gene flow and enormous effective population size act in concert to limit divergence for the majority of loci. Despite the distribution over the entire North Atlantic, the maximum degree of differentiation (F_{ST}) among populations is less than 2%. Against this backdrop of low differentiation, there are significant allele frequency changes associated with a gradual salinity cline extending from the North Sea to the far reaches of the Baltic where salinity declines to three parts per thousand. This finding appears robust to a variety of analytical approaches and sequencing techniques. F_{ST} outlier analysis for genetic markers generated by SNP chips, whole genome sequence and exome capture discover outlier loci in both pairwise comparisons between Baltic and Atlantic populations (Limborg et al. 2012; Martinez Barrio et al. 2016) and among populations distributed across this salinity cline within the Baltic (Lamichhaney et al. 2012, 2017; Limborg et al. 2012). Candidate loci identified as outliers are also identified in parallel by environmental association analysis in this system (Limborg et al. 2012; Martinez Barrio et al. 2016). Similar marine species with large effective population sizes, high gene flow, and correspondingly low genetic differentiation but high genetic diversity including Atlantic cod, European hake, European anchovy, American lobster, pacific Lamprey, and red abalone show similar patterns (see Table 1 for references); in spite of high gene flow, allele frequencies correlate with environmental gradients expected to drive selection

after controlling for demography and candidate loci bear population genetic signatures of selection.

Of course, the stereotypical marine species with large continuously distributed populations and resultant low genetic differentiation poorly represents the diversity of demographies observed in marine species. Species with highly structured populations, small effective population sizes, and even entirely panmictic species are observed in the ocean. A review of the literature suggests that candidate loci for clinal adaptation are discovered across a wide range of gene flow regimes. Studies on salmonids suggest that clinal adaptation occurs among highly spatially structured species (Bourret et al. 2013; Fraser et al. 2011; Hecht et al. 2015; Jeffery et al. 2017). Owing to their natal homing and demersal eggs, anadromous salmon species have reduced gene flow along their extensive ranges, producing strong patterns IBD compared to the species considered so far. In Atlantic salmon, populations are structured hierarchically, within separate lineages (Jeffery et al. 2017). This hierarchical structure makes it difficult to discern outlier loci potentially underlying clinal adaptation from spurious outliers owing to secondary recontact or coupling of endogenous and exogenous selection (Excoffier et al. 2009). This challenge is highlighted by a finding that strongly implicates endogenous selection; outlier loci are dispersed along the genome, rather than clustered within high LD regions (Bourret et al. 2013). However, the finding that some of these clinal outliers occur in parallel across strongly diverged lineages on either side of the Atlantic suggest that at least some may be true targets of selection (Jeffery et al. 2017). To address a similar problem, Hecht et al. (2015) leverage variance partitioning in redundancy analysis of range-wide genetic variation of Chinook salmon. They find that much of the genetic variation that correlates with environmental variables is collinear with the neutral structure among separate lineages and therefore may be due to endogenous barriers to gene flow or secondary recontact, but some genetic variation is best explained by environmental variables alone, strongly implicating a role for selection due to environmental gradients in shaping the population genetic structure of this species.

At the other end of the gene flow spectrum, outlier analyses and EAA studies also identify candidate loci among completely panmictic species or among microhabitats within panmictic populations. In these cases, local adaptation does not occur as a balance between the homogenizing effect of gene flow and the diversifying effect of selection. Instead recurrent spatially variable selection reestablishes locally adaptive divergence along environmental gradients each generation. In the American eel and its sister species, the European eel, all breeding individuals migrate to a communal spawning ground and produce complete panmixia among their offspring (Cote et al. 2013; Pujolar et al. 2014). Despite this panmixia, there is evidence of local adaptation along the species range including gene expression and organismal fitness measurements (Cote et al. 2014; Kalujnaia et al. 2007), and candidate loci for spatially varying selection are found using both F_{ST} outlier and environmental association analysis approaches (Gagnaire et al. 2012; Pujolar et al. 2014). These findings are corroborated by results at much smaller spatial scales. Some authors have observed F_{ST} outliers among microhabitats within single panmictic populations

(Bay and Palumbi 2014; Wagner et al. 2017), highlighting the role of single generation spatially varying selection may play in maintaining genetic diversity.

Together, discovery of candidate loci among these studies suggests that clinal adaptation can occur in the ocean across a wide range of gene flow regimes. Interestingly, Guo et al. (2015) explicitly compare candidate loci underlying clinal adaptation in Baltic threespine stickleback under high gene flow to those from studies of divergence of marine ancestors and isolated freshwater lake populations in this species. There is striking parallelism of candidate loci across these two extremes of gene flow.

But are candidate loci true targets of selection or at least in linkage with true targets? Both demography and alternative selective scenarios, such as background selection and endogenous selection, can produce clinal patterns of genetic variation that can lead to spurious identification of candidates. Most studies attempting to uncover the molecular basis of clinal adaptation make some attempt to validate their candidate loci using multiple independent analyses. Candidates identified as F_{ST} outliers are often subsequently subjected to environmental association analysis, but these tests are not independent in systems with collinearity between environment and neutral structure, e.g., IBD along an environmental gradient (Meirmans 2012), or coupling of endogenous with exogenous selection or an environmental barrier to gene flow (Bierne et al. 2011). Stronger evidence stems from comparisons of spatial multivariate analysis of outlier and non-outlier datasets where outliers demonstrate a stronger signal of environmental dependence than non-outliers (Riginos et al. 2016), but the number of studies that apply this approach in marine clinal adaptation is limited (Benestan et al. 2016; Vandamme et al. 2014). Similarly, variance partitioning of RDA results parses orthogonal and collinear portions of genetic variation explained by environmental and neutral spatial factors (Peres-Neto et al. 2006). Studies that apply this technique to marine species discover that while neutral and putatively adaptive genetic variation are correlated across space, a portion of genetic variation is explained by environmental factors alone (Hecht et al. 2015; Vandamme et al. 2014). Genome scan studies also commonly apply gene annotation enrichment studies as a means of candidate validation. However, as with sequential application of outlier and EAA approaches, these two analytical approaches may not be independent, because genes in the same functional pathways are sometimes clustered along the genome in functional genomic neighborhoods (Pavlidis et al. 2012).

Genome scans of clinal adaptation sometimes recover candidate loci discovered in independent assays of natural selection. For example, the EDA locus responsible for lateral plating in stickleback is discovered as a candidate in outlier analysis and EAA studies (Guo et al. 2015; Pedersen et al. 2017). While this finding does not validate other candidate loci, it does demonstrate the sets of candidate loci at least contain true targets of selection. Another source of validation for candidate loci is the discovery of shared candidates across evolutionarily independent lineages, but low differentiation and high gene flow in most marine species preclude the study of independent lineages, and the polygenic nature of ecologically relevant traits (Le Corre and Kremer 2012; Rockman 2012) suggests there are many potential

targets of selection and convergent evolution may rely on different combinations of genes even across similar selective gradients (Laporte et al. 2015). While the shared origins of adaptive alleles in convergent evolution reduce the strength of adaptive inference, convergence of candidate loci does provide some corroboration.

Ultimately, validation of candidate loci in population genomic studies requires subsequent studies characterizing the functional significance of candidates. To date, such follow-up studies have not been conducted for the minority of studies that apply genome scan approaches to identify the molecular basis of clinal adaptation in marine species, perhaps because functional genetic studies, QTL analyses, and common garden or reciprocal transplant studies are challenging to accomplish in marine species (Sotka 2012).

4.2 Genetic Basis of Adaptation

Assuming that candidates identified in these studies contain true targets of selection, marine populations provide key insights into the molecular basis of adaptation. Does clinal adaptation rely on large- or small-effect alleles, new mutations or standing genetic variation, hard sweeps or soft sweeps? Single locus, biallelic models of the relationship between gene flow and selection are thoroughly developed and make clear predictions about the mechanisms that permit local adaptation despite the homogenizing effect of gene flow. When the migration rate is greater than the strength of selection, polymorphism will be lost (Lenormand 2002). However, it is unlikely that ecologically relevant traits subject to selection along an environmental gradient demonstrate such simple, oligogenic architectures. Instead results from genome-wide association, quantitative genetics, and experimental evolution all point to polygenic architectures for most traits (Boyle et al. 2017; Rockman 2012). Advances in recent theory have extended these predictions to traits with polygenic architecture.

Theoretical predictions for spatial patterns of allele frequency at the loci underlying clinal adaptation (QTL) differ depending on the population genetic assumptions of the model. Polygenic models with continuously distributed individuals over a shallow environmental gradient predict many discordant fixations or strong steep genetic clines over very short spatial distance relative to the scale of the environmental gradient (Barton 1999). Because each locus has a small effect, these successive, short, stepped clines extending across the environmental gradient produce a smooth trajectory of trait values that track the local optimum. In contrast, theoretical predictions based on an island model and polygenic trait architecture suggest gradual changes in allele frequency at individual loci across the subdivided metapopulation. As the number of loci contributing to the trait increases, the degree of differentiation at the QTL decreases relative to differentiation at neutral loci until differentiation at neutral and adaptive genomic regions are indistinguishable (Le Corre and Kremer 2012). Therefore, as the number of genes that control a trait approaches the infinitesimal model, local adaptation is determined more strongly by

genetic covariance of allelic effects than by differentiation (Kremer and Le Corre 2012; McKay and Latta 2002). While these results suggest that clinal adaptation is possible even with polygenic trait architectures and subtle allele frequency differences, they do not explain how such genetic covariance is maintained in spite of high gene flow characteristic of marine populations. Indeed, the probability that an adaptive allele will be lost by swamping is determined by the relative strength of selection and migration; therefore as effect size of individual alleles decrease with more polygenic architectures, adaptive alleles should become more prone to swamping (Yeaman and Otto 2011).

Under high gene flow and polygenic trait architectures, local adaptation is still possible under one of two scenarios (Tigano and Friesen 2016; Yeaman 2015). First, with sufficient genetic variation, transient covariance of adaptive alleles across the landscape can rapidly produce local adaptation (Yeaman and Whitlock 2011). Local adaptation by alleles susceptible to swamping is maintained because as allelic effect size decreases, these alleles demonstrate reduced divergence and the effect of migration on within-population trait genetic variance is decreased (Yeaman 2015). Second, the strength of selection at an individual locus is also determined by the effects of physically linked loci. Therefore, selection is more effective on combinations of adaptive alleles that are closely linked (Feder and Nosil 2010). In this way, clusters of small-effect alleles segregate as de facto large-effect alleles (Remington 2015). This process is particularly powerful if recombination rates are reduced in the region through structural variation (e.g., inversions) or chromosomal location (Kirkpatrick and Barton 2006).

Therefore, there are three primary population genetic expectations for clinal adaptation under high gene flow and polygenic traits: (1) large allele frequency shifts at many loci of small to moderate effects that are discordant across space (Barton 1999); (2) transient, subtle allele frequency variation at adaptive QTL; and (3) strong genetic clines among supergenes or genomic islands of divergence (Kirkpatrick 2010; Schwander et al. 2014). EAA and F_{ST} outlier approaches differ in their ability to recognize loci with these distinct spatial patterns. F_{ST} outlier analysis is strongly biased toward the discovery of large allele frequency changes across populations, while the majority of EAA approaches assume a linear relationship between allele frequency and environmental variables under selection (Rellstab et al. 2015). Therefore, ascertainment bias precludes the results from each class of analyses from definitely resolving this question. For example, the frequent discovery by EAA of candidate genes that demonstrate a gradual change in allele frequency over environmental gradient does not provide insight into the frequency of stepped genetic clines because they are relatively underpowered to detect these changes.

The literature does not strongly support theory that spatially discordant, successive fixations at many loci drive clinal adaptation, nor does it unequivocally refute this mode of clinal adaptation. While studies rarely address this question directly, the role of adaptive fixation can be inferred from the F_{ST} value of candidate loci identified in outlier analysis (Bernatchez 2016). Maximum outlier F_{ST} values near one are rarely reported for outlier analyses conducted along marine clines (Table 1), despite the observation that this approach is biased toward identification of loci with

extreme F_{ST} values. However, absence of evidence is not evidence of absence. Outlier analysis in clinal marine populations is rarely conducted at high enough genomic resolution to effectively cover the entirety of the genome (Lowry et al. 2017); therefore the outlier SNP may only be in partial linkage with the causative locus and demonstrate a reduced signature of selection relative to the true target (Schrider et al. 2015).

The role of transient, subtle shifts in allele frequency across environmental gradients has stronger support in the literature. At face value, spatial variation in allele frequency at candidate loci identified by EAA is evidence of gradual adaptive gradients of allele frequency at individual loci. High explanatory power of constrained ordinations of genetic variation also provide circumstantial evidence for this genetic mode of clinal adaptation, because it represents a correlation between polygenic allele frequency variation (ordination of genetic variation response variables) with environmental gradients after controlling for spatial effects. For example, Hecht et al. (2015) are able to explain up to 22% of genetic variation within a lineage of Chinook salmon after controlling for spatial effects, but very few studies have used this approach so far. The transient nature of these subtle allele frequency clines has both theoretical and limited empirical support in marine populations. Theoretically, large effective size in marine species should support large standing genetic variation through high efficiency of balancing selection mediated by reduced loss of alleles to drift and recurrent mutation (Gagnaire and Gaggiotti 2016). A spatiotemporal analysis of genetic variation in Atlantic cod from museum samples spanning 80 years demonstrates that loci identified as EAA and F_{ST} outliers are temporally dynamic, with no locus identified as an adaptive candidate across the temporal range of the study (Therkildsen et al. 2013a), strongly implicating a role for fluctuating adaptive genetic architectures.

Finally, there is extensive evidence from marine populations that chromosomal rearrangements and genomic cold spots of recombination act to protect genomic islands of divergence from the homogenizing effects of gene flow. Analysis of genomic divergence between sympatric ecotypes demonstrates that groups of adaptive alleles are held together in tight linkage by inversions in Atlantic herring (Martinez Barrio et al. 2016), Atlantic cod (Berg et al. 2016), and stickleback (Marques et al. 2016; Roesti et al. 2015). However, studies in Atlantic cod (Barney et al. 2017; Barth et al. 2017; Sodeland et al. 2016) and the seaweed fly *Coelopa frigida* (Wellenreuther et al. 2017) have highlighted that this phenomenon is not isolated to highly structured populations but also occurs among continuously distributed populations that are clinally adapted to environmental gradients. The frequency of inversions in Atlantic cod demonstrates large shifts across a thermal gradient with alternative forms nearly fixed across warm and cold environments.

In addition to the scale of allele frequency variation across space and genomic islands of divergence, population genomic studies of clinally adapted marine organisms provide insight into the relative roles of hard and soft sweeps and whether adaptation proceeds through selection on the standing genetic variation or on new mutations. Effective population size is a strong predictor of the prevalence of hard sweeps from new mutations vs. soft sweeps from the standing genetic variation

(Hermisson and Pennings 2005; Jensen 2014); therefore the expectation is that hard sweeps should be rare in marine species. Circumstantial evidence for a role of soft sweeps and evolution from the standing genetic variation comes from the absence of allelic fixations discovered by F_{ST} outlier analyses (Bernatchez 2016). However, this can be due to an incomplete portrait of genomic variation in the study and spurious identification of the signal of soft sweeps due to "soft shoulders" surrounding the adaptive haplotype (discussed above) (Schrider et al. 2015). Instead, robust inference of the origins of adaptive alleles must stem from haplotype-based outlier analysis or an in-depth analysis of nucleotide diversity surrounding candidate loci (Pavlidis and Alachiotis 2017). While site frequency spectrum and nucleotide diversity statistics are sometimes reported for candidate loci in EAA and F_{ST} outlier analysis, only one study directly addresses the role of soft sweeps in marine clinal adaptation. This study is also the only follow-up study on a set of candidates identified in a genome scan for marine clinal adaptation that was discovered in a search of the literature. Pespeni and Palumbi (2013) examined nucleotide diversity, site frequency spectrum, and spatial variation in allele frequency and candidate loci identified in a previous genome scan for selection the purple sea urchin. Although their analysis confirms that candidate loci bear signatures of selection, they do not detect reduced nucleotide diversity or alteration in the site frequency spectrum surrounding candidate loci, strongly implicating that clinal adaptation in the clinally adapted purple sea urchin is driven by spatially varying, balancing selection on the standing genetic variation producing patterns of soft sweeps.

5 Future Directions

Marine clinal genetics is dominated by genome scans for selection, despite the possibility that many studies are enriched for false positives owing to inaccurate approximation of null/neutral models. While these studies have provided key insights (see above), future work should place an emphasis on more strongly inferring adaptive significance of candidate loci contributing to clinal adaptation. A promising avenue for development in this area is better integrating the phenotype into analyses by combining association genetics and/or quantitative genetic analysis with genome scans for selection (Berg and Coop 2014; Gagnaire and Gaggiotti 2016).

The genetic architecture of traits influences how allele frequencies at underlying QTL respond to selection (Le Corre and Kremer 2012) and therefore the effectiveness of genome scans. If genetic architectures are highly polygenic, the signal of selection at individual loci decreases. In the case of clinally adapted marine populations, spatially varying selection with strong gene flow and large population sizes is predicted to have a specific set of responses: either tight linkage de facto large-effect alleles with strong allele frequency changes or polygenic adaptation that has a transient genetic basis owing to dynamic equilibrium of allele loss due to swamping but recurring adaptive mutations owing to large population size (Yeaman

2015). These architectures are not mutually exclusive and the evidence presented above suggest that clinal adaptation can depend on both. Therefore, the effect size distribution of adaptive QTL in clinal marine populations is likely to have a high variance (Gagnaire and Gaggiotti 2016), and genome scan results are likely to identify only a portion of adaptive variation.

Integrating the phenotype into genome scans for selection may offer a solution to identifying the signal of polygenic selection. If the genes underlying a trait are known, trait values can be predicted for individuals or populations given their genotypes or distribution of genotypes respectively. Correlation between these genetic values and environmental variables reveal the action of selection on genetic covariance on allelic effects that is expected as a response to selection under highly polygenic architectures, rather than relying on correlation of individual alleles with environmental factors as in EAA or outlier analysis. Such an approach has been developed by Berg and Coop (2014) that relies on additive effect size estimates of individual loci from genome-wide association study results and incorporates a model of demographic effects such as drift on the genetic trait value.

Of course, genome-wide association studies suffer from the same limitation as genome scans for selection. Even with large sample sizes and dense genotyping, univariate associations between genetic markers and traits can only identify causal variants with moderate to large phenotypic effects, and the resources to conduct GWAS are not available for marine species. However, statistical developments in the field of gene-trait association identification and estimation of heritability have improved in recent years (Wellenreuther and Hansson 2016). Developments of the latter include chromosome partitioning, regional heritability mapping, and improvements to pedigree-free estimation of kinship. Combining these results with those from genome scans for selection will allow researchers to determine whether their candidates represent a complete picture of adaptive genetic variation. With respect to identifying gene-trait associations, increasing recognition of classification and regression algorithms from machine learning has also improved statistical inference for genome-wide association analysis. For example, random forest is a nonparametric regression and classification algorithm that is applicable to high-dimensionality datasets where the number of predictor variables (genes) far outnumbers the response variables (phenotype) (Huang and Boutros 2016). In contrast to univariate association tests, random forest can model the joint effects of many predictor variables together, increasing power to identify both polygenic trait architectures and predictor interactions (i.e., epistasis) (Winham et al. 2012). The use of random forest in genome-wide association analysis has produced a wide body of literature on its sensitivity to population structure, cryptic relatedness among samples, and varying trait architectures (Boulesteix et al. 2012; Chen and Ishwaran 2012; Goldstein et al. 2011; Stephan et al. 2015).

As classification and regression algorithms, machine learning techniques can also be applied directly to EAA. Although the sensitivity of these approaches to confounding factors such as demography have yet to be rigorously explored in the context of EAA, several studies have begun to identify candidate loci associated

with environmental variables using random forest (Brieuc et al. 2015; Laporte et al. 2016; Pavey et al. 2015).

6 Conclusions

Local adaptation of marine species distributed across environmental gradients is no longer a paradox. There is evidence that some genomic regions exhibit substantial differentiation with respect to the rest of the genome and that much of this differentiation correlates with environmental variation that may drive selection. This finding is robust to a variety of analytical techniques and demographic scenarios. Assuming the candidate loci identified in such analyses contain true targets of selection, marine clinal population genomic studies suggest that adaptation depends, at least in part, on both transient, small-effect loci and chromosomal rearrangements and differentiation at large-effect "supergenes." Importantly, discovery bias among analytical techniques prevents inference into the relative importance of these genetic architectures of adaptation.

Despite these great strides in our understanding of the genetic basis of adaptation in the ocean, our understanding of the mechanisms by which variation at the level of SNPs discovered as candidates in genome scans translates into variation in traits under selection is unclear. Future studies should focus on closing this gap of knowledge, through a better integration of the phenotype in the identification of candidates and through rigorous verification of candidates where it is possible.

References

Adams SM, Lindmeier JB, Duvernell DD. Microsatellite analysis of the phylogeography, pleistocene history and secondary contact hypotheses for the killifish, Fundulus heteroclitus. Mol Ecol. 2006;15:1109–23.

Aguillon S, Fitzpatrick JW, Bowman R, et al. Deconstructing isolation-by-distance: the genomic consequences of limited dispersal. bioRxiv. 2017.

Assis J, Serrao EA, Claro B, Perrin C, Pearson GA. Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. Mol Ecol. 2014;23:2797–810.

Barney BT, Munkholm C, Walt DR, Palumbi SR. Highly localized divergence within supergenes in Atlantic cod (Gadus morhua) within the Gulf of Maine. BMC Genomics. 2017;18:271.

Barth JMI, Berg PR, Jonsson PR, et al. Genome architecture enables local adaptation of Atlantic cod despite high connectivity. Mol Ecol. 2017;26:4452–66.

Barton NH. Clines in polygenic traits. Genet Res. 1999;74:223–36.

Barton NH, Hewitt GM. Adaptation, speciation and hybrid zones. Nature. 1989;341:497–503.

Bay RA, Palumbi SR. Multilocus adaptation associated with heat resistance in reef-building corals. Curr Biol. 2014;24:2952–6.

Beaumont MA, Nichols RA. Evaluating loci for use in the genetic analysis of population structure. Proc R Soc B Biol Sci. 1996;263:1619–26.

Benestan L, Quinn BK, Maaroufi H, et al. Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (Homarus americanus). Mol Ecol. 2016;25:5073–92.
Berg JJ, Coop G. A population genetic signal of polygenic adaptation. PLoS Genet. 2014;10:e1004412.
Berg PR, Jentoft S, Star B, et al. Adaptation to low salinity promotes genomic divergence in Atlantic cod (Gadus morhua l.). Genome Biol Evol. 2015;7:1644–63.
Berg PR, Star B, Pampoulie C, et al. Three chromosomal rearrangements promote genomic divergence between migratory and stationary ecotypes of Atlantic cod. Sci Rep. 2016;6:23246.
Bernatchez L. On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. J Fish Biol. 2016;89:2519–56.
Bierne N. The distinctive footprints of local hitchhiking in a varied environment and global hitchhiking in a subdivided population. Evolution. 2010;64:3254–72.
Bierne N, Welch J, Loire E, Bonhomme F, David P. The coupling hypothesis: why genome scans may fail to map local adaptation genes. Mol Ecol. 2011;20:2044–72.
Bierne N, Bonhomme F, Arnaud-Haond S. Editorial dedicated population genomics for the silent world: the specific questions of marine population genetics. Curr Zool. 2016;62:545–50.
Blanchet FG, Legendre P, Borcard D. Modelling directional spatial processes in ecological data. Ecol Model. 2008;215:325–36.
Bonhomme M, Chevalet C, Servin B, et al. Detecting selection in population trees: the Lewontin and Krakauer test extended. Genetics. 2010;186(1):241–62.
Boulesteix A-L, Janitza S, Kruppa J, König IR. Overview of random forest methodology and practical guidance with emphasis on computational biology and bioinformatics. Wiley Interdiscip Rev Data Mining Knowl Discov. 2012;2:493–507.
Bourret V, Kent MP, Primmer CR, et al. SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (Salmo salar). Mol Ecol. 2013;22:532–51.
Boyle EA, Li YI, Pritchard JK. An expanded view of complex traits: from polygenic to omnigenic. Cell. 2017;169:1177–86.
Bradburd G, Coop G, Ralph P. 2017.
Bradbury IR, Hubert S, Higgins B, et al. Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. Proc Biol Sci. 2010;277:3725–34.
Brieuc MS, Ono K, Drinan DP, Naish KA. Integration of Random Forest with population-based outlier analyses provides insight on the genomic basis and evolution of run timing in Chinook salmon (Oncorhynchus tshawytscha). Mol Ecol. 2015;24:2729–46.
Brommer JE. Whither Pst? The approximation of Qst by Pst in evolutionary and conservation biology. J Evol Biol. 2011;24:1160–8.
Burford MO, Scarpa J, Cook BJ, Hare MP. Local adaptation of a marine invertebrate with a high dispersal potential: evidence from a reciprocal transplant experiment of the eastern oyster Crassostrea virginica. Mar Ecol Prog Ser. 2014;505:161–75.
Charlesworth B, Nordborg M, Charlesworth D. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. Genet Res. 1997;70:155–74.
Chen X, Ishwaran H. Random forests for genomic data analysis. Genomics. 2012;99:323–9.
Conover DO, Clarke LM, Munch SB, Wagner GN. Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. J Fish Biol. 2006;69:21–47.
Corander J, Majander KK, Cheng L, Merila J. High degree of cryptic population differentiation in the Baltic Sea herring Clupea harengus. Mol Ecol. 2013;22:2931–40.
Cote CL, Gagnaire PA, Bourret V, et al. Population genetics of the American eel (Anguilla rostrata): Fst = 0 and North Atlantic oscillation effects on demographic fluctuations of a panmictic species. Mol Ecol. 2013;22:1763–76.

Cote CL, Castonguay M, Kalujnaia MS, Cramb G, Bernatchez L. In absence of local adaptation, plasticity and spatially varying selection rule: a view from genomic reaction norms in a panmictic species (Anguilla rostrata). BMC Genomics. 2014;15:403.

Crawford DL, Powers DA. Molecular basis of evolutionary adaptation at the lactate dehydrogenase-b locus in the fish Fundulus heteroclitus. Proc Natl Acad Sci. 1989;86:9365–9.

de Villemereuil P, Frichot E, Bazin E, Francois O, Gaggiotti OE. Genome scan methods against more complex models: when and how much should we trust them? Mol Ecol. 2014;23:2006–19.

de Villemereuil P, Gaggiotti OE, O'Hara RB. A new FST-based method to uncover local adaptation using environmental variables. Methods Ecol Evol. 2015;6:1248–58.

De Wit P, Palumbi SR. Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and local adaptation. Mol Ecol. 2013;22:2884–97.

Defaveri J, Merila J. Evidence for adaptive phenotypic differentiation in Baltic Sea sticklebacks. J Evol Biol. 2013;26:1700–15.

DiMichele L, Powers DA. Physiological basis for swimming endurance differences between LDH-B genotypes of Fundulus heteroclitus. Science. 1982;216:1014–6.

Dray S, Legendre P, Peres-Neto PR. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol Model. 2006;196:483–93.

Drury C, Dale KE, Panlilio JM, et al. Genomic variation among populations of threatened coral: Acropora cervicornis. BMC Genomics. 2016;17:286.

Duforet-Frebourg N, Bazin E, Blum MG. Genome scans for detecting footprints of local adaptation using a Bayesian factor model. Mol Biol Evol. 2014;31:2483–95.

Eldon B, Riquet F, Yearsley J, Jollivet D, Broquet T. Current hypotheses to explain genetic chaos under the sea. Curr Zool. 2016;62:551–66.

Endler JA. Geographic variation, speciation, and clines. Princeton: Princeton University Press; 1977.

Excoffier L, Hofer T, Foll M. Detecting loci under selection in a hierarchically structured population. Heredity. 2009;103:285.

Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 2003;164:1567–87.

Feder JL, Nosil P. The efficacy of divergence hitchhiking in generating genomic islands during ecological speciation. Evolution. 2010;64:1729–47.

Flanagan SP, Rose E, Jones AG. Population genomics reveals multiple drivers of population differentiation in a sex-role-reversed pipefish. Mol Ecol. 2016;25:5043–72.

Foll M, Gaggiotti O. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics. 2008;180:977–93.

Forester BR, Jones MR, Joost S, Landguth EL, Lasky JR. Detecting spatial genetic signatures of local adaptation in heterogeneous landscapes. Mol Ecol. 2016;25:104–20.

Frantz AC, Cellina S, Krier A, Schley L, Burke T. Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? J Appl Ecol. 2009;46:493–505.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity (Edinb). 2011;106:404–20.

Frichot E, Schoville SD, Bouchard G, Francois O. Testing for associations between loci and environmental gradients using latent factor mixed models. Mol Biol Evol. 2013;30:1687–99.

Gaggiotti OE, Bekkevold D, Jorgensen HB, et al. Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: Atlantic herring as a case study. Evolution. 2009;63:2939–51.

Gagnaire P-A, Gaggiotti OE. Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. Curr Zool. 2016;62:603–16.

Gagnaire PA, Minegishi Y, Zenboudji S, et al. Within-population structure highlighted by differential introgression across semipermeable barriers to gene flow in Anguilla marmorata. Evolution. 2011;65:3413–27.

Gagnaire PA, Normandeau E, Cote C, Moller Hansen M, Bernatchez L. The genetic consequences of spatially varying selection in the panmictic American eel (Anguilla rostrata). Genetics. 2012;190:725–36.
Gagnaire PA, Broquet T, Aurelle D, et al. Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. Evol Appl. 2015;8:769–86.
Galindo HM, Pfeiffer-Herbert AS, McManus MA, et al. Seascape genetics along a steep cline: using genetic patterns to test predictions of marine larval dispersal. Mol Ecol. 2010;19:3692–707.
Goldstein BA, Polley EC, Briggs FB. Random forests for genetic association studies. Stat Appl Genet Mol Biol. 2011;10:32.
Gosset CC, Bierne N. Differential introgression from a sister species explains high f(st) outlier loci within a mussel species. J Evol Biol. 2013;26:14–26.
Gunther T, Coop G. Robust identification of local adaptation from allele frequencies. Genetics. 2013;195:205–20.
Guo B, DeFaveri J, Sotelo G, Nair A, Merila J. Population genomic evidence for adaptive differentiation in Baltic Sea three-spined sticklebacks. BMC Biol. 2015;13:19.
Hauser L, Carvalho GR. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 2008;9:333–62.
Hecht BC, Matala AP, Hess JE, Narum SR. Environmental adaptation in Chinook salmon (Oncorhynchus tshawytscha) throughout their North American range. Mol Ecol. 2015;24:5573–95.
Hermisson J, Pennings PS. Soft sweeps: molecular population genetics of adaptation from standing genetic variation. Genetics. 2005;169:2335–52.
Hess JE, Campbell NR, Close DA, Docker MF, Narum SR. Population genomics of pacific lamprey: adaptive variation in a highly dispersive species. Mol Ecol. 2013;22:2898–916.
Hice LA, Duffy TA, Munch SB, Conover DO. Spatial scale and divergent patterns of variation in adapted traits in the ocean. Ecol Lett. 2012;15:568–75.
Hoban S, Kelley JL, Lotterhos KE, et al. Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. Am Nat. 2016;188:379–97.
Hu XS, He F. Background selection and population differentiation. J Theor Biol. 2005;235:207–19.
Huang BF, Boutros PC. The parameter sensitivity of random forests. BMC Bioinformatics. 2016;17:331.
Jeffery NW, Stanley RRE, Wringe BF, et al. Range-wide parallel climate-associated genomic clines in Atlantic salmon. R Soc Open Sci. 2017;4:171394.
Jensen JD. On the unfounded enthusiasm for soft selective sweeps. Nat Commun. 2014;5:5281.
Jensen JD, Kim Y, DuMont VB, Aquadro CF, Bustamante CD. Distinguishing between selective sweeps and demography using DNA polymorphism data. Genetics. 2005;170:1401–10.
Jones FC, Grabherr MG, Chan YF, et al. The genomic basis of adaptive evolution in threespine sticklebacks. Nature. 2012;484:55–61.
Jones MR, Forester BR, Teufel AI, et al. Integrating landscape genomics and spatially explicit approaches to detect loci under selection in clinal populations. Evolution. 2013;67:3455–68.
Joost S, Vuilleumier S, Jensen JD, et al. Uncovering the genetic basis of adaptive change: on the intersection of landscape genomics and theoretical population genetics. Mol Ecol. 2013;22:3659–65.
Kalujnaia S, McWilliam I, Zaguinaiko V, et al. Salinity adaptation and gene profiling analysis in the European eel (Anguilla anguilla) using microarray technology. Gen Comp Endocrinol. 2007;152:274–80.
Kawecki TJ, Ebert D. Conceptual issues in local adaptation. Ecol Lett. 2004;7:1225–41.
Kirkpatrick M. How and why chromosome inversions evolve. PLoS Biol. 2010;8:e1000501.
Kirkpatrick M, Barton N. Chromosome inversions, local adaptation and speciation. Genetics. 2006;173:419–34.
Klopfstein S, Currat M, Excoffier L. The fate of mutations surfing on the wave of a range expansion. Mol Biol Evol. 2006;23:482–90.

Kosloski ME, Dietl GP, Handley JC. Anatomy of a cline: dissecting anti-predatory adaptations in a marine gastropod along the U.S. Atlantic Coast. Ecography. 2017;40(11):1285–99.

Kremer A, Le Corre V. Decoupling of differentiation between traits and their underlying genes in response to divergent selection. Heredity (Edinb). 2012;108:375–85.

Kruuk LE, Baird SJ, Gale KS, Barton NH. A comparison of multilocus clines maintained by environmental adaptation or by selection against hybrids. Genetics. 1999;153:1959–71.

Kumar SK, Feldman MW, Rehkopf DH, Tuljapurkar S. Limitations of GCTA as a solution to the missing heritability problem. Proc Natl Acad Sci. 2016;113:E61–70.

Lamichhaney S, Martinez Barrio A, Rafati N, et al. Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. Proc Natl Acad Sci U S A. 2012;109:19345–50.

Lamichhaney S, Fuentes-Pardo AP, Rafati N, et al. Parallel adaptive evolution of geographically distant herring populations on both sides of the North Atlantic Ocean. Proc Natl Acad Sci U S A. 2017;114:E3452–61.

Laporte M, Rogers SM, Dion-Cote AM, et al. RAD-QTL mapping reveals both genome-level parallelism and different genetic architecture underlying the evolution of body shape in lake whitefish (*Coregonus clupeaformis*) species pairs. G3 (Bethesda). 2015;5:1481–91.

Laporte M, Pavey SA, Rougeux C, et al. Rad sequencing reveals within-generation polygenic selection in response to anthropogenic organic and metal contamination in North Atlantic eels. Mol Ecol. 2016;25:219–37.

Le Corre V, Kremer A. The genetic differentiation at quantitative trait loci under local adaptation. Mol Ecol. 2012;21:1548–66.

Le Moan A, Gagnaire PA, Bonhomme F. Parallel genetic divergence among coastal-marine ecotype pairs of European anchovy explained by differential introgression after secondary contact. Mol Ecol. 2016;25:3187–202.

Ledoux JB, Aurelle D, Bensoussan N, et al. Potential for adaptive evolution at species range margins: contrasting interactions between red coral populations and their environment in a changing ocean. Ecol Evol. 2015;5:1178–92.

Legendre P, Legendre LF. Numerical ecology. Amsterdam: Elsevier; 2012.

Leinonen T, Cano JM, Makinen H, Merila J. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. J Evol Biol. 2006;19:1803–12.

Lenormand T. Gene flow and the limits to natural selection. Trends Ecol Evol. 2002;17:183–9.

Leong W, Sun PY, Edmands S. Latitudinal clines in temperature and salinity tolerance in tidepool copepods. J Hered. 2017;109:71–7.

Limborg MT, Helyar SJ, De Bruyn M, et al. Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (Clupea harengus). Mol Ecol. 2012;21:3686–703.

Lotterhos KE, Whitlock MC. Evaluation of demographic history and neutral parameterization on the performance of FST outlier tests. Mol Ecol. 2014;23:2178–92.

Lotterhos KE, Whitlock MC. The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. Mol Ecol. 2015;24:1031–46.

Lowry DB, Hoban S, Kelley JL, et al. Breaking rad: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. Mol Ecol Resour. 2017;17:142–52.

Ludt WB, Rocha LA, Ali J. Shifting seas: the impacts of pleistocene sea-level fluctuations on the evolution of tropical marine taxa. J Biogeogr. 2015;42:25–38.

Maggs CA, Castilho R, Foltz D, et al. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology. 2008;89:S108–22.

Manel S, Holderegger R. Ten years of landscape genetics. Trends Ecol Evol. 2013;28:614–21.

Mariani S, Peijnenburg K, Weetman D. Independence of neutral and adaptive divergence in a low dispersal marine mollusc. Mar Ecol Prog Ser. 2012;446:173–87.

Marko PB. 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. Mol Ecol. 2004;13:597–611.

Marques DA, Lucek K, Meier JI, et al. Genomics of rapid incipient speciation in sympatric threespine stickleback. PLoS Genet. 2016;12:e1005887.

Martinez Barrio A, Lamichhaney S, Fan G, et al. The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. Elife. 2016;5:e12081.

McKay JK, Latta RG. Adaptive population divergence: markers, QTL and traits. Trends Ecol Evol. 2002;17:285–91.

McKenzie JL, Dhillon RS, Schulte PM. Steep, coincident, and concordant clines in mitochondrial and nuclear-encoded genes in a hybrid zone between subspecies of Atlantic killifish, Fundulus heteroclitus. Ecol Evol. 2016;6:5771–87.

McManus MA, Woodson CB. Plankton distribution and ocean dispersal. J Exp Biol. 2012;215:1008–16.

Meirmans PG. The trouble with isolation by distance. Mol Ecol. 2012;21:2839–46.

Milano I, Babbucci M, Cariani A, et al. Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (Merluccius merluccius). Mol Ecol. 2014;23:118–35.

Orsini L, Vanoverbeke J, Swillen I, Mergeay J, De Meester L. Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. Mol Ecol. 2013;22:5983–99.

Parsons KJ, Concannon M, Navon D, et al. Foraging environment determines the genetic architecture and evolutionary potential of trophic morphology in cichlid fishes. Mol Ecol. 2016;25:6012–23.

Pavey SA, Gaudin J, Normandeau E, et al. Rad sequencing highlights polygenic discrimination of habitat ecotypes in the panmictic American eel. Curr Biol. 2015;25:1666–71.

Pavlidis P, Alachiotis N. A survey of methods and tools to detect recent and strong positive selection. J Biol Res (Thessalon). 2017;24:7.

Pavlidis P, Jensen JD, Stephan W. Searching for footprints of positive selection in whole-genome SNP data from nonequilibrium populations. Genetics. 2010;185:907–22.

Pavlidis P, Jensen JD, Stephan W, Stamatakis A. A critical assessment of storytelling: gene ontology categories and the importance of validating genomic scans. Mol Biol Evol. 2012;29:3237–48.

Payseur BA. Using differential introgression in hybrid zones to identify genomic regions involved in speciation. Mol Ecol Resour. 2010;10:806–20.

Pedersen SH, Ferchaud AL, Bertelsen MS, Bekkevold D, Hansen MM. Low genetic and phenotypic divergence in a contact zone between freshwater and marine sticklebacks: gene flow constrains adaptation. BMC Evol Biol. 2017;17:130.

Peres-Neto PR, Legendre P, Dray S, Borcard D. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology. 2006;87:2614–25.

Pespeni MH, Palumbi SR. Signals of selection in outlier loci in a widely dispersing species across an environmental mosaic. Mol Ecol. 2013;22:3580–97.

Pespeni MH, Ladner JT, Moczek AP. Signals of selection in conditionally expressed genes in the diversification of three horned beetle species. J Evol Biol. 2017;30:1644–57.

Prairie JC, Sutherland KR, Nickols KJ, Kaltenberg AM. Biophysical interactions in the plankton: a cross-scale review. Limnol Oceanogr Fluid Environ. 2012;2:121–45.

Pujol B, Wilson AJ, Ross RI, Pannell JR. Are q(st)-f(st) comparisons for natural populations meaningful? Mol Ecol. 2008;17:4782–5.

Pujolar JM, Jacobsen MW, Als TD, et al. Genome-wide single-generation signatures of local selection in the panmictic European eel. Mol Ecol. 2014;23:2514–28.

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. A practical guide to environmental association analysis in landscape genomics. Mol Ecol. 2015;24:4348–70.

Remington DL. Alleles versus mutations: understanding the evolution of genetic architecture requires a molecular perspective on allelic origins. Evolution. 2015;69:3025–38.

Riginos C, Crandall ED, Liggins L, Bongaerts P, Treml EA. Navigating the currents of seascape genomics: how spatial analyses can augment population genomic studies. Curr Zool. 2016;62:581–601.
Rockman MV. The QTN program and the alleles that matter for evolution: all that's gold does not glitter. Evolution. 2012;66:1–17.
Roesti M, Kueng B, Moser D, Berner D. The genomics of ecological vicariance in threespine stickleback fish. Nat Commun. 2015;6:8767.
Sanford E, Kelly MW. Local adaptation in marine invertebrates. Annu Rev Mar Sci. 2011;3:509–35.
Schmidt PS, Serrão EA, Pearson GA, et al. Ecological genetics in the North Atlantic: environmental gradients and adaptation at specific loci. Ecology. 2008;89:S91–S107.
Schrider DR, Mendes FK, Hahn MW, Kern AD. Soft shoulders ahead: spurious signatures of soft and partial selective sweeps result from linked hard sweeps. Genetics. 2015;200:267–84.
Schwander T, Libbrecht R, Keller L. Supergenes and complex phenotypes. Curr Biol. 2014;24:R288–94.
Selkoe KA, Gaines SD, Caselle JE, Warner RR. Current shifts and kin aggregation explain genetic patchiness in fish recruits. Ecology. 2006;87:3082–94.
Selkoe KA, Watson JR, White C, et al. Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. Mol Ecol. 2010;19:3708–26.
Serre D, Pääbo S. Evidence for gradients of human genetic diversity within and among continents. Genome Res. 2004;14:1679–85.
Slatkin M. Gene flow and selection in a cline. Genetics. 1973;75:733–56.
Slatkin M, Wiehe T. Genetic hitch-hiking in a subdivided population. Genet Res. 1998;71:155–60.
Smith JM, Haigh J. The hitch-hiking effect of a favourable gene. Genet Res. 1974;23:23–35.
Smith CT, Nelson RJ, Wood CC, Koop BF. Glacial biogeography of North American coho salmon (Oncorhynchus kisutch). Mol Ecol. 2001;10:2775–85.
Sodeland M, Jorde PE, Lien S, et al. "Islands of divergence" in the Atlantic cod genome represent polymorphic chromosomal rearrangements. Genome Biol Evol. 2016;8:1012–22.
Sotka EE. Natural selection, larval dispersal, and the geography of phenotype in the sea. Integr Comp Biol. 2012;52:538–45.
Stephan J, Stegle O, Beyer A. A random forest approach to capture genetic effects in the presence of population structure. Nat Commun. 2015;6:7432.
Strand AE, Williams LM, Oleksiak MF, Sotka EE. Can diversifying selection be distinguished from history in geographic clines? A population genomic study of killifish (Fundulus heteroclitus). PLoS One. 2012;7:e45138.
Tepolt CK, Palumbi SR. Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. Mol Ecol. 2015;24:4145–58.
Therkildsen NO, Hemmer-Hansen J, Als TD, et al. Microevolution in time and space: SNP analysis of historical DNA reveals dynamic signatures of selection in Atlantic cod. Mol Ecol. 2013a;22:2424–40.
Therkildsen NO, Hemmer-Hansen J, Hedeholm RB, et al. Spatiotemporal SNP analysis reveals pronounced biocomplexity at the northern range margin of Atlantic cod Gadus morhua. Evol Appl. 2013b;6:690–705.
Tigano A, Friesen VL. Genomics of local adaptation with gene flow. Mol Ecol. 2016;25:2144–64.
Van Wyngaarden M, Snelgrove PV, DiBacco C, et al. Identifying patterns of dispersal, connectivity and selection in the sea scallop, *Placopecten magellanicus*, using RADseq-derived SNPs. Evol Appl. 2017;10:102–17.
Vandamme SG, Maes GE, Raeymaekers JA, et al. Regional environmental pressure influences population differentiation in turbot (Scophthalmus maximus). Mol Ecol. 2014;23:618–36.
Vasemagi A. The adaptive hypothesis of clinal variation revisited: single-locus clines as a result of spatially restricted gene flow. Genetics. 2006;173:2411–4.

Vitti JJ, Grossman SR, Sabeti PC. Detecting natural selection in genomic data. Annu Rev Genet. 2013;47:97–120.
Wagner DN, Baris TZ, Dayan DI, et al. Fine-scale genetic structure due to adaptive divergence among microhabitats. Heredity (Edinb). 2017;118:594–604.
Waples RS, Punt AE, Cope JM. Integrating genetic data into management of marine resources: how can we do it better? Fish Fish. 2008;9:423–49.
Wellenreuther M, Hansson B. Detecting polygenic evolution: problems, pitfalls, and promises. Trends Genet. 2016;32:155–64.
Wellenreuther M, Rosenquist H, Jaksons P, Larson KW. Local adaptation along an environmental cline in a species with an inversion polymorphism. J Evol Biol. 2017;30:1068–77.
Winham SJ, Colby CL, Freimuth RR, et al. SNP interaction detection with random forests in high-dimensional genetic data. BMC Bioinformatics. 2012;13:164.
Wright S. Isolation by distance. Genetics. 1943;28:114–38.
Yeaman S. Local adaptation by alleles of small effect. Am Nat. 2015;186(Suppl 1):S74–89.
Yeaman S, Otto SP. Establishment and maintenance of adaptive genetic divergence under migration, selection, and drift. Evolution. 2011;65:2123–9.
Yeaman S, Whitlock MC. The genetic architecture of adaptation under migration-selection balance. Evolution. 2011;65:1897–911.

The Population Genomics of Parallel Adaptation: Lessons from Threespine Stickleback

Paul A. Hohenlohe and Isabel Santos Magalhaes

Abstract Threespine stickleback fish (*Gasterosteus aculeatus*) have long been an ecological and evolutionary model system. Stickleback exhibit remarkable patterns of parallel adaptation among populations across their range, most notably repeated colonization and adaptation in freshwater habitats from ancestral marine or anadromous forms and repeated diversification into different freshwater ecotypes such as lake/stream and benthic/limnetic. The phenotypic traits involved in this adaptive evolution include physiology, behavior, life history, pigmentation, and numerous aspects of body size, shape, and morphology, the genetic basis of which has been elucidated through laboratory-based genetic mapping. With the advent of next-generation sequencing and the availability of a well-assembled reference genome for the species, numerous studies have identified genomic regions exhibiting signatures of selection in natural populations. The combination of these approaches has established numerous linkages among genotype, phenotype, environment, and adaptation. Here we review these results and assess alternative modes for the genetic basis of parallel phenotypic adaptation in terms of the genetic architecture of the traits and the source of adaptive variation across populations. We highlight examples ranging from single genes of major effect to polygenic traits and from reuse of allelic variation shared among populations to independent mutations across loci. Demographic scenarios such as serial colonization and adaptation, along with genomic features such as inversion polymorphism, provide insights into how widespread parallel adaptation in multiple phenotypes can occur. The diversity of genetic mechanisms for parallel evolution in stickleback leads to the "Everyone Wins" principle of biology—nearly any alternative mechanism plays a role in at least some cases, and often multiple mechanisms act concurrently. Because of the wealth of natural evolutionary experiments and the ever-expanding set of genomics and

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other tools available in this species, threespine stickleback will likely remain a key model system for population genomics studies of adaptation.

Keywords Adaptive radiation · *Gasterosteus aculeatus* · Genome scan · GWAS · Parallel evolution · QTL mapping

1 Adaptive Evolution in Threespine Stickleback

1.1 Ecological Diversity and Parallel Evolution

With a nearly circumpolar marine distribution in the Northern Hemisphere and ongoing repeated colonization of freshwater habitats, threespine stickleback fish (*Gasterosteus aculeatus*) exhibits remarkable diversity in a wide range of phenotypes (Fig. 1). This diversity, combined with their local abundance and ease of collection, has made threespine stickleback an evolutionary model system for the study of rapid and dramatic evolution of morphological, physiological, and behavioral adaptations to new environments and, in particular, replicated colonization and adaptation to a set of habitats distributed across the species range. Early studies attempting to characterize the species were presented with a bewildering amount of phenotypic diversity that challenged taxonomic groupings (Bell and Foster 1994). Further research in the twentieth century began correlating phenotypic diversity with habitat, suggesting that natural selection could probably account for much of the observed variation (Hagen and McPhail 1970; Bell 1976). Stickleback became a model system for adaptive radiation and sympatric diversification with the discovery of evidence for character displacement in lakes where morphologically distinct types coexisted, with links between morphology and resource use, and reduced gene flow between morphotypes even in sympatry (Schluter and McPhail 1992; Schluter 1993, 1996).

A remarkable feature of stickleback diversity is that similar phenotypes can be found repeatedly in similar environments in geographically isolated locations across the species range, suggesting independent but phenotypically parallel adaptation. For instance, Lavin and McPhail (1993) found repeated phenotypic differences between lake and stream populations in British Columbia, arguing that phenotypic similarities could be the result of parallel evolution. Since then the number of studies of parallel evolution and adaptive radiation in stickleback has continued to grow, along multiple phenotypic and environmental axes. With the advent of genomics and other experimental tools that are readily applied in this species, many researchers have combined various types of data to address the connections among genotype, phenotype, environment, and adaptation. Table 1 presents a representative set of publications focused on parallel evolution in threespine stickleback, based on a simple and far-from-exhaustive web search. Not only has the frequency of such publications steadily increased, but they are well-cited in the broader literature, showing the influence of stickleback research on the field of evolutionary biology (Fig. 2).

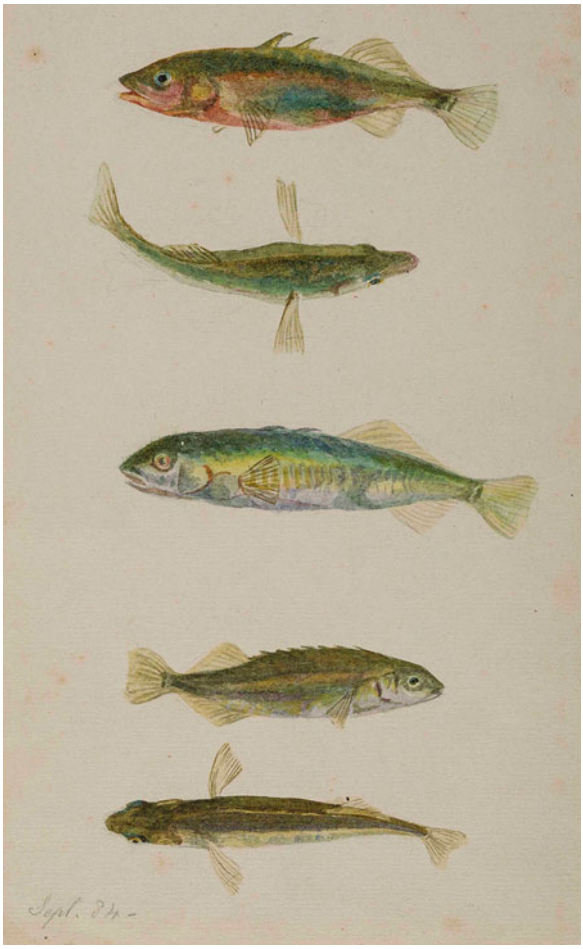

Fig. 1 Phenotypic variation and popular appreciation of threespine stickleback (*Gasterosteus aculeatus*; top three fish), as well as ninespine stickleback (*Pungitius pungitius*; bottom two individuals). Illustration by children's book author and naturalist Beatrix Potter (1866–1943), reproduced courtesy of the Armitt Trust

Three main pairwise comparisons have been the focus of studies of stickleback evolution and divergence: (1) marine versus freshwater, (2) stream versus lake, and (3) benthic versus limnetic forms. It is generally accepted that extant marine populations represent the ancestral threespine stickleback form (Bell 1977; Schluter and McPhail 1992; Bell and Foster 1994; but see Morris et al. 2018), and the diverse freshwater ecotypes are derived. There is also differentiation between truly marine and anadromous populations, although this distinction is poorly known (Ahnelt 2018). Many studies have focused on external morphology, particularly the reduction in armor traits, such as lateral plates and spines, which often occurs with colonization of freshwater from marine habitats (Bell et al. 2004). Marine stickleback nearly always have three dorsal spines, two pelvic spines (one on each side of the body), an armored pelvis, and a full set of lateral armor plates running from behind the head to the tail. Freshwater populations, however, exhibit a wide variation in the reduction in the number of dorsal spines, pelvic armor and spines, and

Table 1 A non-exhaustive survey of studies of parallel evolution in threespine stickleback, showing the region in which populations were sampled, the primary axis of comparison, and phenotypic and genetic data collected

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Rudman et al. (2019)	British Columbia, Canada	Marine, freshwater	Lateral plate morph (high, low), ionome, calcium uptake, excretions	–
Verta and Jones (2019)	Scotland; British Columbia, Canada	Marine, freshwater	Gene expression	RNAseq
Miller et al. (2019)	British Columbia, Canada	Sculpin presence, absence	Body shape, armor traits	mtDNA, WGS
Haenel et al. (2019)	Scotland	Marine, acid lakes, alkaline lakes	Armor traits	Pooled RADseq
Xie et al. (2019)	California, Alaska, USA; British Columbia, Canada	Marine, freshwater	Pelvic armor	Mutational mechanism
Kitano et al. (2019)	Japan; British Columbia, Canada	Marine, stream	Gene expression	eQTL from targeted SNP genotyping
Liu et al. (2018)	Denmark; Greenland	Marine, freshwater	Lateral plates, keel plates	RADseq
Bassham et al. (2018)	Alaska, USA	Marine, freshwater	–	RADseq
Nelson and Cresko (2018)	Alaska, USA	Marine, freshwater	–	RADseq
Hanson et al. (2017)	British Columbia, Canada	Lake, stream	Gene expression	Transcriptome sequencing
Pujolar et al. (2017)	Denmark	Marine, freshwater	Lateral plates	Targeted SNP genotyping
Mobley et al. (2016)	British Columbia, Canada	Benthic, limnetic	Mating preference	–
Erickson et al. (2016)	British Columbia, Canada	Marine, benthic freshwater	Skeletal morphology, armor traits	GBS
Hanson et al. (2016)	British Columbia, Canada	Lake, stream	Sexual maturity (body color for males, gravidity for females)	–
Oke et al. (2016)	British Columbia, Canada	Lake, stream	Morphological measurements, gill rakers	–

(continued)

Table 1 (continued)

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Conte et al. (2015)	British Columbia	Benthic, limnetic	Body shape, armor traits, gill rakers	Targeted SNP genotyping
Mazzarella et al. (2015)	Norway	Salinity	Body shape	–
Hirase et al. (2014)	California, Washington, Alaska, USA; British Columbia, Nova Scotia, Canada; Japan; Germany; Norway; Scotland; Iceland	Marine, freshwater	Gene copy number	WGS
Glazer et al. (2014)	British Columbia, Canada; Washington, Alaska, USA	Marine, freshwater	Gill rakers	Microsatellites, InDel markers
Lucek et al. (2013)	Switzerland	Lake, stream	Body shape, armor traits, gill rakers	Microsatellites
Ravinet et al. (2013)	Northern Ireland	Lake, stream	Body shape, armor traits, gill rakers, diet	Microsatellites
Moser et al. (2012)	Germany; Austria; Switzerland	Lake, stream	Otoliths, body shape, fecundity, stomach content, lateral plates	Microsatellites, mtDNA
Natsopoulou et al. (2012)	Iceland	Rocky, lava, mud substrates	Parasite load	MHC diversity by SSCP
Deagle et al. (2012)	British Columbia, Canada	Lake, stream	Morphological measurements, lateral plates	Targeted SNP genotyping
Dalziel et al. (2012)	British Columbia, Canada	Marine, stream	Gill rakers, ventricular and pectoral muscle, hemoglobin concentration, hematocrit	–
Hohenlohe et al. (2012)	Alaska, USA	Marine, freshwater	–	RADseq
Kaeuffer et al. (2012)	British Columbia, Canada	Lake, stream	Body shape, armor traits, gill rakers, diet, trophic position	Microsatellites
Kimmel et al. (2012)	Alaska, Oregon, USA; British Columbia, Canada; Iceland	Marine, freshwater	Opercle morphology	–

(continued)

Table 1 (continued)

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Jones et al. (2012)	California, Washington, Alaska, USA; British Columbia, Nova Scotia, Canada; Japan; Germany; Norway; Scotland; Iceland	Marine, freshwater, benthic, limnetic	Body shape	WGS
Hohenlohe et al. (2010)	Alaska, USA	Marine, freshwater	–	RADseq
Ólafsdóttir and Snorrason (2009)	Iceland	Rocky, lava, mud substrates	Microhabitat, body shape, armor traits	Microsatellites
Chan et al. (2009)	British Columbia, Canada; Alaska, USA; Japan	Marine, freshwater	Pelvic armor	Microsatellites, targeted sequencing of *PitxI*, transgenics
Marchinko (2009)	British Columbia, Canada	Marine, freshwater	Armor traits	Targeted genotyping of *Eda*
Miller et al. (2007)	British Columbia, Canada; Washington, California, USA; Japan	Marine, freshwater	Gill and skin pigmentation	Microsatellites
Coyle et al. (2007)	Scotland	Lake, stream	Pelvic girdle and pelvic spine	Microsatellites
Marchinko and Schluter (2007)	British Columbia, Canada	Marine, freshwater	Lateral plates, growth rate	–
Ólafsdóttir et al. (2007)	Iceland	Marine, freshwater	Spine length and lateral plate morphology, microsatellite loci	–
Colosimo et al. (2005)	British Columbia, Canada	Marine, freshwater	Lateral plates	Microsatellites, targeted sequencing of *Eda*
Boughman et al. (2005)	British Columbia, Canada	Benthic, limnetic	Behavior (courtship), gill raker, armor plate numbers	–
Colosimo et al. (2004)	California, USA; British Columbia, Canada	Marine, freshwater	Lateral plates	Microsatellites
Cresko et al. (2004)	Alaska, USA	Marine, freshwater	Armor traits	Microsatellites
Rundle et al. (2000)	British Columbia, Canada	Benthic, limnetic	Spawning probability	–
Thompson et al. (1997)	British Columbia, Canada	Lake, stream	–	mtDNA

(continued)

Table 1 (continued)

Publication	Region	Habitat comparison	Phenotypes	Genetic data
Lavin and McPhail (1993)	British Columbia, Canada	Lake, stream	Gill rakers, body shape	–

We searched Web of Science for *TITLE*: (stickleback∗) *AND TITLE*: (parallel∗ or repeat∗) *NOT TITLE*: (nine∗) as of June 2019. Ten publications were removed as the word "repeated" in the title did not refer to parallel evolution, leaving a total of 44 publications. This excludes relevant studies without the specific keywords in the title (e.g., Raeymaekers et al. 2017; Stuart et al. 2017)
mtDNA mitochondrial DNA sequence, *RADseq* restriction site-associated DNA sequencing, *eQTL* expression quantitative trait locus, *SNP* single-nucleotide polymorphism, *WGS* whole-genome sequencing, *MHC* major histocompatibility complex, *SSCP* single-stranded conformation polymorphism

lateral plates, with some populations having lost nearly all of these. Early comparisons of lake and stream populations focused on the ecology of adaptive radiations (Schluter and McPhail 1992; Schluter 1993, 1996) and parallel evolution (Lavin and McPhail 1993), particularly in body shape phenotypes.

Both marine/freshwater and lake/stream stickleback population pairs are widespread across the species range, including both sides of the North Atlantic and North Pacific. In contrast, the coexistence of distinct bottom-dwelling (benthic) and open-water (limnetic) forms within freshwater habitats is much less common, with examples primarily from a few lakes in British Columbia. Their rarity may be explained by the fact that the pairs most likely resulted from double invasions facilitated by fluctuations in sea level in this region (Schluter 1996): the first oceanic colonizers of these lakes evolved into a freshwater form, and then a second invasion by oceanic sticklebacks displaced the first population to the benthic niche while adapting to the alternative open-water limnetic niche (Taylor and McPhail 1999). These pairs have provided examples of divergence in size, shape, feeding morphology, body armor, mate preference, and behavior, which confer fitness advantages when tested in the corresponding benthic and limnetic environments (Schluter and McPhail 1992; Erickson et al. 2016).

1.2 Threespine Stickleback as a Model System

The threespine stickleback has become a model system for adaptive evolution from multiple perspectives (Hendry et al. 2013). While the taxonomic implications of diversification have been a continuing source of debate, the mechanisms of ecotype formation and evolution of partial or complete reproductive isolation between stickleback forms provide a model for understanding the processes of adaptive radiation and speciation (Foster et al. 1998; McKinnon et al. 2004). Because of the adaptation to different habitats and ecological niches in stickleback diversification, the species has played a key role in the concept of ecological speciation—speciation

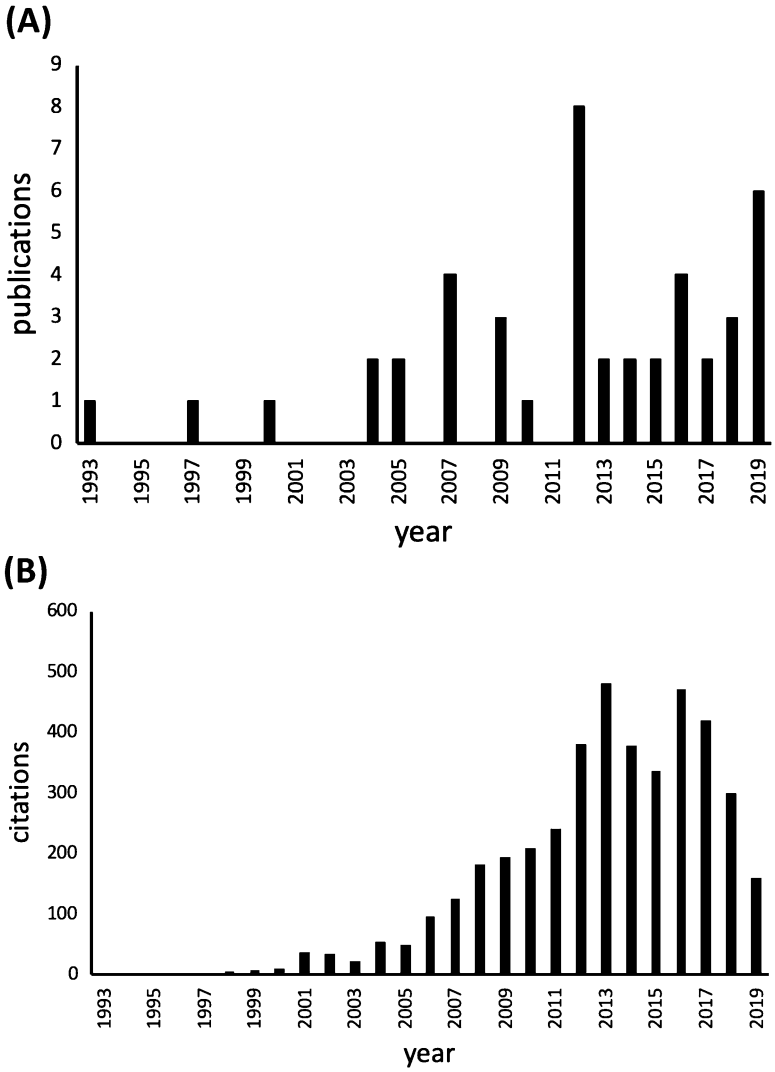

Fig. 2 (**a**) Annual counts of published studies of parallel evolution in threespine stickleback as of June 2019 that are shown in Table 1. (**b**) Total number of times that all of these publications have been cited per year

in which reproductive isolation occurs as a by-product of phenotypic divergence resulting from adaptation to different ecological roles (Schluter 2001, 2009; Nosil 2012). Ecological communities feel the effects of these processes. For example, divergence into benthic and limnetic ecotypes has been shown to have cascading ecological effects on prey community structure, primary productivity, and dissolved organic material (Harmon et al. 2009). Conversely, the collapse of ecotypes into

a single interbreeding population (termed "reverse speciation" or "introgressive extinction") can also have ecological consequences. For instance, Rudman and Schluter (2016) found that when benthic and limnetic forms combined into a single intermediate form, effects on relative abundances of prey included changes in the pupating aquatic insects that emerged into the surrounding terrestrial environment.

A number of genomics and laboratory tools and resources have facilitated stickleback research. The genome of the threespine stickleback is of a tractable size (~460 Mb, in 21 chromosomes), and a high-quality reference genome assembly has been available for some time (Kingsley et al. 2004; Jones et al. 2012). With the advent of next-generation sequencing, threespine stickleback have been the focus of early empirical studies in the field of population genomics (Hohenlohe et al. 2010; Jones et al. 2012). They are also easily raised in the lab and subject to experimental manipulation for developmental or physiological studies. To the extent that research can uncover the developmental genetic basis of traits that play important roles in ecology and parallel adaptation, stickleback can be a model for connecting evolutionary patterns to developmental processes ("evo-devo"; Cresko et al. 2007; Miller et al. 2014).

Despite the strong evidence that phenotypic changes in stickleback have evolved in response to environmental conditions, particularly along the ecological axes described above, there are comparatively few studies of the specific environmental drivers of divergence. Most of these have focused on predation, parasites, and salinity and/or pH. A few studies have found positive associations between spine length and predation intensity (Moodie et al. 1973; Gross 1978; Reimchen 1995). Large variation in pH and calcium among lakes has been linked to the evolution of body size or armor in stickleback in these lakes (Giles 1983; Spence et al. 2013; MacColl and Aucott 2014). Studies of environmental drivers of stickleback adaptation have traditionally focused on the relationship between a single environmental factor and the evolution of one or a small number of traits (Vamosi and Schluter 2002; Marchinko 2009), although this has begun to change (Bourgeois et al. 1994; Raeymaekers et al. 2017; Stuart et al. 2017). The understanding of stickleback diversity can benefit from viewing both the environment and phenotype as highly multivariate and with complex relationships to fitness.

1.3 Genetics of Parallel Evolution

Parallel phenotypic evolution has been observed in a number of taxa, such as cichlid and salmonid fishes (Elmer and Meyer 2011) and *Anolis* lizards (Mahler et al. 2013). Several authors (Arendt and Reznick 2008; Elmer and Meyer 2011; Rosenblum et al. 2014; Bolnick et al. 2018) have addressed the distinction between "parallel" and "convergent" evolution, which depends on how two populations or lineages arrived at a similar phenotypic state; parallel implies a similar starting point (i.e., more recent common ancestor or similar genetic basis), while convergent implies different

starting points (i.e., distant and phenotypically distinct common ancestor or different genetic mechanisms). The wealth of natural experiments and genomics tools in stickleback allow direct investigation of the genetic basis of adaptive phenotypes. However, the genetic basis of phenotypes shared among populations can be similar or different in a multitude of ways (Arendt and Reznick 2008). First, it is important to consider the level of biological organization at which the relevant genetic variation occurs (e.g., nucleotide, gene, network) (Rosenblum et al. 2014). For example, mutations that affect different nucleotide positions within the same gene and thus result in similar phenotypes could be considered convergent at the gene level but not at the nucleotide level (Fig. 3). Variation may be inherited from a common ancestor,

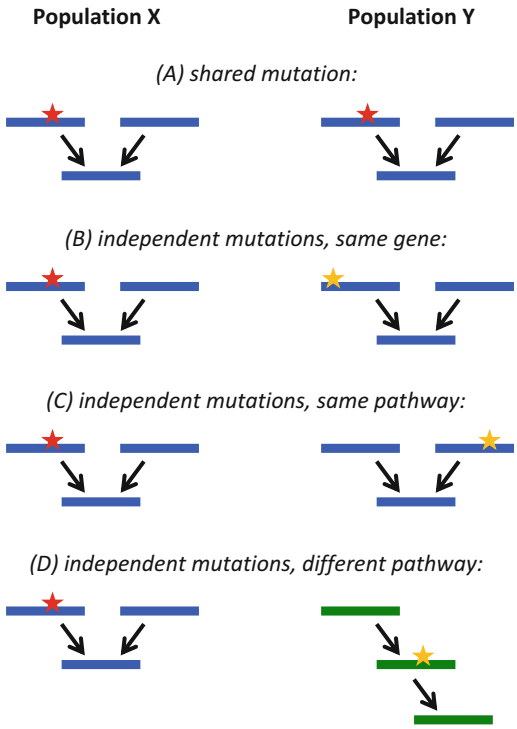

Fig. 3 Alternative genetic scenarios for parallel phenotypic evolution (Elmer and Meyer 2011; Rosenblum et al. 2014). Similar phenotypes evolve in similar habitats in two independent populations X and Y (e.g., stickleback in two freshwater bodies) from a divergent common ancestor (e.g., marine). Bars represent genes interacting with each other in a pathway that affects the phenotype, and stars represent any type of mutation (nucleotide substitution, insertion/deletion, etc.) that affects either regulatory or coding regions of the gene. (**a**) A single mutation in one gene creates an allele that is present in the ancestral population, and selection acts on this allele in both the descendant populations. (**b**) Two different mutations in the same gene lead to similar phenotypes in each population. (**c**) Two different mutations in different genes produce similar phenotypes by affecting the same genetic pathway. (**d**) Independent mutations affect genes in different pathways, but nonetheless have similar phenotypic effects. In the case of polygenic phenotypes, some combination of any or all of these scenarios may play a role together

thus providing the shared genetic mechanism of parallel evolution, in the case of adaptation from standing variation (Barrett and Schluter 2008). Alternatively, it may reflect independent genetic changes between populations or lineages. Phenotypic variation may also be polygenic, so that parallel phenotypic change may depend on genetic changes in overlapping suites of loci or pathways. To address these cases, and to make use of quantitative trait locus mapping studies, Conte et al. (2012) developed a metric of proportional similarity to reflect the proportional contributions of genes to parallel phenotypes.

Whether parallel phenotypic evolution relies on one or many genes, or independent variants versus shared ancestral variation, depends on a large number of factors that are specific to the genetic basis of the phenotype and the demographic history of the populations (Rosenblum et al. 2014). It is possible that different phenotypes show different patterns within the same set of populations or that parallel evolution of a polygenic phenotype reflects a mixture of shared ancestral variation and independent mutations (Fig. 3). Indeed, examples of all of these scenarios can be found in threespine stickleback. Below we describe the primary population genomics approaches that have been taken to understand the genetics of adaptation in stickleback, highlight examples of the various genetic modes of parallel phenotypic evolution, and discuss how demographic and genomic conditions can facilitate repeated, rapid adaptation in this species. With the power of population genomics, threespine stickleback continue to reveal insights into the genetics of adaptation.

2 Identifying Functional Loci in Stickleback

The advent of molecular population genetics has enabled direct investigations of important factors in the evolution of threespine stickleback and the relationships among genotype, phenotype, fitness, and the environment (Hendry et al. 2013). Two broad areas of focus have been most widely applied to understand the genetic basis of adaptation: first, genetic mapping of traits—identifying loci in the genome that explain some proportion of variation in a particular phenotype, directly linking genotype to phenotype. Second, genome scans for selection or genotype–environment association (GEA)—identifying loci that show either evidence of a response to selection or correlation with environmental variables in natural populations, linking genotype to fitness or the environment. Mapping studies can be grouped as traditional genetic mapping approaches, which use a laboratory cross of individuals with divergent phenotypes and identify marker loci that segregate with phenotypic variation, termed quantitative trait loci (QTL), and genome-wide association studies (GWAS), which identify associations between marker loci and phenotypic variation in an outbred population (Wellenreuther and Hansson 2016). Genetic markers, such as microsatellites, can be used for traditional mapping because the relatively large linkage blocks present in a laboratory cross can be genotyped with fewer markers. However, GWAS, genome scans for selection, and GEA require larger numbers of

markers to survey the entire genome, because they use information from outbred populations in which linkage blocks are much smaller and a higher density of markers is required to detect functional loci.

2.1 Mapping

Accordingly, the first major insights into the genetic basis of parallel adaptation in stickleback grew out of traditional mapping studies using laboratory crosses of phenotypically divergent individuals. For instance, Peichel et al. (2001) created a linkage map based on 227 informative microsatellite markers and used it to map traits involved in benthic–limnetic differentiation in freshwater stickleback in British Columbia. Shapiro et al. (2004) used an overlapping set of markers to link the pelvic armor phenotype to the gene *Pitx1*, which also affects hind limb development in mice. Other genes identified in QTL studies of stickleback are also known to have similar functions in widely divergent model organisms (Miller et al. 2007). The well-studied lateral plate phenotype, which typically diverges rapidly between marine and freshwater habitats, was mapped to a single Mendelian locus of major effect and traced to the gene *Ectodysplasin* (*Eda*) in a series of microsatellite-based mapping studies (Colosimo et al. 2004, 2005; Cresko et al. 2004). Other studies have used microsatellite or single-nucleotide polymorphism (SNP) markers to identify QTL for multivariate phenotypes such as body shape (Albert et al. 2008; Liu et al. 2014), skeletal morphology (Kimmel et al. 2005; Miller et al. 2014), and pigmentation (Greenwood et al. 2011). The advent of genomics tools has greatly increased the number of genetic markers that can be efficiently genotyped in non-model organisms (Ellegren 2014; Kratochwil and Meyer 2015). This means that the density of markers possible across the stickleback genome with tools such as restriction site-associated DNA sequencing (RADseq) (Hohenlohe et al. 2010) is sufficient to identify the relatively small linkage blocks present in outbred populations (Hohenlohe et al. 2018). For instance, while much of the repeated evolution of lateral plate number in freshwater stickleback populations involves substantial reduction in number of lateral plates, it is quite rare for lateral plates to be lost altogether (Magalhaes et al. 2016). Mazzarella et al. (2016) used RADseq in a GWAS framework to identify the genetic basis of the plateless phenotype in Norwegian stickleback populations, finding this trait to be polygenic.

A few general conclusions can be reached about the genetic basis of phenotypic variation in stickleback from the results of QTL mapping studies. Not surprisingly, there are relatively few loci with a large effect on phenotypic variation and many more loci with small effect (Peichel and Marques 2016). QTL also appear to be clustered across the genome; for instance, chromosomes IV and XXI have a higher-than-expected number of QTL across phenotypic traits after accounting for chromosome size and gene number (Peichel and Marques 2016). Of course it should be noted that the phenotypes that have been the subject of QTL mapping studies in stickleback are not a random sample of variable phenotypes but instead are focused

on ecologically important traits and those that exhibit parallel evolution across stickleback ecotypes.

To compare across replicate natural populations, phenotypic variance can be attributed to multiple loci and their proportional effect quantified. The growing number of comparable mapping studies allows for meta-analysis of the degree of overlap in loci that contribute to parallel phenotypic evolution (Conte et al. 2012; Peichel and Marques 2016). For instance, in a pair of comprehensive QTL mapping studies of parallel evolution in benthic freshwater sticklebacks in British Columbia, Conte et al. (2015) and Erickson et al. (2016) mapped multiple phenotypic traits and compared overlap of QTL across lakes. Traits included body shape morphometrics and skeletal meristic traits, including lateral plates, and the authors genotyped a large number of SNP markers in each case. Again the results were mixed (Fig. 4); Conte et al. (2015) found that at nearly half of the QTL, alleles showed the same association with the same trait in the same direction. At other loci, genotype was associated with the trait in one lake but not the other. At a smaller number of QTL, the genotype was associated with the same phenotype in both lakes but in opposite directions, suggesting different patterns of linkage disequilibrium between the marker and the causative mutation. Erickson et al. (2016) found that just over half of the QTL were unique to one of the three lakes tested, although some loci were significant for all three.

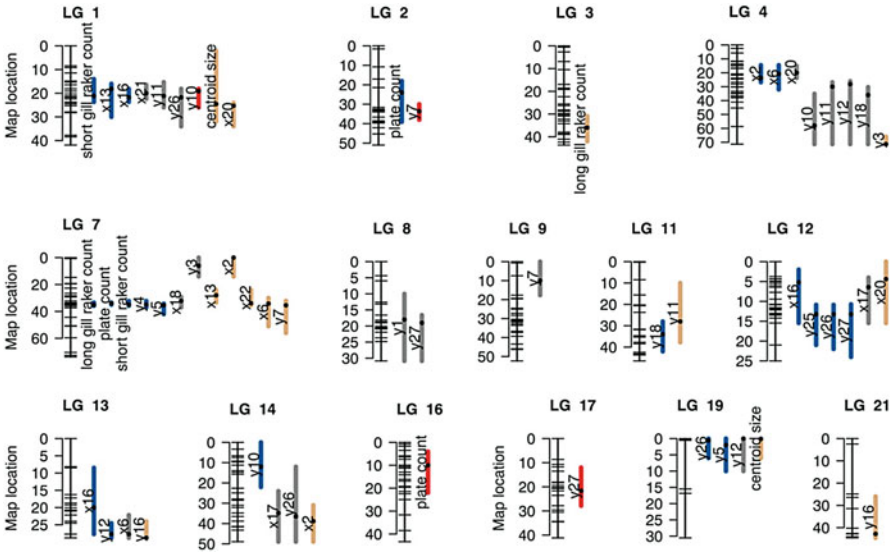

Fig. 4 Overlapping QTL associated with phenotypic differentiation between benthic and limnetic stickleback forms in two lakes in British Columbia. Only linkage groups (chromosomes) on which QTL were found are shown, and the trait is given next to each QTL. Blue indicates parallel effects in both the lakes; gray indicates effects in one lake but not the other; red indicates effects in opposite directions in each lake; tan indicates QTL for which two or more models cannot be distinguished. Reproduced with permission from Conte et al. (2015)

To extend from QTL mapping to selection and parallel evolution in nature, QTL identified in a laboratory cross can be tested for association with the same phenotype or signatures of selection across multiple natural populations, using targeted marker genotyping or sequencing. Examples can be found between the ecotypes of estuarine and freshwater (Raeymaekers et al. 2007), lake and stream (Berner et al. 2014), or benthic and limnetic comparisons (Erickson et al. 2016). The results are mixed. Many QTL in these studies do not show consistent patterns across populations, suggesting that parallel phenotypic evolution is not attributable to divergence at these loci. However, many other QTL, particularly those with large phenotypic effect, do show consistent association with particular phenotypes or signatures of selection across populations. Perhaps the most striking and well-studied example is *Eda*, discussed in more detail below.

2.2 Genome Scans for Selection

Genome scans for selection assess the patterns of allelic variation, haplotype structure, and other features across populations to identify signatures of natural selection acting on the genome (Fig. 5). Genome scans in threespine stickleback have made use of both reduced representation genomic sequencing techniques like RADseq (e.g., Hohenlohe et al. 2010; Liu et al. 2018) and whole-genome sequencing (e.g., Jones et al. 2012). These genomics techniques provide a dense set of markers across the genome. When placed on the periodically improving stickleback genome assembly (Glazer et al. 2015), a genomic set of markers can identify significant genomic regions either by finding clusters of significant markers such as SNPs or by using sliding window analyses (e.g., Fig. 5d shows both individual SNPs and a smoothed sliding window average), and then candidate genes can often be identified in the chromosomal neighborhood of such significant regions. This illustrates the value of a physical map of the genome in population genomics research (Luikart et al. 2018). Because of the role of adaptive divergence between habitats in stickleback evolution, genome scans for selection have most commonly searched for outlier loci—loci with differentiation (often quantified by F_{ST}) between populations significantly greater than the genome-wide background. Most commonly, these studies have tested for outliers between replicate habitat pairs such as marine–freshwater (Fig. 5b, e), lake–stream (Fig. 5c, d, f), or benthic–limnetic, while a few have focused on biotic factors such as the presence of prickly sculpin (*Cottus asper*), which is both a predator and a competitor (Miller et al. 2019).

Outlier-based genome scans do not directly indicate which phenotype or environmental variable is associated with the genetic signature of selection. In contrast, genotype–environment association (GEA) analyses specifically test for relationships between loci and specific environmental variables, such as temperature or salinity (Hoban et al. 2016). For example, Guo et al. (2015) surveyed 10 stickleback populations across temperature and salinity gradients in the Baltic Sea and used RADseq to genotype a large number of SNP markers. They identified several loci

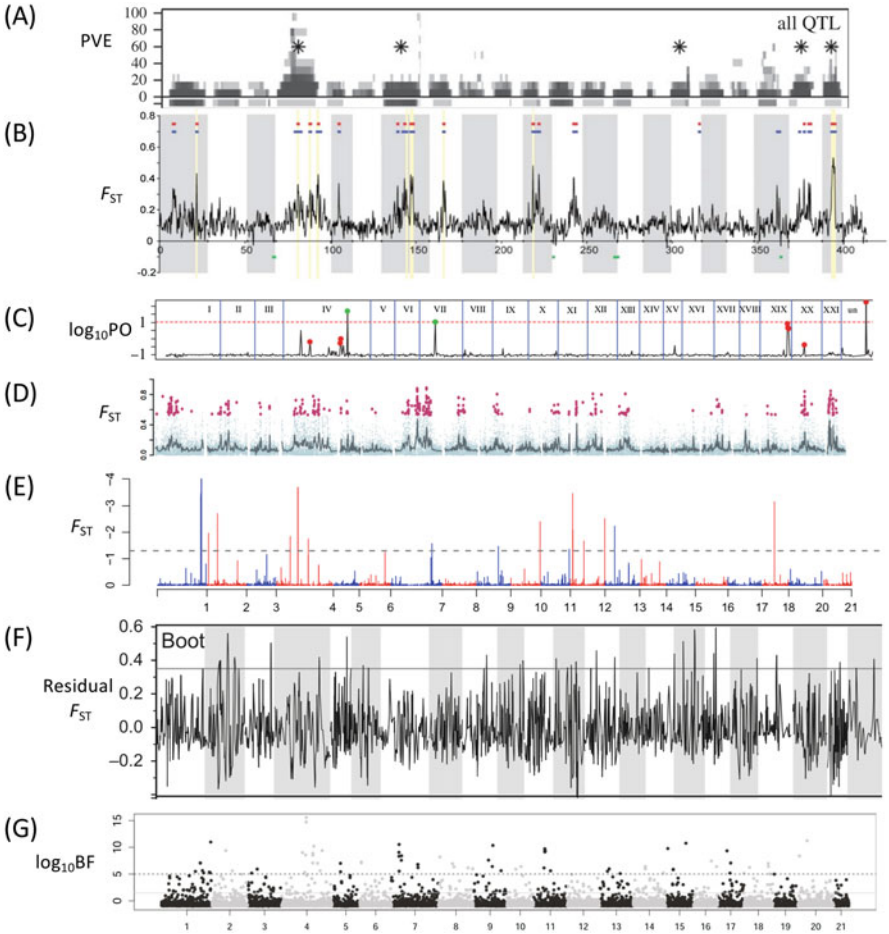

Fig. 5 Examples of genetic mapping and genome scan studies in threespine stickleback. In all cases, chromosomes I through XXI are aligned along the horizontal axis, and plots are rescaled to correspond with each other; (**b**, **c**, and **f**) also show unassembled scaffolds, and (**d** and **e**) have removed the sex chromosome XIX. (**a**) Percent variance explained (PVE) by QTL across multiple studies (Peichel and Marques 2016). (**b**) Differentiation (F_{ST}) between three independent freshwater and two marine populations in Alaska (Hohenlohe et al. 2010). (**c**) Significance of selection signatures in a Bayesian analysis (log of posterior odds, $\log_{10}$PO) in lake–stream comparisons in British Columbia (Deagle et al. 2012). (**d**) Differentiation (F_{ST}) between lake and stream populations in Alaska (Feulner et al. 2015). (**e**) Differentiation (F_{ST}) between marine and freshwater populations in Denmark (Liu et al. 2018). (**f**) Differentiation (residual F_{ST}) between lake and stream populations in British Columbia (Roesti et al. 2012). (**g**) Genotype–environment association with salinity (log of Bayes factors) in populations across the Baltic Sea (Guo et al. 2015)

across the genome associated with each of these environmental variables (Fig. 5g), including several that matched outlier loci found in previously published genome scans of marine–freshwater comparisons. Rennison et al. (2019) combined a genome scan for outlier genomic windows of differentiation between lake and stream populations with GEA methods to detect associations between genotype and both environmental variables and morphology. Multiple genomic regions were associated with lake–stream differentiation, abiotic environmental factors, diet, and morphology, and these regions exhibited some clustering on particular chromosomes, such as IV and VII (as evident in Fig. 5). However, while there was some overlap among categories, it was not significant at a genome-wide scale. Less overlap in genomic regions associated with adaptation to salinity was observed between threespine and ninespine stickleback (*Pungitius pungitius*) (Raeymaekers et al. 2017).

The large and growing number of mapping and genome scan studies in stickleback allows for comparative meta-analyses. Multiple studies have identified concentrations of functional loci in the stickleback genome (Peichel and Marques 2016). For instance, note the prevalence across the studies in Fig. 5 of significant loci on chromosome IV, which is the chromosome containing *Eda*. Many of the same tools have been shared by the stickleback research community and applied across QTL mapping, genome scan, and GEA studies, allowing direct comparison of the same loci across a large number of populations and studies. For instance, a largely overlapping set of microsatellite loci has been used following Peichel et al. (2001) (Table 1). Most RADseq-based studies, starting with Hohenlohe et al. (2010), have applied a similar protocol even down to the restriction enzyme used (SbfI), which again means that a common set of loci are interrogated. As whole-genome sequencing becomes more prevalent (Jones et al. 2012), this pattern of comparability among studies continues.

3 Population Genomics of Parallel Adaptation

3.1 Shared Variation at a Gene of Major Effect

The best-known example of a gene of major effect in threespine stickleback is *Ectodysplasin* (*Eda*), associated with the repeated reduction in lateral plates commonly seen in adaptation to freshwater habitats. A region of linkage group IV was first linked to the lateral plate phenotype by genetic mapping (Colosimo et al. 2004; Cresko et al. 2004). Laboratory complementation studies established that the same gene was involved in parallel adaptation to freshwater habitats (Cresko et al. 2004), and fine-scale mapping and sequencing determined that repeated evolution of the low-plated phenotype resulted from a shared allele at *Eda* estimated to be 2 million years old (Fig. 6; Colosimo et al. 2005). Further work established that selection on the *Eda* region, particularly evidenced by elevated differentiation at this locus between marine and freshwater populations, was widespread across the Atlantic (Mäkinen et al. 2008) and Pacific Ocean basins (DeFaveri et al. 2011). Subsequent

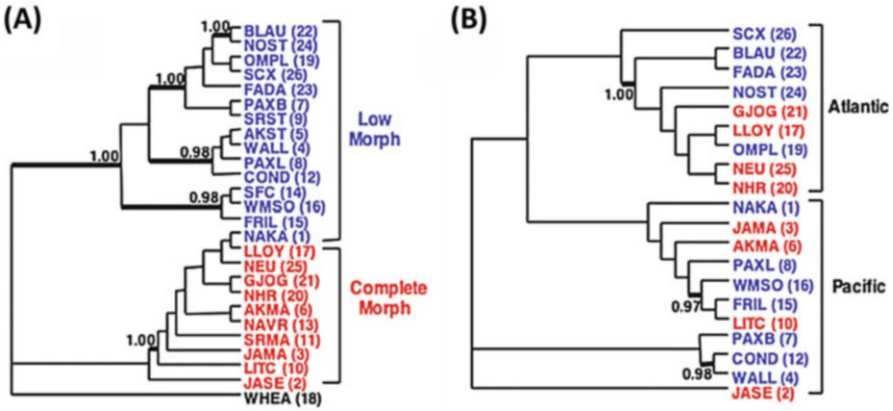

Fig. 6 Widespread reuse of a shared haplotype at the *Eda* gene across the threespine stickleback range in adaptation to freshwater habitats. (**a**) Relationships among sequences at *Eda* show two distinct clades corresponding to lateral plate morph, and the "low morph" allele is shared across ocean basins. (**b**) Relationships among sequences at an unrelated locus reflect geographic region rather than plate morph. Reproduced with permission from Colosimo et al. (2005)

genome scans for selection between marine and freshwater populations have found elevated genetic differentiation around *Eda* across the range of the species (Hohenlohe et al. 2010; Jones et al. 2012; Terekhanova et al. 2014; Ferchaud and Hansen 2016). Roesti et al. (2015) also found elevated differentiation in the chromosomal region around *Eda* in lake–stream comparisons, also corresponding to differences in lateral plate phenotypes.

The repeated use of a shared, ancient allele at *Eda* in lateral plate reduction demonstrates that this parallel evolution relies on standing genetic variation present in the marine population (Fig. 3a). Evolution from standing genetic variation can be remarkably rapid, occurring over just a few decades (Lescak et al. 2015; Marques et al. 2018), demonstrating the strength of selection in newly colonized freshwater habitats. However, the situation is more complex than it may seem. Selection for reduced lateral plates has been linked to predation by insect larvae in freshwater (Marchinko 2009), but *Eda* haplotypes also appear linked to growth rate (Barrett et al. 2008, 2009a), behavior including propensity to migrate between saltwater and freshwater habitats (Barrett et al. 2009b), and immune system function (Robertson et al. 2017). In some cases, for example in northern Europe, it appears that standing variation at *Eda* is not available, and a similar phenotype is achieved by reduction in lateral plate size (Leinonen et al. 2012) or by genetic changes at other loci (Pujolar et al. 2017) (Fig. 3c, d).

3.2 Independent Mutations at a Large-Effect Gene

In contrast to the ancient *Eda* haplotype clade shared in stickleback populations across the species range, a different major-effect locus shows evidence of repeated independent mutations with similar phenotypic effects (Fig. 3b). The pelvic girdle and spines found in marine stickleback led to the genus name *Gasterosteus* ("bony stomach"), but like the lateral plates, pelvic armor is often reduced in the adaptation of stickleback to newly colonized freshwater habitats from ancestral marine populations (Bell and Foster 1994). Also like lateral plates, loss of pelvic armor may be linked to changes in vertebrate and invertebrate predators as well as calcium ion availability in the different habitats (Shapiro et al. 2004). Laboratory crosses found pelvic armor to be a Mendelian trait, and genetic mapping traced the variation to the *pituitary homeobox transcription factor I* (*PitxI*), which is remarkable because of this gene's role in hind limb development in mice (Cresko et al. 2004; Shapiro et al. 2004). Stickleback with reduced pelvic structures exhibited reduced expression of *PitxI* in pelvic precursor tissue during development (Shapiro et al. 2004), and *PitxI* expression was implicated in pelvic reduction in both Atlantic and Pacific stickleback populations (Cresko et al. 2004; Shapiro et al. 2004; Coyle et al. 2007).

Chan et al. (2010) tested allele-specific expression patterns in F1 crosses and used fine-mapping and transgenic techniques to determine that multiple independent deletion mutations in a tissue-specific enhancer of *PitxI* had resulted in the parallel phenotype of loss of pelvic structures. These mutations were positively selected during adaptation to freshwater habitats, meaning that parallel evolution in this case was driven by independent mutations that nonetheless had very similar genetic mechanisms leading to similar phenotypes. This genomic region appears to be particularly prone to a high rate of double-stranded DNA breaks and deletion mutation (Chan et al. 2010; Xie et al. 2019), so this is an example of mutational bias facilitating parallel evolution (Rosenblum et al. 2014).

3.3 Polygenic Adaptation

Single genes of major effect on an important phenotype, such as *Eda* and *PitxI*, may be more the exception than the rule in adaptive evolution of stickleback. A large number of QTL mapping studies and genome scans have generally identified a larger number of loci contributing to phenotypic variation and adaptation. In their review of QTL studies, Peichel and Marques (2016) found a similar pattern of few genes of large effect and many genes of smaller effect, and this pattern held across traits related to feeding, body shape, defense, and other categories. Similarly, genome scans for local adaptation typically identify multiple outlier loci or loci associated with environmental variables (Fig. 5). While there may be some clustering of genes that contribute to ecologically relevant phenotypes, multiple genes across multiple chromosomes still contribute to phenotypic variation that is under selection during

colonization of novel habitats. This is particularly true for complex, multivariate traits, for example, the body shape differences that play an important role in benthic–limnetic divergence (Schluter 1993; Erickson et al. 2016).

4 Mechanisms of Rapid, Parallel Evolution

4.1 Recurrent Colonization and Standing Genetic Variation

Several factors may play a role in the remarkable parallel evolution observed in stickleback. As described above, parallel phenotypic evolution in stickleback appears to rely on a mix of independent mutations that produce similar phenotypes and shared ancestral variation that is subject to repeated selection in independent populations. Evolution from standing genetic variation can occur quickly because there is no waiting time for mutations to appear, and so the remarkably rapid evolution observed in some stickleback populations (e.g., Lescak et al. 2015; Marques et al. 2018) depends on selection acting on existing alleles.

Schluter and Conte (2009) proposed the "transporter hypothesis" to explain this phenomenon, named for the transporter in the television series *Star Trek*, in which humans or objects could be disintegrated in one place and transported to another location, where they are then rapidly reassembled. Under this model, adaptation to freshwater habitats involves alleles at multiple loci affecting traits such as morphology, life history, and behavior, so that a freshwater-adapted genotype is a multi-locus combination. Because most marine stickleback are either anadromous or breed in estuarine or coastal habitats, despite some reproductive isolation, there is still opportunity for gene flow between freshwater and marine populations. This will carry freshwater alleles into the marine population, where the multi-locus, freshwater-adapted genotypes will be broken up by recombination in subsequent generations and exist at low frequency, potentially subject to negative selection. Nonetheless, colonization into new freshwater habitats will carry some of these alleles, where they will again be favored by selection. The actions of selection and recombination will reassemble the multi-locus freshwater genotype, and the transporter process is then complete. The rapid evolution observed in stickleback from marine to freshwater, and subsequently into multiple different freshwater ecotypes, relies on an ancestral marine population that is relatively old, large, and able to maintain high levels of genetic diversity, with repeated colonizations into relatively new freshwater habitats (Liu et al. 2016).

This demographic model of standing variation is consistent with several observations (Terekhanova et al. 2014; Marques et al. 2018; Haenel et al. 2019). For instance, the low-plated *Eda* haplotype that has contributed to parallel evolution is known to occur in marine stickleback, although at very low frequencies (Colosimo et al. 2005; Barrett et al. 2008). Many of the freshwater-adapted alleles, including *Eda*, are known to be very old and much older than many of the freshwater habitats in which stickleback are currently found, such as those that appeared only after the

Pleistocene glaciation (Nelson and Cresko 2018). In fact, variants that characterize marine–freshwater divergence average several million years old, suggesting that they have persisted through multiple recurrent cycles of selection in freshwater habitats and gene flow back into the marine population (Nelson and Cresko 2018). Roesti et al. (2014) described a characteristic pattern of genomic variation around these recurrently selected loci, a peak-valley-peak pattern of F_{ST}, which is predicted based on population genomic models and observed in marine–freshwater stickleback comparisons. Barrett et al. (2009b) even found the intriguing result that the *Eda* haplotype is associated with migration behavior, facilitating the movement of this freshwater-adapted allele back into the marine population where it can contribute to subsequent freshwater colonization. Finally, the results described above in which multi-trait parallel evolution tends to involve a mix of shared and non-shared variation among derived populations are also consistent with the transporter model. Because freshwater-adapted alleles are at low frequency in the marine ancestor, each new colonization of freshwater habitat may by chance include some and not others in the founding individuals, and this will drive the degree of parallelism (Leinonen et al. 2012; Pujolar et al. 2017).

4.2 Genomic Mechanisms

Several genomic features may also facilitate parallel evolution in stickleback by making it easier for a population to respond to selection in a newly colonized habitat. Multi-locus genotypes can be maintained in several ways, so that selection does not need to act independently on each locus; instead, if multiple favored alleles co-occur in individuals, selection on multiple phenotypic traits can act synergistically. First, for a few generations after gene flow from a derived (e.g., freshwater) population back into the ancestral (e.g., marine) one, freshwater-adapted alleles will continue to be in linkage disequilibrium (LD) with each other, meaning that they are statistically more likely to co-occur than the expectation based on their frequencies in the population. Even when they are on different chromosomes with free recombination between them, LD between these alleles decays in an exponential process, and there is evidence that freshwater-adapted alleles exist to some extent in LD with each other in the marine stickleback population even across chromosomes (Hohenlohe et al. 2012). Within chromosomes, many freshwater alleles appear clustered as described above, which will reduce the recombination rate between them and allow LD to persist for longer periods. In fact, especially with the recurrent colonization of the transporter model, there is a theoretical reason to expect that these loci will become clustered over evolutionary time (Yeaman 2013). Rates of recombination also vary across the genome, so that freshwater alleles that are co-localized in regions of low recombination will be maintained longer in LD, and indeed several authors (Roesti et al. 2013; Marques et al. 2016; Samuk et al. 2017) have found that regions of low recombination contribute to rapid adaptation. Finally, chromosomal inversions greatly reduce the rate of recombination, and there is ample evidence that inversion

polymorphisms are common in stickleback and that they contain clusters of functionally important loci contributing to parallel adaptation (Jones et al. 2012; Feulner et al. 2013; Bassham et al. 2018). All of these genomic features suggest that the transporter model of rapidly reassembling multi-locus genotypes adapted to a newly colonized habitat is not as unlikely as it may seem.

5 Conclusions: The "Everyone Wins" Principle of Biology

Threespine stickleback have become a model system in evolutionary biology and population genomics for a number of reasons. Aside from being amenable to genetic and laboratory studies, they exhibit remarkable patterns of parallel evolution across a number of phenotypic and environmental axes at multiple spatial and temporal scales, giving biologists a wealth of replicate natural experiments to investigate. Stickleback have been the focus of multiple genome scans for selection, using reduced representation sequencing approaches like RADseq, helping to refine these techniques and improve their applicability to non-model taxa (Jensen et al. 2015). Stickleback also provide an example for how genome scans can be extended with specific data on environmental variables, ecology, or specific phenotypes (Haasl and Payseur 2015). Although the taxonomy of stickleback ecomorphs may continue to be a source of debate, the divergence of stickleback forms has informed our understanding of speciation processes (Schluter 2009; Hendry et al. 2013; Lackey and Boughman 2017).

A large number of studies have identified the genetic mechanisms of parallel evolution in threespine stickleback, ranging from single genes of major effect to highly polygenic phenotypes, from shared variation to novel mutations, and from single-nucleotide changes to structural variations such as inversion polymorphisms. This leads to what we might call the "Everyone Wins" principle: When multiple plausible mechanisms are proposed to explain some biological pattern, it is likely that all of them play a role in at least some instances, and further they are likely sometimes to co-occur with interesting and important interactions. Threespine stickleback exemplify this view, as they show examples of nearly all the mechanisms of parallel evolution proposed (Rosenblum et al. 2014; Bolnick et al. 2018). This is certainly in part because of the research effort that has been directed toward this taxon, but we suggest that the Everyone Wins principle is more generally an inherent outcome of the complexities of biological systems.

6 Future Directions

Threespine stickleback are likely to continue to be a valuable evolutionary model system. As the costs of DNA sequencing continue to drop, we anticipate that more studies will use whole-genome sequencing rather than the reduced representation

approaches that have been applied. To date, whole-genome sequencing has been used on a relatively small number of representative individuals (e.g., Jones et al. 2012; Liu et al. 2016), but it is now becoming feasible for studies that require genetic data on larger numbers of individuals across many populations. This will allow fine-mapping of causal variants in a single experiment, identification of genomic structural variation, and more. Because stickleback are relatively easy to work with in the laboratory, they are amenable to the ever-expanding toolkit of genetic manipulation and developmental and physiological studies. This will allow continued understanding of the mechanistic basis of stickleback phenotypes and direct linkages between mechanism and adaptation in natural populations.

A few avenues are promising for future work in threespine stickleback. Studies that explicitly combine data on genotype, phenotype, fitness, and the environment (e.g., Rennison et al. 2019) are best-suited to illuminate all the interactions among these factors and gain a comprehensive understanding. Expanding from DNA sequencing to transcriptomics to provide direct estimates of gene expression, as well as directly assessing the role of phenotypic plasticity, will further reveal important aspects of genetic variation (Morris et al. 2014). The role of epigenetics, particularly in rapid parallel evolution, is still relatively unknown but may be critical (Heckwolf et al. 2019). Behavior is a notoriously difficult phenotype to unravel, in part because of the potential roles of plasticity and epigenetics in addition to genetics, but stickleback are a tractable system for behavioral genomics, particularly for behaviors related to mate choice and parental care (e.g., Mobley et al. 2016; Stein and Bell 2019). Finally, the microbiome is a fairly unexplored area that may have a substantial impact on stickleback phenotypes and adaptation (Small et al. 2017; Steury et al. 2019).

These research directions will keep threespine stickleback relevant into the future for continuing progress in understanding the processes of evolution. Specific knowledge about the genetic modes of adaptation, such as the saltwater–freshwater transition, can be extended to related species, such as other fish taxa facing similar environmental challenges. More generally, because stickleback exhibit such a diversity of modes of adaptation across replicate populations, they will continue as a model for understanding the interactions among multiple genetic processes during adaptation to novel or recurrent environments.

References

Ahnelt H. Imprecise naming: the anadromous and the sea spawning threespine stickleback should be discriminated by names. Biologia. 2018;73:389–92.

Albert AYK, Sawaya S, Vines TH, Knecht AK, Miller CT, Summers BR, et al. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. Evolution. 2008;62:76–85.

Arendt J, Reznick D. Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? Trends Ecol Evol. 2008;23:26–32.

Barrett RDH, Schluter D. Adaptation from standing genetic variation. Trends Ecol Evol. 2008;23:38–44.

Barrett RDH, Rogers SM, Schluter D. Natural selection on a major armor gene in threespine stickleback. Science. 2008;322:255–7.

Barrett RDH, Rogers SM, Schluter D. Environment specific pleiotropy facilitates divergence at the ectodysplasin locus in threespine stickleback. Evolution. 2009a;63:2831–7.

Barrett RDH, Vines TH, Bystriansky JS, Schulte PM. Should I stay or should I go? The *Ectodysplasin* locus is associated with behavioural differences in threespine stickleback. Biol Lett. 2009b;5:788–91.

Bassham S, Catchen J, Lescak E, von Hippel FA, Cresko WA. Repeated selection of alternatively adapted haplotypes creates sweeping genomic remodeling in stickleback. Genetics. 2018;209:921–39.

Bell MA. Evolution of phenotypic diversity in *Gasterosteus aculeatus* superspecies on the Pacific coast of North America. Syst Zool. 1976;25:211–27.

Bell MA. A late Miocene marine threespine stickleback, *Gasterosteus aculeatus aculeatus*, and its zoogeographic and evolutionary significance. Copeia. 1977;1977(2):277–82.

Bell MA, Foster SA. The evolutionary biology of the threespine stickleback. Oxford: Oxford University Press; 1994.

Bell MA, Aguirre WE, Buck NJ. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution. 2004;58:814–24.

Berner D, Moser D, Roesti M, Buescher H, Salzburger W. Genetic architecture of skeletal evolution in European lake and stream stickleback. Evolution. 2014;68:1792–805.

Bolnick DI, Barrett RDH, Oke KB, Rennison DJ, Stuart YE. (Non)parallel evolution. Annu Rev Ecol Evol Syst. 2018;49:303–30.

Boughman JW, Rundle HD, Schluter D. Parallel evolution of sexual isolation in sticklebacks. Evolution. 2005;59:361–73.

Bourgeois JF, Bluow DM, Koenings JP, Bell MA. Multivariate analysis of geographic covariance between phenotypes and environments in the threespine stickleback, *Gasterosteus aculeatus*, from the Cook Inlet area, Alaska. Can J Zool. 1994;72:1497–509.

Chan YF, Villareal G, Marks M, Shapiro M, Jones F, Petrov D, et al. From trait to base pairs: Parallel evolution of pelvic reduction in three-spined sticklebacks occurs by repeated deletion of a tissue-specific pelvic enhancer at PitxI. Mech Dev. 2009;126:S14–5.

Chan YF, Marks ME, Jones FC, Villarreal G, Shapiro MD, Brady SD, et al. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. Science. 2010;327:302–5.

Colosimo PF, Peichel CL, Nereng K, Blackman BK, Shapiro MD, Schluter D, Kingsley DM. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. PLoS Biol. 2004;2:0635–41.

Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G, Dickson M, Grimwood J, et al. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science. 2005;307:1928–32.

Conte GL, Arnegard ME, Peichel CL, Schluter D. The probability of parallelism and convergence in natural populations. Proc R Soc B. 2012;279:5039–47.

Conte GL, Arnegard ME, Best J, Chan YF, Jones FC, Kingsley DM, Schluter D, Peichel CL. Extent of QTL reuse during repeated phenotypic divergence of sympatric threespine stickleback. Genetics. 2015;201:1189–200.

Coyle SM, Huntingford FA, Peichel CL. Parallel evolution of *PitxI* underlies pelvic reduction in Scottish threespine stickleback (*Gasterosteus aculeatus*). J Hered. 2007;98:581–6.

Cresko WA, Amores A, Wilson C, Murphy J, Currey M, Phillips P, et al. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. Proc Natl Acad Sci U S A. 2004;101:6050–5.

Cresko WA, McGuigan KL, Phillips PC, Postlethwait JH. Studies of threespine stickleback developmental evolution: progress and promise. Genetica. 2007;129:105–29.

Dalziel AC, Ou M, Schulte PM. Mechanisms underlying parallel reductions in aerobic capacity in non-migratory threespine stickleback (*Gasterosteus aculeatus*) populations. J Exp Biol. 2012;215:746–59.

Deagle BE, Jones FC, Chan YF, Absher DM, Kingsley DM, Reimchen TE. Population genomics of parallel phenotypic evolution in stickleback across stream-lake ecological transitions. Proc R Soc B. 2012;279:1277–86.

DeFaveri J, Shikano T, Shimada Y, Goto A, Merilä J. Global analysis of genes involved in freshwater adaptation in threespine sticklebacks (*Gasterosteus aculeatus*). Evolution. 2011;65:1800–7.

Ellegren H. Genome sequencing and population genomics in non-model organisms. Trends Ecol Evol. 2014;29:51–63.

Elmer KR, Meyer A. Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends Ecol Evol. 2011;26:298–306.

Erickson PA, Glazer AM, Lillingbeck EE, Agoglia RM, Baek J, Carsanaro SM, et al. Partially repeatable genetic basis of benthic adaptation in threespine sticklebacks. Evolution. 2016;70:887–902.

Ferchaud A-L, Hansen MM. The impact of selection, gene flow and demographic history on heterogeneous genomic divergence: three-spine sticklebacks in divergent environments. Mol Ecol. 2016;25:238–59.

Feulner PGD, Chain FJJ, Panchal M, Eizaguirre C, Kalbe M, Lenz TL, et al. Genome-wide patterns of standing genetic variation in a marine population of three-spined sticklebacks. Mol Ecol. 2013;22:635–49.

Feulner PGD, Chain FJJ, Panchal M, Huang Y, Eizaguirre C, Kalbe M, et al. Genomics of divergence along a continuum of parapatric population differentiation. PLoS Genet. 2015;11: e1004966.

Foster SA, Scott RJ, Cresko WA. Nested biological variation and speciation. Phil Trans R Soc Lond B. 1998;353:207–18.

Guo B, DeFaveri J, Sotelo G, Nair A, Merilä J. Population genomic evidence for adaptive differentiation in Baltic Sea three-spined sticklebacks. BMC Biol. 2015;13:19.

Giles N. Behavioural effects of the parasite *Schistocephalus solidus* (Cestoda) on an intermediate host the three-spined stickleback *Gasterosteus aculeatus* L. Anim Behav. 1983;31:1192–4.

Glazer AM, Cleves PA, Erickson PA, Lam AY, Miller CT. Parallel developmental genetic features underlie stickleback gill raker evolution. EvoDevo. 2014;5:19.

Glazer AM, Killingbeck EE, Mitros T, Rokhsar DS, Miller CT. Genome assembly improvement and mapping convergently evolved skeletal traits in sticklebacks with genotyping-by-sequencing. G3 Genes Genomes Genet. 2015;5:1463–72.

Greenwood AK, Jones FC, Chan YF, Brady SD, Absher DM, Grimwood J, et al. The genetic basis of divergent pigment patterns in juvenile threespine sticklebacks. Heredity. 2011;107:155–66.

Gross HP. Natural selection by predators on the defensive apparatus of the three-spined stickleback, *Gasterosteus aculeatus* L. Can J Zool. 1978;56:398–413.

Haasl RJ, Payseur BA. Fifteen years of genomewide scans for selection: trends, lessons and unaddressed genetic sources of complication. Mol Ecol. 2015;25:5–23.

Haenel Q, Roesti M, Moser D, MacColl ADC, Berner D. Predictable genome-wide sorting of standing genetic variation during parallel adaptation to basic versus acidic environments in stickleback fish. Evol Lett. 2019;3:28–42.

Hagen DW, McPhail JD. The species problem within *Gasterosteus aculeatus* on the Pacific coast of North America. J Fish Res Bd Can. 1970;27:147–55.

Hanson D, Barrett RDH, Hendry AP. Testing for parallel allochronic isolation in lake-stream stickleback. J Evol Biol. 2016;29:47–57.

Hanson D, Hu J, Hendry AP, Barrett RDH. Heritable gene expression differences between lake and stream stickleback include both parallel and antiparallel components. Heredity. 2017;119:339–48.

Harmon LJ, Matthews B, Des Roches S, Chase JM, Shurin JB, Schluter D. Evolutionary diversification in stickleback affects ecosystem functioning. Nature. 2009;458:1167–70.

Heckwolf MJ, Meyer BS, Häsler R, Höppner MP, Eizaquirre C, Reusch TBH. DNA methylation facilitates local adaptation and adaptive transgenerational plasticity. bioRxiv. 2019:649574.

Hendry AP, Peichel CL, Matthews B, Boughman JW, Nosil P. Stickleback research: the now and the next. Evol Ecol Res. 2013;15:1–31.

Hirase S, Ozaki H, Iwasaki W. Parallel selection on gene copy number variations through evolution of three-spined stickleback genomes. BMC Genomics. 2014;15:735.

Hoban S, Kelley JL, Lotterhos KE, Antolin MF, Bradburd G, Lowry DB, et al. Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. Am Nat. 2016;188:379–97.

Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genet. 2010;6: e1000862.

Hohenlohe PA, Bassham S, Currey M, Cresko WA. Extensive linkage disequilibrium and parallel adaptive divergence across threespine stickleback genomes. Philos Trans R Soc B. 2012;367:395–408.

Hohenlohe PA, Hand BK, Andrews KR, Luikart G. Population genomics provides key insights in ecology and evolution. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer; 2018.

Jensen JD, Foll M, Bernatchez L. The past, present and future of genomic scans for selection. Mol Ecol. 2015;25:1–4.

Jones FC, Grabherr MG, Chan YF, Russell P, Mauceli E, Johnson J, et al. The genomic basis of adaptive evolution in threespine sticklebacks. Nature. 2012;484:55–61.

Kaeuffer R, Peichel CL, Bolnick DI, Hendry AP. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. Evolution. 2012;66:402–18.

Kimmel CB, Ullmann B, Walker C, Wilson C, Currey M, Phillips PC, et al. Evolution and development of facial bone morphology in threespine sticklebacks. Proc Natl Acad Sci U S A. 2005;102:5791–6.

Kimmel CB, Cresko WA, Phillips PC, Ullmann B, Currey M, von Hippel F, Kristjánsson BK, Gelmond O, McGuigan K. Independent axes of genetic variation and parallel evolutionary divergence of opercle bone shape in threespine stickleback. Evolution. 2012;66:419–34.

Kingsley D, Zhu B, Osoegawa K, deJong PJ, Schein J, Marra M, et al. New genomic tools for molecular studies of evolutionary change in sticklebacks. Behaviour. 2004;141:1331–44.

Kitano J, Ishikawa A, Kusakabe M. Parallel transcriptome evolution in stream threespine sticklebacks. Dev Growth Differ. 2019;61:104–13.

Kratochwil CF, Meyer A. Emerging technologies for evolutionary genetics in ecological model vertebrate systems. Bioessays. 2015;37:213–26.

Lackey ACR, Boughman JW. Evolution of reproductive isolation in stickleback fish. Evolution. 2017;71:357–72.

Lavin PA, McPhail JD. Parapatric lake and stream sticklebacks on northern Vancouver Island: disjunct distribution or parallel evolution? Can J Zool. 1993;71:11–7.

Leinonen T, McCairns RJS, Herczeg G, Merilä J. Multiple evolutionary pathways to decreased lateral plate coverage in freshwater threespine sticklebacks. Evolution. 2012;66:3866–75.

Lescak EA, Bassham SL, Catchen J, Gelmond O, Sherbick ML, von Hippel FA, Cresko WA. Evolution of stickleback in 50 years on earthquake-uplifted islands. Proc Natl Acad Sci U S A. 2015;112:E7204–12.

Liu J, Shikano T, Leinonen T, Cano JM, Li M-H, Merilä J. Identification of major and minor QTL for ecologically important morphological traits in three-spined sticklebacks (*Gasterosteus aculeatus*). G3 Genes Genomes Genet. 2014;4:595–604.

Liu S, Hansen MM, Jacobsen MW. Region-wide and ecotype-specific differences in demographic histories of threespine stickleback populations, estimated from whole genome sequences. Mol Ecol. 2016;25:5187–202.

Liu S, Ferchaud A-L, Grønkjaer P, Nygaard R, Hansen MM. Genomic parallelism and lack thereof in contrasting systems of three-spined sticklebacks. Mol Ecol. 2018;27:4725–43.

Lucek K, Sivasundar A, Roy D, Seehausen O. Repeated and predictable patterns of ecotypic differentiation during a biological invasion: lake-stream divergence in parapatric Swiss stickleback. J Evol Biol. 2013;26:2691–709.

Luikart G, Kardos M, Hand BK, Rajora OP, Aitken SN, Hohenlohe PA. Population genomics: advancing understanding of nature. In: Rajora OP, editor. Population genomics: concepts, approaches and applications. Cham: Springer; 2018.

MacColl ADC, Aucott B. Inappropriate analysis does not reveal the ecological causes of evolution of stickleback armour: a critique of Spence et al. 2013. Ecol Evol. 2014;4(18):3509–13.

Magalhaes IS, D'Agostino D, Hohenlohe PA, MacColl ADC. The ecology of an adaptive radiation of three-spined stickleback from North Uist, Scotland. Mol Ecol. 2016;25:4319–36.

Mahler DL, Ingram T, Revell LJ, Losos JB. Exceptional convergence on the macroevolutionary landscape in island lizard radiations. Science. 2013;341:292–5.

Mäkinen HS, Cano JM, Merilä J. Identifying footprints of directional and balancing selection in marine and freshwater three-spined stickleback (*Gasterosteus aculeatus*) populations. Mol Ecol. 2008;17:3565–82.

Marchinko KB. Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. Evolution. 2009;63:127–38.

Marchinko KB, Schluter D. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution. 2007;61:1084–90.

Marques DA, Lucek K, Meier JI, Mwaiko S, Wagner CE, Excoffier L, Seehausen O. Genomics of rapid incipient speciation in sympatric threespine stickleback. PLoS Genet. 2016;12:e1005887.

Marques DA, Jones FC, Di Palma F, Kingsley DM, Reimchen TE. Experimental evidence for rapid genomic adaptation to a new niche in an adaptive radiation. Nat Ecol Evol. 2018;2:1128.

Mazzarella AB, Voje KL, Hansson TH, Taugbøl A, Fischer B. Strong and parallel salinity-induced phenotypic plasticity in one generation of threespine stickleback. J Evol Biol. 2015;28:667–77.

Mazzarella AB, Boessenkool S, Østbye K, Vøllestad LA, Trucchi E. Genomic signatures of the plateless phenotype in the threespine stickleback. Ecol Evol. 2016;6:3161–73.

McKinnon JS, Mori S, Blackman BK, David L, Kingsley DM, Jamieson L, Chou J, Schluter D. Evidence for ecology's role in speciation. Nature. 2004;429:294–8.

Miller CT, Beleza S, Pollen AA, Schluter D, Kittles RA, Shriver MD, Kingsley DM. *cis*-regulatory changes in *Kit ligand* expression and parallel evolution of pigmentation in sticklebacks and humans. Cell. 2007;131:1179–89.

Miller CR, Glazer AM, Summers BR, Blackman BK, Norman AR, Shapiro MD, et al. Modular skeletal evolution in sticklebacks is controlled by additive and clustered quantitative trait loci. Genetics. 2014;197:405–20.

Miller SE, Roesti M, Schluter D. A single interacting species leads to widespread parallel evolution of the stickleback genome. Curr Biol. 2019;29:1–8.

Mobley RB, Tillotson ML, Boughman JW. Olfactory perception of mates in ecologically divergent stickleback: population parallels and differences. Evol Ecol Res. 2016;17:551–64.

Moodie GEE, McPhail JD, Hagen DW. Experimental demonstration of selective predation on *Gasterosteus aculeatus*. Behaviour. 1973;47:95–105.

Morris MRJ, Richard R, Leder EH, Barrett RDH, Aubin-Horth N, Rogers SM. Gene expression plasticity evolves in response to colonization of freshwater lakes in threespine stickleback. Mol Ecol. 2014;23:3226–40.

Morris MR, Bowles E, Allen BE, Jamniczky HA, Rogers SM. Contemporary ancestor? Adaptive divergence from standing genetic variation in Pacific marine threespine stickleback. BMC Evol Biol. 2018;18:113.

Moser D, Roesti M, Berner D. Repeated lake-stream divergence in stickleback life history within a Central European lake basin. PLoS One. 2012;7:e50620.

Natsopoulou ME, Pálsson S, Ólafsdóttir GÁ. Parasites and parallel divergence of the number of individual MHC alleles between sympatric three-spined stickleback *Gasterosteus aculeatus* morphs in Iceland. J Fish Biol. 2012;81:1696–714.

Nelson TC, Cresko WA. Ancient genomic variation underlies repeated ecological adaptation in young stickleback populations. Evol Lett. 2018;2:9–21.

Nosil P. Ecological speciation. Oxford: Oxford University Press; 2012.

Oke KB, Bukhari M, Kaeuffer R, Rolshausen G, Räsänen K, Bolnick DI, Peichel CL, Hendry AP. Does plasticity enhance or dampen phenotypic parallelism? A test with three lake-stream stickleback pairs. J Evol Biol. 2016;29:126–43.

Ólafsdóttir GÁ, Snorrason SS. Parallels, nonparallels, and plasticity in population differentiation of threespine stickleback within a lake. Biol J Linn Soc. 2009;98:803–13.

Ólafsdóttir GÁ, Snorrason SS, Ritchie MG. Parallel evolution? Microsatellite variation of recently isolated marine and freshwater three-spined stickleback. J Fish Biol. 2007;70:125–31.

Peichel CL, Marques DA. The genetic and molecular architecture of phenotypic diversity in sticklebacks. Philos Trans R Soc B. 2016;372:20150486.

Peichel CL, Nereng KS, Ohgi KA, Cole BLE, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM. The genetic architecture of divergence between threespine stickleback species. Nature. 2001;414:901–5.

Pujolar JM, Ferchaud A-L, Bekkevold D, Hansen MM. Non-parallel divergence across freshwater and marine three-spined stickleback *Gasterosteus aculeatus* populations. J Fish Biol. 2017;91:175–94.

Raeymaekers JAM, Chaturvedi A, Hablützel PI, Verdonck I, Hellemans B, Maes GE, De Meester L, Volckaert FAM. Adaptive and non-adaptive divergence in a common landscape. Nat Comms. 2017;8:267.

Raeymaekers JAM, Van Houdt JKJ, Larmuseau MHD, Geldof S, Volckaert FAM. Divergent selection as revealed by P_{ST} and QTL-based F_{ST} in three-spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-inland gradient. Mol Ecol. 2007;16:891–905.

Ravinet M, Prodöhl PA, Harrod C. Parallel and nonparallel ecological, morphological and genetic divergence in lake-stream stickleback from a single catchment. J Evol Biol. 2013;26:186–204.

Reimchen TE. Predator-induced cyclical changes in lateral plate frequencies of *Gasterosteus*. Behaviour. 1995;132:1079–94.

Rennison DJ, Stuart YE, Bolnick DI, Peichel CL. Ecological factors and morphological traits are associated with repeated genomic differentiation between lake and stream stickleback. Philos Trans R Soc B. 2019;374:20180241.

Robertson S, Bradley JE, MacColl ADC. Eda haplotypes in three-spined stickleback are associated with variation in immune gene expression. Sci Rep. 2017;7:42677.

Roesti M, Hendry AP, Salzburger W, Berner D. Genome divergence during evolutionary diversification as revealed in replicate lake-stream stickleback population pairs. Mol Ecol. 2012;21:2852–62.

Roesti M, Moser D, Berner D. Recombination in the threespine stickleback genome – patterns and consequences. Mol Ecol. 2013;22:3014–27.

Roesti M, Gavrilets S, Hendry AP, Salzburger W, Berner D. The genomic signature of parallel adaptation from shared genetic variation. Mol Ecol. 2014;23:3944–56.

Roesti M, Kueng B, Moser D, Berner D. The genomics of ecological vicariance in threespine stickleback fish. Nat Comms. 2015;6:8767.

Rosenblum EB, Parent CE, Brandt EE. The molecular basis of phenotypic convergence. Annu Rev Ecol Evol Syst. 2014;45:203–26.

Rudman SM, Schluter D. Ecological impacts of reverse speciation in threespine stickleback. Curr Biol. 2016;26:490–5.

Rudman SM, Goos JM, Burant JB, Brix KV, Gibbons TC, Brauner CJ, Jeyasingh PD. Ionome and elemental transport kinetics shaped by parallel evolution in threespine stickleback. Ecol Lett. 2019;22:645–53.

Rundle HD, Nagel L, Boughman JW, Schluter D. Natural selection and parallel speciation in sympatric sticklebacks. Science. 2000;287:306–8.

Samuk K, Owens G, Delmore K, Miller S, Rennison D, Schluter D. Gene flow and selection interact to promote adaptive divergence in regions of low recombination. Mol Ecol. 2017;26:4378–90.

Schluter D. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. Ecology. 1993;74:699–709.

Schluter D. Ecological causes of adaptive radiation. Am Nat. 1996;148:S40–64.
Schluter D. Ecology and the origin of species. Trends Ecol Evol. 2001;16:372–80.
Schluter D. Evidence for ecological speciation and its alternative. Science. 2009;323:737–41.
Schluter D, Conte GL. Genetics and ecological speciation. Proc Natl Acad Sci U S A. 2009;106:9955–62.
Schluter D, McPhail JD. Ecological character displacement and speciation in sticklebacks. Am Nat. 1992;140:85–108.
Shapiro MD, Marks ME, Peichel CL, Blackman BK, Nereng KS, Jónsson B, Schluter D, Kingsley DM. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. Nature. 2004;428:717–23.
Small CM, Milligan-Myhre K, Bassham S, Guillemin K, Cresko WA. Host genotype and microbiota contribute asymmetrically to transcriptional variation in the threespine stickleback gut. Genome Biol Evol. 2017;9:504–20.
Spence R, Wootton RJ, Barber I, Przybylski M, Smith C. Ecological causes of morphological evolution in the three-spined stickleback. Ecol Evol. 2013;3:1717–26.
Stein LR, Bell AM. The role of variation and plasticity in parental care during the adaptive radiation of three-spined sticklebacks. Evolution. 2019;73:1037–44.
Steury RA, Currey MC, Cresko WA, Bohannan BJ. Population genetic divergence and environment influence the gut microbiome in Oregon threespine stickleback. Genes (Basel). 2019;10:484.
Stuart YE, Veen T, Weber JN, Hanson D, Ravinet M, Lohman BK, et al. Contrasting effects of environment and genetics generate a continuum of parallel evolution. Nat Ecol Evol. 2017;1:158.
Taylor EB, McPhail JD. Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (*Gasterosteus*): insights from mitochondrial DNA. Biol J Linn Soc. 1999;66:271–91.
Terekhanova NV, Logacheva MD, Penin AA, Neretina TV, Barmintseva AE, Bazykin GA, Kondrashov AS, Mugue NS. Fast evolution from precast bricks: genomics of young freshwater populations of threespine stickleback *Gasterosteus aculeatus*. PLoS Genet. 2014;10:e1004696.
Thompson CE, Taylor EB, McPhail JD. Parallel evolution of lake-stream pairs of threespine sticklebacks (*Gasterosteus*) inferred from mitochondrial DNA variation. Evolution. 1997;51:1955–65.
Vamosi SM, Schluter D. Impacts of trout predation on fitness of sympatric sticklebacks and their hybrids. Proc R Soc B. 2002;269:923–390.
Verta JP, Jones FC. Predominance of *cis*-regulatory changes in parallel expression divergence of sticklebacks. Elife. 2019;8:e43785.
Wellenreuther M, Hansson B. Detecting polygenic evolution: problems, pitfalls, and promises. Trends Genet. 2016;32:155–64.
Xie KT, Wang G, Thompson AC, Wucherpfennig JI, Reimchen TE, MacColl ADC, et al. DNA fragility in the parallel evolution of pelvic reduction in stickleback fish. Science. 2019;363:81–4.
Yeaman S. Genomic rearrangements and the evolution of clusters of locally adaptive loci. Proc Natl Acad Sci U S A. 2013;110:E1743–51.

Mechanisms of Adaptive Divergence and Speciation in *Littorina saxatilis*: Integrating Knowledge from Ecology and Genetics with New Data Emerging from Genomic Studies

Kerstin Johannesson, Roger K. Butlin, Marina Panova, and Anja M. Westram

Abstract New opportunities to understand marine speciation and evolution of local adaptation come with genomic approaches and with the development of comprehensive model systems. The marine snail *Littorina saxatilis* is one example of a developing marine model for investigating genetic mechanisms of rapid divergence and evolution in natural systems. This species is strongly polymorphic and shows formation of local ecotypes throughout its distribution. Support is strong for primary (in situ) and parallel formation of reproductively semi-isolated ecotypes with contact zones between heterogeneous intertidal microhabitats. This makes this species an ideal organism for gaining new insights into the interplay of divergent selection, gene flow and genetic drift during local adaptation and speciation. A relatively well-resolved draft genome and a genetic map describing 17 linkage groups ("chromosomes") are key tools for investigating the role of structural genomic variation, such as inversions, gene duplications and translocations. Whole genome re-sequencing of pools of individuals and the first comprehensive study of a contact zone contribute direct information on selection and barriers to gene flow present in specific regions of the genome. Linking selection at the phenotypic level to patterns obser ved in the genome is under way by quantitative

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trait loci mapping and annotation of candidate genes, while the role of single mutations on individual fitness will have to await development of gene manipulation tools. The features of the snail system facilitate the study of local adaptation and speciation and its genomic basis, but the underlying evolutionary processes are expected to be similar in other organisms, and hence this species is a useful model.

Keywords Ecotypes · Hybrid zones · Local adaptation · Marine snail · Reference genome · Speciation

1 Genomics Offers New Opportunities to Understand Marine Evolution

While we are witnessing a global wave of species extinction and presumably also extensive loss of genetic diversity within species on land and in the seas, we still cannot fully explain how new biodiversity is generated. In open and highly connected marine systems where high numbers of local physical barriers are hard to perceive, the question of how species evolve is perhaps even more intriguing than on land. Speciation and local adaptation, two major components of evolution, are connected and need to be understood in light of fundamental genetic mechanisms such as mutation, selection, gene flow and drift. In addition, these mechanisms interact with the spatial and temporal changes of the environment. Under the current pressure of local and global environmental change, increased knowledge on the rate of evolutionary change in relation to the rate of environmental change is critical to our understanding of the rate of population and species extinction in the near future. To study evolutionary change in natural populations, we need efficient methods to map genetic variation over spatial and temporal scales, and we need suitable model systems.

With our current genetic toolbox, including comprehensive sequencing and bioinformatics approaches, we may investigate both the structure of the genomic landscape at the level of individuals and the structure of the genetic landscape at the levels of populations and species. Adding to this the strength of today's modelling tools, evolutionary research has gained increased power and is currently making rapid progress. While keeping in mind that we know essentially nothing for most of all the millions of species, not least in the marine realm, for a tiny fraction of all species, new knowledge is now emerging at an unprecedented rate. For very good reasons, the marine snail *Littorina saxatilis* is one of this vanguard.

This species has been extensively studied using now-classical genetic markers (allozymes, microsatellites, sequencing fragments of mitochondrial or nuclear genes, AFLP), and these methods are being replaced by genome-wide approaches sequencing tens of thousands of fragments throughout the genome (RAD, capture sequencing, transcriptome sequencing, whole genome re-sequencing). The new methods expand our opportunities for investigating fundamental biological questions in evolution and genetics using *Littorina saxatilis* and related species as a model system, including the understanding of how new biodiversity evolves. In this

chapter, we review the knowledge background for the snail system, generated by pre-genomic approaches, and we present new results emerging from the use of genomic approaches together with glimpses from ongoing work. Where relevant, we suggest how the new genomic resources may be used in future studies to advance our knowledge even further.

2 A Convenient Model System for Studies of Evolutionary Divergence

The intertidal snail *Littorina saxatilis*, a very common north Atlantic gastropod, is one of the species for which genomic data is currently accumulating at a high rate. A major reason for the large interest in this species is the prominent evolution of locally adapted morphs – ecotypes – that differ in size, shell shape, colour, behaviour and physiology and are partly separated by reproductive barriers in contact areas (e.g. Sundell 1985; Johannesson and Johannesson 1996; Reid 1996; Johannesson et al. 2008). The absence of a free-floating larva and the sedentary lifestyle of juvenile and adult snails result in dispersal ranges of only a few metres over a generation (Janson 1983; Erlandsson et al. 1998). The complex environment of the intertidal zone varies at the same spatial scale, resulting in populations being linked to specific physical environments shaped by a mosaic of steep abiotic and biotic selection gradients (Janson and Ward 1984). This results in local adaptation and striking phenotypic differentiation, sometimes present at a very small spatial scale (Fig. 1). The snail presents a uniquely amenable system but is not unusual in terms of the trade-offs among evolutionary forces, even if scales of dispersal and selection gradients are very compressed. These features of the snail system facilitate the study of local adaptation and its genomic basis, but the underlying evolutionary processes are expected to be similar in other organisms.

Earlier, the many ecotypes of *L. saxatilis* generated a confused taxonomy based on shell morphology and colour, the traditional traits used in mollusc taxonomy. Indeed, this species with its various adult sizes, shell shapes and colours is said to have the world record in fooling taxonomists, which is not surprising as more than 100 named taxa have been described over the years (Reid 1996). With a more comprehensive morphological approach more focused on the soft parts, and with additional support from allozymes, it became obvious during the 1990s that most of these differently named taxa were connected by gene flow and differences were generated by divergent selection (reviewed in Johannesson 2015). Interestingly, the one taxon that actually turned out to be a separate species (*L. arcana*) was indistinguishable from one of the *L. saxatilis* ecotypes based on shell morphology and ecology (Hannaford Ellis 1979; Ward and Warwick 1980).

The suggestion of habitat-specific ecotypes evolved by divergent selection but connected by weak gene flow has been supported by many later studies using random molecular markers such as microsatellites, mitochondrial DNA, AFLP and SNPs (Mäkinen et al. 2008; Wilding et al. 2001; Quesada et al. 2007; Tirado

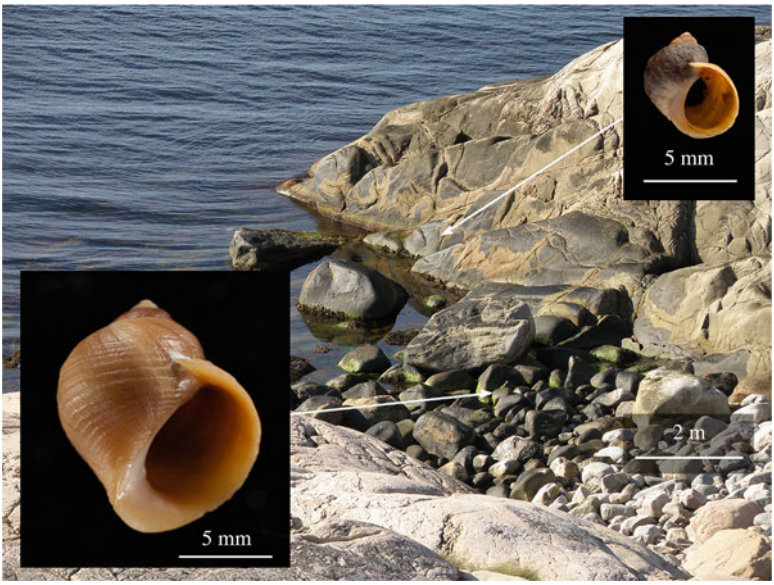

Fig. 1 The crab (*left*) and wave (*right*) ecotypes of *Littorina saxatilis* from a Swedish rocky shore. The crab ecotype is confined to the boulder part of the shore where predation from shore crabs is strong during summer and autumn. The wave ecotype inhabits the cliff surfaces exposed to wave action during windy days. Close-ups illustrate differences in shell form and size of ecotypes (Shell photos: Fredrik Pleijel)

et al. 2016). Various ecotypes have been investigated using molecular tools, but most studies on the formation of divergent ecotypes have concentrated on two common forms, the crab ecotype and the wave ecotype. The crab ecotype is locally adapted to crab-rich patches of rocky shores (e.g. boulder areas or upper shore crevices and rock pools) and is characterized by a large size, a thick shell and a shy behaviour. The wave ecotype is adapted to wave-exposed cliff surfaces and has a small size, a thin shell and a bold behaviour. Contact zones (hybrid zones) are formed where the two microhabitats intergrade (Fig. 1), and here hybrid phenotypes are present.

The crab-wave ecotype setting, with the micro-scale ecotone transitions between contrasting microenvironments, is maintained by strong divergent selection. The survival rate of adult snails moved to the contrasting environment is reduced to a fraction of their predicted survival in their home environment (Janson 1983; Rolán-Alvarez et al. 1997). This strong selection counteracts the homogenizing effects of gene flow, and estimates of gene flow made from neutral markers show a substantial reduction over the contact zone (Rolán-Alvarez et al. 1996; Grahame et al. 2006; Panova et al. 2006; Galindo et al. 2009) (Fig. 2). It is important to note that strong selection and a reduced gene flow may be complemented by strong plasticity as found between populations of dog whelks in wave-exposed and crab-exposed environments (Appleton and Palmer 1988). For example, the formation of a thicker

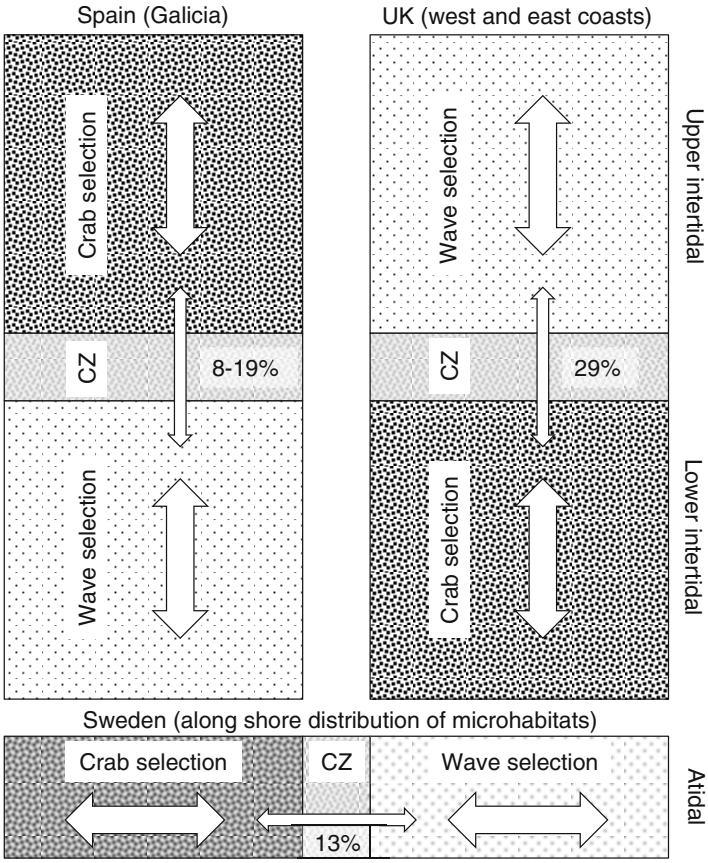

Fig. 2 Crab and wave habitats appear in different parts of the shore in Spain, Britain and Sweden. Upper shore rock pools and crevices in Spain are occupied by night-active predatory crabs, while physical stress from the open Atlantic waves increases risk of snail dislodgement in the low shore. In Britain, vertical upper shore cliffs of sedimentary rocks are exposed to wave action during high tide, while below the cliffs are boulders and blocks that provide hiding places for predatory shore crabs. In Sweden, boulder fields are crab habitats, while adjacent protruding cliff surfaces are strongly exposed to wave action but without crabs (see also Fig. 1). Estimates of gene flow over contact zones as a proportion of within habitat gene flow are indicated for the different countries (from Johannesson et al. 2010)

shell and a smaller aperture in dog whelks is triggered by the smell of crabs and crushed snails. In *L. saxatilis*, plasticity induced by the environment adds to the phenotypic variation, but a large proportion of the ecotype difference is explained by genetic variation (e.g. Janson 1982; Johannesson and Johannesson 1996; Hollander et al. 2006). In addition, the capacity for induced phenotypic change is under selection and effects of plasticity are largely supportive of local adaptation of phenotypes (Hollander and Butlin 2010).

Interestingly, *L. saxatilis* separates into crab and wave ecotypes in different geographic areas, such as in Sweden, Britain and Spain, even though the distribution of the microhabitats may be very different (horizontal in Sweden, vertical in Britain and in Spain but in opposite orders; see Fig. 2). In all of these three settings, snails present in either crab- or wave-dominated microenvironments are phenotypically surprisingly similar but with some differences in colour and shell ornamentation (Butlin et al. 2014) (Fig. 3).

The snail system is remarkable in that it provides replication of ecotype formation at various geographical scales: within the same bay, between bays within the same region, between regions in the same country and between distant areas, e.g. Sweden vs. Spain. This provides us with an outstanding opportunity to test genetic mechanisms involved in local adaptation of similar traits and evolution of barriers to gene flow among populations with similar levels of phenotypic divergence. We may ask whether the mutations involved in adaptive divergence have single origins or if the same mutations appear repeatedly in different locations. In addition, it allows us to test whether the genomic architecture of divergence is shared between geographically distant locations, that is, if genomic rearrangements and genomic regions of increased or decreased divergence are the same throughout the species' distribution. Below we will expand on each of these topics, both presenting the knowledge we have from traditional genetic studies and describing how new genomic approaches

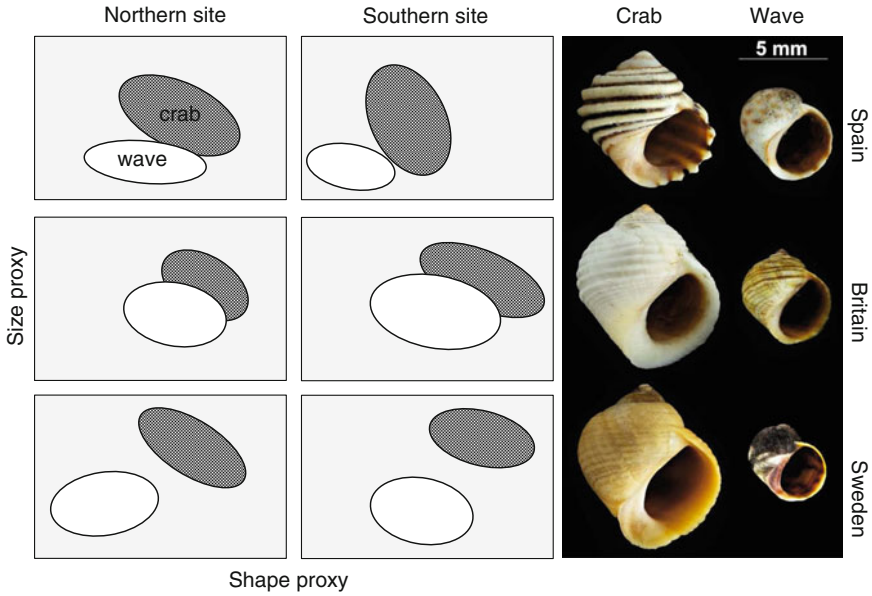

Fig. 3 Simplified outline of shell shape and size variation for 32 individuals of crab and wave ecotype from a northern and a southern site within each country. For the original illustration with additional details, see Butlin et al. (2014) (Photos: Fredrik Pleijel)

now move us beyond earlier knowledge. But, before doing so, we will briefly introduce the genomic resources now becoming available for *Littorina saxatilis*.

3 Genomic Resources Now Emerging for the Snail System

For *L. saxatilis*, work with allozymes, mitochondrial markers and microsatellites has a long and comprehensive history (e.g. Janson and Ward 1984; Johannesson and Tatarenkov 1997; Quesada et al. 2007; Mäkinen et al. 2008). In addition, AFLP data have been used for demographic inference and one of the first outlier scans (Wilding et al. 2001; Butlin et al. 2014). Now, with the availability of next-generation sequencing technology, extensive genomic resources are being developed for the snail system (Canbäck et al. 2012; Panova, Larsson et al. in prep.). There is currently a first version of an assembled draft genome (genome size ~1.3 Gb) of a single, crab ecotype *L. saxatilis* from Sweden, sequenced using paired-end Illumina reads from several libraries (insert size from 160 to about 8,000 bp) to a depth of more than 500×. The assembly has been improved by scaffolding with mate-pair libraries and Pacific Biosciences long-read data. A reference transcriptome, a reference genome assembly for a wave ecotype individual and genome re-sequencing data for outgroup species are available. In addition, a genetic map has been generated using genotyping-by-sequencing markers of a large crab ecotype family (>180 offspring). Moreover, whole-genome re-sequencing datasets from pools of 100 individuals of crab-wave ecotype pairs from geographically distant sites in Spain, France, Britain and Sweden with an average coverage of 75× have been added to the resources. Annotation is ongoing, and although it is expected that the functions will remain unknown for a large proportion of all genes (as in other mollusc genomes), these resources will provide a large leap forward to interpret sequence differences found in recent and ongoing population genomic studies. With these genomic resources at hand, the basic knowledge already generated for the *Littorina* system using classical population genetic approaches can now be considerably advanced.

4 The Demographic History Shapes the Large-Scale Genetic Structure

The species' present distribution covers most coastal areas of both sides of the Northern Atlantic where it occupies a variety of intertidal habitats from the temperate zone to the Arctic (Reid 1996). From phylogeographic studies using mtDNA markers, it seems that this trans-Atlantic distribution has persisted over extended periods of time (Doellman et al. 2011), with survival in widespread glacial refugia in the eastern and western Atlantic, and on northern islands, during the last glacial maximum (Panova et al. 2011). From these local refugia, postglacial expansion

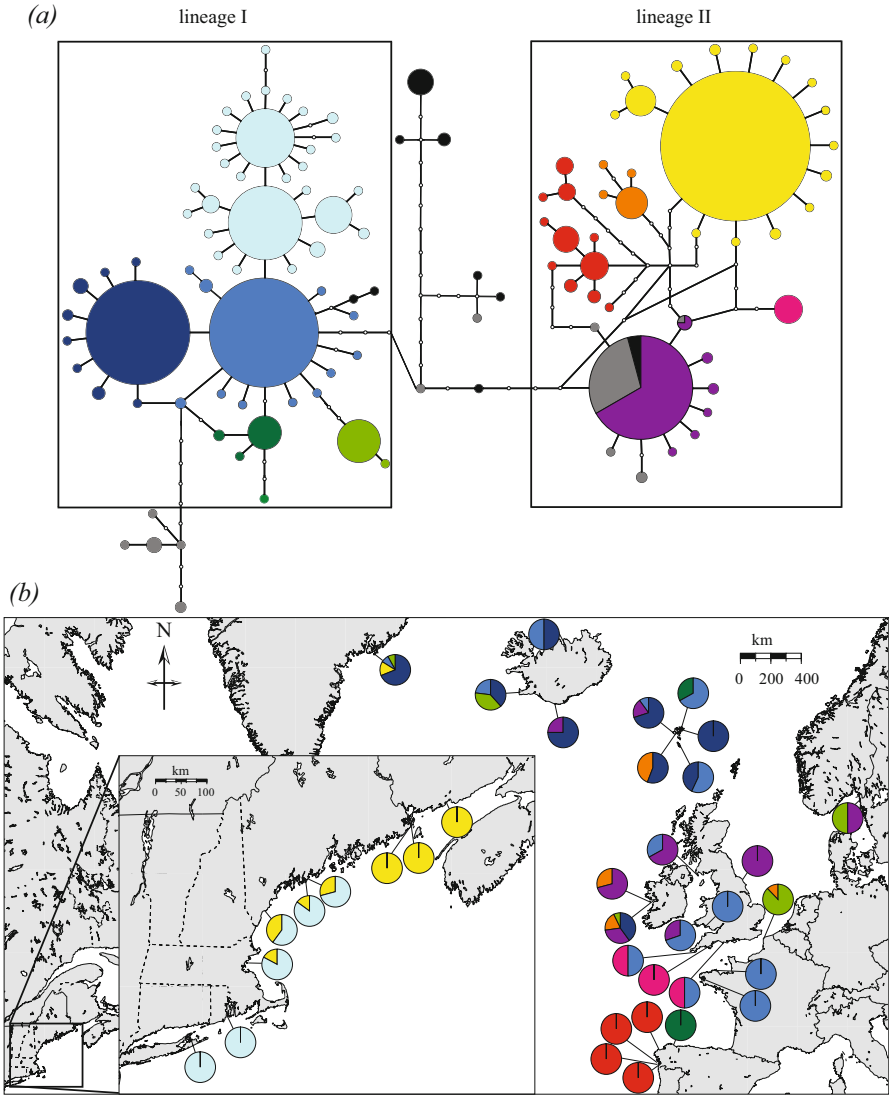

Fig. 4 (**a**) Maximum parsimony network for *L. saxatilis* (colours), *L. arcana* (*grey*) and *L. compressa* (*black*). Circle size is proportional to the number of individuals (1–66). (**b**) Geographic distribution of *L. saxatilis* haplotypes over its main area of distribution showing the complex large-scale geographic pattern with western-eastern and southern-northern structures (Figure reproduced from Doellman et al. 2011 with permission)

resulted in a wide distribution, a high level of genetic heterogeneity and the rather complex large-scale population genetic structure observed today (Fig. 4). For example, in addition to the east-west Atlantic separation, there are north-south divisions on both sides of the Atlantic that are remarkably sharp. On the European

side, this division in the Bay of Biscay overlaps with that of other shallow water species (Hoarau et al. 2007; Kemppainen et al. 2009; Olsen et al. 2010), suggesting historical barriers to gene flow of large significance for many marine species.

With the extended genomic resources now available for this species, the phylogeographic patterns suggested by the mtDNA markers can be compared with patterns from a high number of nuclear genetic markers to test robustness. Such a general phylogeographic pattern (a population tree) will be a useful reference against which to mirror gene trees for single gene loci or genomic regions to suggest the origin and history of genes under selection.

Notably, part of the species' history can be ascribed to the paradox that despite the lack of a pelagic spreading phase, the species has a very high capacity to colonize and establish new populations in remote areas following occasional transportation of single females. Using microsatellite markers suitable for parental analysis, it was found that female snails may store sperm from ~20 males simultaneously (Panova et al. 2010). Obviously, this substantially increases the genetic variation brought by a single female colonizer during a founder event (Rafajlović et al. 2013), and together with the fact that females also store viable sperm for up to a year upon isolation (Johannesson et al. 2016), this increases the probability of a successful founder event after a long-distance journey of a single female on driftwood or a piece of macroalga. Moreover, long-term sperm storage will supply a female with enough sperm to release several hundred offspring in the new environment. The lack of a pelagic larval stage will, in addition, prevent spread of the offspring over a very large area. This will facilitate mate finding in the next generation and the establishment of a new population (Johannesson 1988). The successful colonization of small and very remote Atlantic islands such as Rockall shows that postglacial long-distance colonization events have occurred, and it seems likely that these processes may have contributed to shaping the current population genetic structure of *L. saxatilis* following the last glacial maximum and may still play a role in the distribution of new favourable mutations.

Migration (and potential gene flow) is continuous at much more local scales. With a distribution restricted to the intertidal zone and no swimming or drifting larval stages, high migration is only possible over distances of a few metres. In addition, low but rather constant levels of migration are expected also at medium distances (>100 m to <100 km). Direct measurements of migration at these distances are difficult. However, a toxic algal bloom in 1988, when all populations established on small, shallow skerries (rocky islets) in the Swedish archipelago were completely wiped out, offered a rare possibility for direct estimates of recolonization rate from larger islands in the archipelago (Johannesson and Johannesson 1995). Yearly visits after 1988 showed that on average 3% of all skerries received a founder population each generation, translating to a migration rate (Nm) of ~0.1 individuals per generation and indicating that stepping-stone migration among suitable habitats over distances of 0.1–1 km is small but definitely not negligible. This corroborates results from studies based on allozymes and microsatellites showing that over distances of 1–100 km there are clear isolation by distance effects (Janson 1987; Mäkinen et al. 2008).

5 Small-Scale Patterns Are Dominated by Local Adaptation

At very small distances, phenotypic differentiation is affected by divergent selection between contrasting microenvironments and associated barriers to gene flow. An early result from analyses of micro-scale variation in allozyme loci was that ecotones act as general barriers to gene flow (Johannesson et al. 1993), and this result was corroborated by later analyses of microsatellite and AFLP markers (Wilding et al. 2001; Panova et al. 2006). In addition, ecological experiments show that strong differential phenotypic selection acts on spatial scales of only tens of metres (Janson 1983; Rolán-Alvarez et al. 1997). For example, reciprocal transplants of snails of crab and wave ecotypes show that relative fitness of one ecotype in the other environment may be only 28–42% of the local ecotype, as estimated in a Swedish site (Janson 1983), or 11–12%, as estimated in Spanish sites (Rolán-Alvarez et al. 1997). Also estimates based on selection on specific traits indicate generally strong spatially varying selection that may be directional or balancing. For example, one allele of an allozyme locus (aspartate aminotransferase) had a selective advantage of 40% over a locally disfavoured allele in a specific microhabitat, while a few metres away, selection was in the other direction (Johannesson et al. 1995a). Non-cryptic colours are found in some habitats, and here balancing selection (probably negative frequency-dependent selection from bird predators) may reduce survival of a non-cryptic snail by up to 10% if the non-cryptic colours increase to high frequencies (Johannesson and Butlin 2017).

Direct experimental tests are necessary to uncover the specific selection factors involved in the directional selection (Rolán-Alvarez et al. 2015), but this is technically challenging to do in the field. However, successful experiments show that crabs select for the traits typically found in the crab morph, that is, a large and thick shell with a relatively smaller aperture (Johannesson 1986; Boulding et al. 2007, 2017). Experimental tests in a laboratory high-speed flume show that snails sampled along a transect from less to more intense wave exposure increasingly risk dislodgement in the water flow unless the relative foot and aperture areas increase, and the shell becomes laterally compressed (Le Pennec et al. 2017). It seems highly likely, but has not been experimentally tested, that inherited differences in snail behaviours (Johannesson and Johannesson 1996) are selected in response to the presence of crabs (a shy behaviour) or increased risk of dislodgement (a bold behaviour).

Recent studies using genomic approaches to study genetic differentiation over the crab-wave ecotones at different locations and in different countries (Spain, Britain and Sweden) first of all contribute with data that show how large a proportion of the genome is involved in the differentiation (e.g. Galindo et al. 2010). Sequencing transcribed parts of the genome (RNA sequencing) in separate pools of crab and wave ecotype snails sampled from either side of local microhabitat ecotones on shores in Sweden, Britain and Spain indicated that a very large proportion (~1/3) of all ~7,000 loci showed ecotype differentiation of 10% or more ($F_{ST} > 0.1$) over the

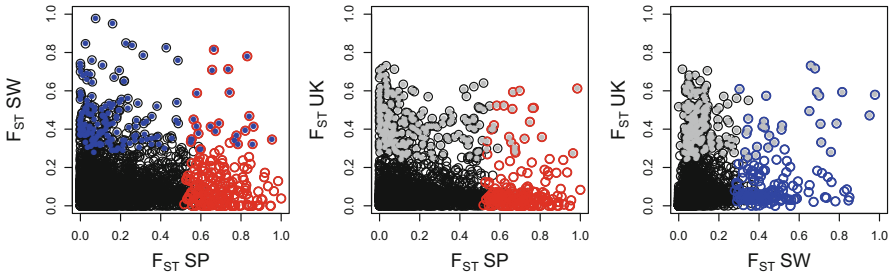

Fig. 5 F_{ST} estimates between ecotype pairs compared among countries (F_{ST} averaged across two replicate estimates within each country). Loci above the 96% quantile of the F_{ST} distribution in both replicate sample pairs ("outliers") are shown in colour (Spain, *red*; Sweden, *blue*; Britain, *grey*), and shared outliers have two colours. $n = 6{,}790$ loci (From Westram et al. 2014)

ecotones and, despite different microhabitat arrangements (Fig. 2), the pattern was more or less consistent over all three countries (Fig. 5, data from Westram et al. 2014). A later study sequencing crab and wave individuals from either side local ecotones in three Swedish sites used a genome scan approach (RADseq) including both transcribed and un-transcribed parts of the genome (Ravinet et al. 2016). With a similar total number of loci after filtering (8,500), about 15% of these showed strong differentiation ($F_{ST} > 0.1$) between ecotypes. Both studies thus convincingly show that the genetic divergence of the crab and wave ecotypes involves differentiation over an extensive part of the snail genome, despite the fact that the physical distances between the crab and wave samples either side of the ecotone were usually less than 30–50 m. Furthermore, crab and wave populations are in direct contact, with opportunities for inter-ecotype matings (Johannesson et al. 1995b), resulting in frequent occurrence of hybrid phenotypes (Janson and Sundberg 1983) and a reduced gene flow (Panova et al. 2006). How many of the differentiated genetic markers are directly or indirectly (through linkage) affected by selection cannot be inferred from these types of studies as differences over hybrid zones can also be present due to historical, demographic and stochastic reasons (Bierne et al. 2011, 2013). New sampling approaches combined with models that generate predictions separating out effects of selection may take us further to an understanding of the role of selection in forming barriers to gene flow over contact zones (see "Evolution of barriers to gene flow" below).

6 Parallel Formation of the Crab and Wave Ecotypes

The repeated occurrence of crab and wave ecotype snails in different local sites within a small geographic area (<10 km), in different regions of a country and even in different countries (Butlin et al. 2014) has two conceptually very different possible explanations. One is that different ecotypes evolved in isolation, during earlier glacial periods, and

spread postglacially into the same geographic area where they established secondary contact zones by hybridization (Grahame et al. 2006). Under such a scenario, we expect many of the genetic differences between the ecotypes to be ancestral and therefore shared among areas. Alternatively, ecotype divergence may have evolved in each local area, in parallel, from one ancestral form similar to one of the present ecotypes, or of a different phenotype (Rolán-Alvarez et al. 2004; Quesada et al. 2007). Various mixed models combining periods of isolation and divergence under gene flow are, of course, also possible (Johannesson et al. 2010).

Modelling the demographic history of a number of European populations of the two ecotypes using sequence data from several types of genetic markers (mtDNA, 4 nuclear genes and 614 AFLP loci) showed strong support for a model of local and parallel divergence of ecotypes under gene flow and weak support for a contrasting model of allopatric origin of the ecotypes (Butlin et al. 2014). Taken together with earlier studies based on allozymes, microsatellites and mtDNA markers that also suggested in situ origin of ecotypes but at more local geographic scales (Johannesson et al. 1993; Panova et al. 2006), these results support evolution of parallel ecotypes under gene flow and at the scale of local microhabitat ecotones.

Importantly, however, these results, including the outcome of the demographic model, do not exclude the possibility that genetic elements that are under selection and involved in the divergence in many of the places are ancestral and may have originated in isolation. Rather, what the results indicate is that a majority of random genetic markers reflect parallel processes of divergence under which similar regimes of selection (crab predation, wave exposure, etc.) shape the available genetic variation into genotypes that by parallel or convergent molecular mechanisms lead to phenotypically similar snails in similar types of microhabitats independent of geographic location. The whole process is very recent (postglacial) and results in primary hybrid or contact zones where divergence takes place in the face of gene flow.

The primary divergence and the parallel evolution are two very important features of the *Littorina* system that make this species a useful model for studies of local adaptation and speciation. For example, differentiation under gene flow emphasizes the role of divergent selection and rejects the possibility that ecotype formation and establishment of reproductive barriers (as will be discussed below) are consequences of stochastic accumulation of differences. Furthermore, as the primary zones of the *Littorina* system have formed multiple times, this system offers possibilities to study the repeatability of the divergence process at various levels of organization (e.g. morphology, metabolic pathways and genetic variation). In addition, the snail system also offers replicated divergence at very different scales of genetic and demographic independence. For example, it is possible to compare demographically independent Swedish populations from different islands that essentially share the same postglacial (~8,000 years) gene pool. Or, one may compare Swedish and Spanish populations of crab and wave ecotypes that have had independent evolutionary histories during the past 50,000–100,000 years (Panova et al. 2011). Using genomic data, this setting can be used to study the role of new mutations vs. already available genetic variation and the role of the genomic architecture. Below we discuss these potentially rewarding future directions of research further.

7 To What Extent Is Evolution Stochastic or Deterministic?

The *Littorina* system may be useful to contribute important understanding to several generic questions in evolutionary biology. One is the role of stochasticity in evolution. As remarked by Patrik Nosil and colleagues "whether evolution is predictable and repeatable is difficult to test yet central to our understanding of biological diversification" (Soria-Carrasco et al. 2014). The *Littorina* system, in which similar phenotypes evolve repeatedly and in parallel at various levels of population independence, from populations separated only a few thousand generations ago to sibling species separated a few million years ago (Reid et al. 2012), can be used to address questions such as: Are the same loci and mutations involved in shaping similar phenotypes, or does parallel evolution at the phenotypic level derive from convergence at the genetic and molecular level? Are some parts of DNA more prone to mutations than others, and at what rate do new mutations contribute to phenotypic evolution? And, finally, are some types of mutations or mutations in some types of genes more likely to contribute to local adaptation than others? For example, are mutations in regulatory genes more important than mutations in structural genes, or are genes with pleiotropic or epistatic effects more involved than those with no such effects, or are genes with effects at various parts of the developmental pathways more or less influential on local adaptation (Seehausen et al. 2014)?

An appealing model is that similar phenotypes evolve from a common pool of genetic variation that is shared among populations by ancestry and/or by gene flow providing the "standing genetic variation" from which various phenotypes may evolve by selection to fit the local conditions (Barrett and Schluter 2008). If local selection regimes are similar, phenotypes will be similar either by recombining the same set of alleles or by combining genetic variation with similar effects on the phenotype. A somewhat related model is that whenever a new favourable mutation is introduced to the species, either by a new mutation in one local population, by long-distance migration from a remote part of the species, or by introgression from a closely related species, this new mutation may spread by selective sweeps among those populations where it is beneficial. In this way, populations occupying similar types of microhabitats will evolve in a concerted way (Johannesson et al. 2010). This mechanism is similar to what Rieseberg and colleagues suggested would explain the "collective evolution" of closely related species (Rieseberg and Burke 2001; Morjan and Rieseberg 2004). And it also appears similar to the "transporter process" suggested to explain the parallel evolution of geographically distant freshwater populations of stickleback (Schluter and Conte 2009). Importantly, strong directional selection at local sites of a given microhabitat would make this possible, even with gene flow that is so low that the spread of neutral variation is generally restricted (Morjan and Rieseberg 2004) – for example, the type of gene flow contributed by an occasional long-distance dispersal of a single snail discussed above.

Both the standing genetic variation hypothesis and the evolution in concert hypothesis make the same important prediction, that the same genetic variation

will be reused in multiple populations that share a recent (postglacial) origin and/or are connected by contemporary gene flow. For such populations, it is expected under both these models that a large proportion of the loci that show local divergence between ecotypes should be the same. Populations of Northern Europe (e.g. in Sweden and Britain) were established after the last glacial maximum and do share a recent common ancestry (Doellman et al. 2011; Panova et al. 2011). Geographic variation in allozyme loci under selection shows the expected pattern with the alleles favoured by selection being shared over both small and large geographic areas in *L. saxatilis* (Johannesson and Johannesson 1989; Johannesson and Tatarenkov 1997). However, for the same allozyme loci, populations from Spain appear to have an allozyme allele that, despite the same mobility on the allozyme gel, has two unique non-synonymous mutations with what seems to be different effects on the phenotype, as this allele is more frequent in another microhabitat (Panova, Mittermayer et al. in prep.). Variation in 306 AFLP loci in British populations is also consistent with divergent loci being shared among locations: 15 out of 20 loci that diverged locally between *L. saxatilis* ecotypes were shared among three populations 4–26 km apart (Wilding et al. 2001).

With genomic approaches, proportions of shared loci can be investigated at numerous loci, which is very relevant for our understanding of the more general mechanisms involved in local adaptation and divergence. In addition, studies performed at different scales of population independence are important, testing the prediction that more sharing will be present among populations with a very recent common origin and/or with recent or contemporary gene flow. Such a genomic study sequencing transcriptome loci showed that at a geographical scale of 100–300 km populations still tended to share the majority of outliers, while this proportion decreased for most markers when populations were separated over larger distances (Fig. 6, and see Westram et al. 2016 for details). Thus these results support a level of sharing that may be explained by ecotypes evolving from standing genetic variation or by evolution in concert. However, in contrast to the majority of results, genotyping population samples from a very small geographic area (<10 km) with RADseq markers showed a less expected result: A very small proportion of all crab-wave divergent SNPs (2–9%) were shared among the three study islands (Ravinet et al. 2016), despite a recent common ancestry and the populations being connected by continuous gene flow. This difference between studies could reflect a problem inherent in genome scans: The threshold used to classify loci into "outliers" vs. "non-outliers" is always arbitrary to some extent, so that in studies applying a stringent threshold, false positive rate is expected to decrease (which is desirable) but outlier sharing is also expected to decrease because fewer loci fall above the threshold in multiple locations. In addition, other mechanisms, not least local demography, may interfere with patterns of divergence caused by differential selection (Bierne et al. 2011). These partly conflicting results highlight the need to obtain independent evidence for selection in order to reliably identify loci affected by selection, while avoiding false positives and false negatives.

However, it seems clear that compared to sharing of outliers at short distances, at large geographical scales (between Sweden, Spain and Britain), outlier sharing

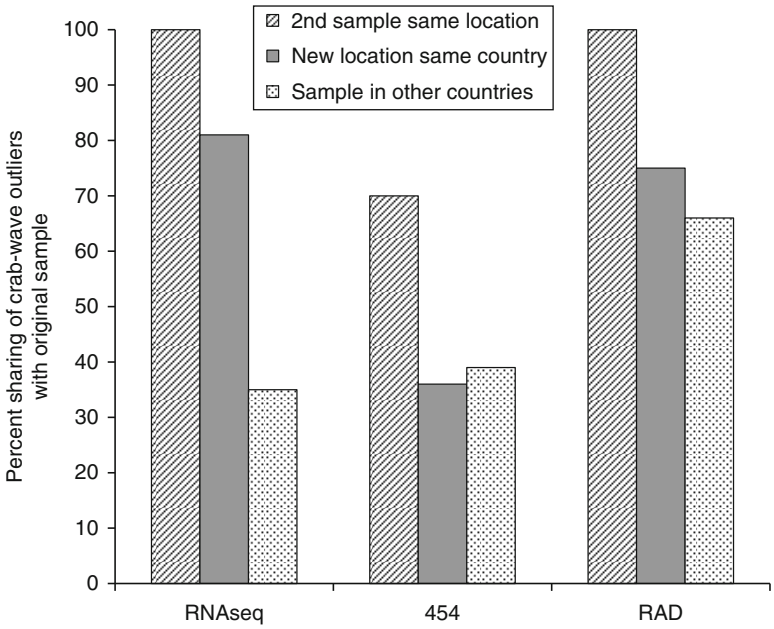

Fig. 6 The proportions of sharing of crab-wave F_{ST} outlier loci from different sequencing studies when the same sites are resampled (hatched bars); comparisons are made with other sites in the same country (>100 km) (*grey* bars) or with sites in other countries. All values are larger than expected by chance, but see the original publication (Westram et al. 2016) for details and a proper statistical evaluation

appears to be low across studies (Fig. 6, and see Butlin et al. 2014; Westram et al. 2014), even when lenient thresholds for outlier detection are applied (Westram et al. 2016), indicating that at large geographical scale, different novel mutations might be involved in divergence or different components of ancestral standing genetic variation might be used to achieve similar adaptations of phenotypes of similar microhabitats.

With a *Littorina saxatilis* reference genome, a genetic map and some first information of QTL positions, it will be possible to trace the position of F_{ST} outliers in relation to genes with strong effect on the phenotype. Associations of outliers with differentiated phenotypes will provide additional evidence for selection acting on candidate loci. Functional annotation of outliers will also be important as, for example, it is possible that parallel phenotypic evolution between different countries is based on different loci, which are however involved in the same molecular functions or pathways (Roda et al. 2013). For the most extreme outliers, it may be possible to trace their evolutionary history and with the help of new gene manipulation tools also study their functions and interactions with other genes. Again, the parallel formation of *Littorina* ecotypes will be useful and serve as a highly replicated evolutionary experiment where the role of different outliers (or the genes they tentatively represent) can be revealed under different levels of population independence and on partly different genetic backgrounds.

8 Evolution of Barriers to Gene Flow

Local adaptation not only optimizes survival for the individual snail in one habitat, but it also lowers survival for the same snail in the other habitat and by that impedes gene flow between the two microhabitats. Ongoing genome-wide analyses of a Swedish contact zone between crab and wave ecotypes of *L. saxatilis* have identified hundreds of gene loci that show significant clines, with allele frequency changes being strongly associated with the ecotone shift from one microhabitat to the other (Westram et al. in prep.). Unless such loci are extremely common and spread throughout the genome, divergent selection over the contact zone will nevertheless mainly only result in local barriers to gene flow at, and near, loci under differential selection (Barton and Bengtsson 1986; Nosil et al. 2009), even if differential selection may be very strong at single loci (see Johannesson et al. 1995a for one example in *L. saxatilis*). Thus, an intriguing question is what makes the overall barriers to gene flow increase, eventually bringing the lineage into a branching process that may lead to speciation. Below we discuss what is known about assortative mating, habitat choice, post-zygotic incompatibilities and suppression of recombination in *Littorina saxatilis* and from there demonstrate how the use of new genomic approaches can support us in reaching a deeper understanding of these key components of speciation.

8.1 Assortative Mating and Habitat Choice

Large differences in adult size between crab and wave ecotypes, combined with male preference for mating females that are somewhat larger than themselves (Hollander et al. 2005; Johannesson et al. 2008), result in size-assortative mating which further reduces the number of matings between crab and wave ecotype snails in the contact zone. The males' preference for females of slightly larger sizes than themselves seems to be an ancestral trait, as it is present also in other littorinids (e.g. *L. littorea*, Erlandsson and Johannesson 1994; *L. fabalis*, Saltin et al. 2013; *Littoraria ardouiniana*, Ng and Williams 2014). The size preference thus constitutes a "one-allele" barrier trait (Felsenstein 1981), that is, in this case the same behavioural trait (preference for slightly larger females) is fixed in both ecotypes (and other species), and this generates a barrier to gene flow. This barrier may, in principle, be genome-wide because no allele is able to recombine away from its effects. However the barrier effect also depends on the size difference, and size is a two-allele trait, requiring divergence between ecotypes. Alleles at neutral loci can recombine away from size alleles, and for this reason, the barrier is localized to genomic regions around the size loci. The situation may be further multifaceted if size, as is often the case, has a complex genetic background involving a large number of genes.

Contact zone snails of *L. saxatilis* are usually not randomly distributed but found in patches of their preferred microhabitat resulting in habitat-induced assortative mating (Johannesson et al. 1995b; Otero-Schmitt et al. 1997). The non-random

distribution may be due to differential survival in different microhabitats or to active habitat choice (Erlandsson et al. 1998; Grahame et al. 2006) and seems from available experimental data to predominantly be due to differential survival (Cruz et al. 2004). If present, habitat choice can operate as a one-allele or a two-allele trait, depending on the way it works (Webster et al. 2012). A one-allele mechanism may be possible if habitat choice is based on matching an individual's phenotype against the background and the positively selected allele improves the matching mechanism or if preference is for the natal habitat of the individual. Alternatively, two alleles may be involved in habitat choice, each allele giving the snail a preference for one of the habitats. In the case of a two-allele mechanism, providing a barrier to gene flow requires linkage disequilibrium between the habitat choice gene (or genes) and genes under divergent selection for fitness in the two ecotypes (Webster et al. 2012). While a barrier effect is more easily obtained from a one-allele mechanism (Felsenstein 1981), the two-allele mechanism may provide a more robust barrier that is less sensitive to environmental modifications (Webster et al. 2012).

Identification of the major loci involved in size, assortative mating, habitat choice and other traits strongly involved in snail fitness is a somewhat challenging undertaking but can be performed using quantitative trait loci mapping (QTL, e.g. see Hawthorne and Via 2001) or genome-wide association study (GWAS). Indeed, both these approaches are currently underway in *L. saxatilis* involving a number of fitness-related shell traits, habitat choice, mate preference and size at maturation (Butlin et al. in prep.; Morales et al. in prep.). If, for example, major loci involved in these traits can be identified and mapped to the reference genome, it will be informative to look for linkage among them.

Both size-assortative mating and habitat selection are likely to contribute to the gene flow barriers but seem insufficient to explain more than minor parts of the observed barriers (Rolán-Alvarez et al. 1999; Cruz et al. 2004). Indeed, based on ~1,000 mating trials in the laboratory, the female/male size relationship explained only 12% of males' mate choice, and ecotype matching did not add to the explanation (Perini et al. in prep.). With general gene flow, estimated from randomly chosen (and tentatively neutral) markers, being reduced to 10–30% over the contact zone compared to gene flow over similar distances elsewhere (reviewed in Johannesson et al. 2010), the effects of differential selection on a large number of loci may indeed contribute significant barrier effects, in addition to the barriers caused by size-assortative mating and habitat choice.

8.2 The Potential Role of Genetic Incompatibilities

Post-zygotic barriers caused by genomic incompatibilities are traditionally associated with secondary hybrid zones, with incompatibilities of Bateson-Dobzhansky-Muller type (DMIs) that originated when populations were isolated (Coyne and Orr 2004; Unckless and Orr 2009). However, arguments have been raised that DMIs may evolve also under gene flow (Gavrilets 2004; Nosil and Flaxman 2011; Bank et al.

2012; Kulmuni and Westram 2017) and may be hard to separate from DMIs evolved in isolation, as the latter also tend to occur in environmental transitions (Bierne et al. 2011). Under gene flow, divergent selection is required to establish DMIs, and the most likely mechanism may be that positive selection favours the establishment of different alleles in different areas, while epistatic effects of the same alleles are incompatible in hybrids. The evidence for post-zygotic intrinsic barriers in *L. saxatilis* is scarce as yet, but observations of deformed shells in offspring of hybrid crosses when raised in the laboratory suggest DMIs that may in fact be lethal in the field: Adult snails with these deformations have never been reported from field samples. Re-sequencing individuals with and without shell deformations from populations raised in the laboratory may shed light on the genetic background of the deformation, and from here it may be possible to generate hypotheses of incompatibilities that may be further tested in strategic crosses, or, in the future, with genetic manipulation (e.g. CRISPR technology, Bono et al. 2015).

Interestingly, it has been suggested that many of the F_{ST} outliers identified by genome scans may be consequences of DMIs (Bierne et al. 2011; Bank et al. 2012; Kulmuni and Westram 2017) and, if so, will not necessarily directly reflect loci under differential selection. Possibly, this may contribute to explaining the relatively low rate of sharing of outliers among pairs of geographically very close populations of crab and wave ecotype in *L. saxatilis* described above (Ravinet et al. 2016). To separate true candidates of selection from loci that emerge as outliers for other reasons (e.g. incompatibilities) in genome scans may, however, need other approaches in addition to genomic analyses (reviewed in Ravinet et al. 2017). Even if genomic regions are correctly identified, the exact mutations causing the effect may not be easily diagnosed. For example, different alleles in an arginine kinase locus known to be under strong divergent selection in the conspecific *Littorina fabalis* ecotypes differ by nine non-synonymous nucleotide substitutions (Panova, Duvetorp et al. in prep.). In addition, despite their efficiency as general barriers to gene flow, one-allele barriers (Felsenstein 1981) will obviously not appear as outliers in a standard genome scan.

8.3 Structural Genome Variation

The genetic basis of adaptive divergence and speciation may include SNPs and small-scale indels but also larger structural variants, such as copy number variations and large insertions and deletions. Furthermore, genome rearrangements (inversions and translocations) may facilitate divergence in multiple loci by impeding recombination rates in addition to the direct effects the rearrangements may have on the divergence. Recently sequenced mollusc genomes reveal expansion of gene families involved in adaptation to environmental stress in the Japanese oyster (Zhang et al. 2012), toxin diversity in *Conus* snails (Hu et al. 2011) and the development of nervous system in *Octopus* (Albertin et al. 2015). Another feature shared by the currently sequenced mollusc genomes is a large diversity of transposable repetitive elements (Murgarella et al. 2016). Transposable elements have been shown to generate genetic polymorphism in the Japanese oyster

(Zhang et al. 2012) and large-scale genome rearrangements in *Octopus* (Albertin et al. 2015).

Problematically, anonymous genome scans have a limited ability to identify these types of variation as, without a very good genome assembly or a genetic map, the genomic locations of highly differentiated SNPs are unknown. However, in *L. saxatilis*, there are several indications that variants other than SNPs might be involved in ecotype divergence. The first outlier scan in the system (Wilding et al. 2001), based on AFLP markers, detected outliers that were associated with insertions of transposable elements, potentially affecting gene expression (Wood et al. 2008). Similarly, several outliers identified in a RNAseq comparison of *Littorina* ecotypes (Galindo et al. 2010) and ecotype-specific loci from a RADseq scan (Ravinet et al. 2016) were annotated as reverse transcriptases from mobile genetic elements. Ongoing annotation of a draft genome of *L. saxatilis* suggests that repetitive elements are common (Panova, Larsson et al. in prep.), and whole-genome re-sequencing data will further reveal to what extent they might underlie ecotype differences.

Gene copy number variation also seems common in the *Littorina* genome. Array comparative genomic hybridization showed that 10% of the expressed genes and 23% of the analysed genomic fragments are present in multiple copies (Panova et al. 2014). Further, there is some evidence that copy number variations and repetitive elements may be associated with ecotype divergence (Panova et al. 2014). Finally, recent work on a Swedish hybrid zone, combining genome-wide cline analysis with a genetic map, indicates that chromosomal rearrangements (inversions or translocations) distinguish ecotypes and contain highly differentiated SNPs, providing an exciting avenue for future research.

9 Will the Ecotypes Evolve into New Species?

The formation of crab and wave ecotypes in *Littorina saxatilis* illustrates a case of partial reproductive isolation that may be followed by speciation. Whether or not speciation will be completed remains, however, a challenging question. Using a mathematical model parameterized with empirical data from the Swedish crab-wave ecotype system, Sadedin and co-authors found support for unanimous and rapid ecotype formation, but speciation followed only in some of the simulations (Sadedin et al. 2009). In fact, one important conclusion was that circumstances that favoured one ecotype to spread over the environmental transition and eventually give rise to the second ecotype, such as gene flow over the contact zone, impeded speciation and vice versa. Models studying formation of clusters of divergent loci ("islands of divergence") in the genome suggest that local genetic barriers may grow through the accumulation of new mutations close to the already established divergence loci under both strong and weak selection, although the mechanism by which this occurs has been somewhat debated (Yeaman and Whitlock 2011; Rafajlović et al. 2016). Following this, it may be interesting to combine the

approaches of the specific *Littorina* model of Sadedin et al. (2009) with a genome model of the Yeaman type, that is, to parameterize the key traits of Sadedin's model with knowledge on the genetics of these traits, and for major genes involved in these traits include estimates of selection, linkage and gene flow.

Empirical data that may be used to test expectations from speciation models may be generated by comparing sequences of genomes at various stages of reproductive isolation along the "speciation continuum" (Seehausen et al. 2014; Roux et al. 2017). Indeed, *Littorina saxatilis* is part of such a continuum with two closely related sister species (*L. arcana* and *L. compressa*, Reid 1996; Panova et al. 2014), one of which is able to hybridize with *L. saxatilis* (Ward et al. 1986; Mikhailova et al. 2009). With the new genomic tools available now for the *Littorina* model, it is possible to analyse gene flow between ecotypes, subspecies and sibling species over much of the genome, for example, using low coverage re-sequencing and mapping of data to the reference genome. This will generate new insights on how barriers to gene flow are arranged over the genome and how they influence isolation at different stages along the speciation continuum. It will, for example, highlight the effects of various isolating mechanisms such as clusters of divergence around loci under strong divergent selection (Feder et al. 2012) and genomic rearrangements including inversions (Navarro and Barton 2003, and see Berg et al. 2016 for an example where an inversion forms a barrier that supports local adaptation in cod in the face of high gene flow). Awareness of other genomic processes that may generate similar genomic signatures to reproductive isolation is a crucial component of this type of analysis (Wolf and Ellegren 2017; Ravinet et al. 2017).

10 Conclusions

Littorina saxatilis, and related species in its speciation continuum, is an ideal model system for investigating genetic mechanisms of local adaptation and speciation. Most importantly, the parallel formation of ecotypes in populations of various magnitudes of evolutionary independence can be extremely useful to study the mechanisms involved in evolution of barriers to gene flow. What stands out for *L. saxatilis* is the primary divergence and local formation of barriers, with some populations having diverged as recently as within the past few thousands of years (Butlin et al. 2014). Furthermore, the possibility to compare ecotype divergence with divergence between closely related sibling species will add power to investigating later steps in the process that take divergence into complete speciation.

Resources that are presently available match, more or less, what has recently been identified as key components of suitable model systems for studies of genome divergence and speciation (Ravinet et al. 2017). For example, a reasonably well-resolved reference genome complemented with a genetic map (Panova, Larsson et al. in prep.) and ecological knowledge from field sampling and phenotypic analyses including selection and migration estimates over contact zones (Janson 1983; Johannesson et al. 1993; Rolán-Alvarez et al. 1997; Grahame et al. 2006;

Boulding et al. 2017; Le Pennec et al. 2017). In addition, new data analyses on contact zones are underway from a comprehensive study of four Swedish localities (Westram, Butlin et al. in prep.). Furthermore, the possibility to crossbreed and raise individuals for several generations in a common garden (a laboratory environment) is a very useful complement in addition to genome sequencing, not only to provide families for genetic maps and QTL analyses but also to separate the influence of habitat, selection and gene flow on trait variation over the contact zone. Into the future, genetic manipulation using CRISPR technology will potentially open possibilities for an era of functional genomics in this species, adding important information on the detailed molecular mechanisms involved in evolution of this intriguingly variable species.

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References

Albertin CB, Simakov O, Mitros T, et al. The octopus genome and the evolution of cephalopod neural and morphological novelties. Nature. 2015;524:220–4.

Appleton RD, Palmer AR. Water born stimuli released by predatory crabs and damaged prey induce more predatory-resistant shells in a marine gastropod. Proc Natl Acad Sci U S A. 1988;85:4387–91.

Bank C, Burger R, Hermisson J. The limits to parapatric speciation: Dobzhansky-Muller incompatibilities in a continent-island model. Genetics. 2012;191:845–63.

Barrett RDH, Schluter D. Adaptation from standing genetic variation. Trends Ecol Evol. 2008;23:38–44.

Barton N, Bengtsson BO. The barrier to genetic exchange between hybridising populations. Heredity. 1986;56:357–76.

Berg PR, Jentoft S, Star B, Ring KH, Knutsen H, Lien S, Jakobsen KS, André C. Adaptation to low salinity promotes genomic divergence in Atlantic cod (*Gadus morhua* L.). Genome Biol Evol. 2016;7:1644–63.

Bierne N, Welch J, Loire E, Bonhomme F, David P. The coupling hypothesis: why genome scans may fail to map local adaptation genes. Mol Ecol. 2011;20:2044–72.

Bierne N, Gagnaire PA, David P. The geography of introgression in a patchy environment and the thorn in the side of ecological speciation. Curr Zool. 2013;59:72–86.

Bono J, Killian EO, Matzkin LM. Connecting genotypes, phenotypes, and fitness: harnessing the power of CRISPR/Cas9 genome editing. Mol Ecol. 2015;24:3810–22.

Boulding EG, Hay T, Holst M, Kamel S, Pakes D, Tie AD. Modelling the genetics and demography of step cline formation: gastropod populations preyed on by experimentally introduced crabs. J Evol Biol. 2007;20:1976–87.

Boulding EG, Rivas MJ, González-Lavin N, Rolán-Alvarez E, Galindo J. Size selection by a gape-limited predator of a marine snail: insights into magic traits for speciation. Ecol Evol. 2017;7:674–88.

Butlin RK, Saura M, Charrier G, Jackson B, André C, Caballero A, Coyne JA, Galindo J, Grahame JW, Hollander J, Kemppainen P, Martínez-Fernández M, Panova M, Quesada H, Johannesson K, Rolán-Alvarez E. Parallel evolution of local adaptation and reproductive isolation in the face of gene flow. Evolution. 2014;68:935–49.

Canbäck B, André A, Galindo J, Johannesson K, Johansson T, Panova M, Tunlid A, Butlin R. The *Littorina* sequence database (LSD) – an online resource for genomic data. Mol Ecol Resour. 2012;12:142–8.

Coyne JA, Orr HA. Speciation. Sunderland: Sinauer Associates, Inc.; 2004.

Cruz R, Vilas C, Mosquera J, García C. Relative contribution of dispersal and natural selection to the maintenance of a hybrid zone in *Littorina*. Evolution. 2004;58:2734–46.

Doellman MM, Trussell GC, Grahame JW, Vollmer SV. Phylogeographic analysis reveals a deep lineage split within North Atlantic *Littorina saxatilis*. Proc R Soc B Biol Sci. 2011;278:3175–83.

Erlandsson J, Johannesson K. Sexual selection on female size in a marine snail, *Littorina littorea* (L). J Exp Mar Biol Ecol. 1994;181:145–57.

Erlandsson J, Rolán-Alvarez E, Johannesson K. Migratory differences between ecotypes of the snail *Littorina saxatilis* on Galician rocky shores. Evol Ecol. 1998;12:913–24.

Feder JL, Gejji R, Yeaman S, Nosil P. Establishment of new mutations under divergence and genome hitchhiking. Philos Trans R Soc B Biol Sci. 2012;367:461–74.

Felsenstein J. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution. 1981;35:124–38.

Galindo J, Morán P, Rolán-Alvarez E. Comparing geographical genetic differentiation between candidate and noncandidate loci for adaptation strengthens support for parallel ecological divergence in the marine snail *Littorina saxatilis*. Mol Ecol. 2009;18:919–30.

Galindo J, Grahame JW, Butlin RK. An Est-based genome scan using 454 sequencing in the marine snail *Littorina saxatilis*. J Evol Biol. 2010;23:2004–16.

Gavrilets S. Fitness landscapes and the origin of species. Princeton, NJ: Princeton Univ Press; 2004.

Grahame JW, Wilding CS, Butlin RK. Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. Evolution. 2006;60:268–78.

Hannaford Ellis C. Morphology of the oviparous rough winkle, *Littorina arcana* Hannaford Ellis, 1978, with notes on taxonomy of the *L. saxatilis* species complex (Prosobranchia: Littorinidae). J Conchol. 1979;30:43–56.

Hawthorne DJ, Via S. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature. 2001;412:904–7.

Hoarau G, Coyer JA, Veldsink JH, Stam WT, Olsen JL. Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. Mol Ecol. 2007;16:3606–16.

Hollander J, Butlin RK. The adaptive value of phenotypic plasticity in two ecotypes of a marine gastropod. BMC Evol Biol. 2010;10:333.

Hollander J, Lindegarth M, Johannesson K. Local adaptation but not geographic separation promotes assortative mating in a snail – support for ecological speciation. Anim Behav. 2005;70:1209–19.

Hollander J, Collyer ML, Adams DC, Johannesson K. Phenotypic plasticity in two marine snails: constraints superseding life-history. J Evol Biol. 2006;19:1861–72.

Hu H, Bandyopadhyay PK, Olivera BM, Yandell M. Characterization of the *Conus bullatus* genome and its venom-duct transcriptome. BMC Genomics. 2011;12:60.

Janson K. Genetic and environmental effects on the growth rate of *Littorina saxatilis* Olivi. Mar Biol. 1982;69:73–8.

Janson K. Selection and migration in two distinct phenotypes of *Littorina saxatilis* in Sweden. Oecologia. 1983;59:58–61.

Janson K. Allozyme and shell variation in two marine snails (*Littorina,* Prosobranchia) with different dispersal abilities. Biol J Linn Soc. 1987;30:245–56.

Janson K, Sundberg P. Multivariate morphometric analysis of two varieties of *Littorina saxatilis* from the Swedish west coast. Mar Biol. 1983;74:49–53.

Janson K, Ward RD. Microgeographic variation in allozyme and shell characters in *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae). Biol J Linn Soc. 1984;22:289–307.

Johannesson B. Shell morphology of *Littorina saxatilis* Olivi: the relative importance of physical factors and predation. J Exp Mar Biol Ecol. 1986;102:183–95.

Johannesson B, Johannesson K. Population differences in behaviour and morphology in *Littorina saxatilis*: phenotypic plasticity or genetic differentiation? J Zool. 1996;240:475–93.

Johannesson K. The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? Mar Biol. 1988;99:507–13.

Johannesson K. What can be learnt from a snail? Evol Appl. 2015;9:153–65.

Johannesson K, Butlin R. What explains rare and conspicuous colours in a snail? – a test of time-series data against models of drift, migration or selection. Heredity. 2017;118:21–30.

Johannesson K, Johannesson B. Differences in allele frequencies of *Aat* between high- and mid-rocky shore populations of *Littorina saxatilis* (Olivi) suggest selection in this enzyme locus. Genet Res Camb. 1989;54:7–11.

Johannesson K, Johannesson B. Dispersal and population expansion in a direct developing marine snail (*Littorina saxatilis*) following a severe population bottleneck. Hydrobiologia. 1995;309:173–80.

Johannesson K, Tatarenkov A. Allozyme variation in a snail (*Littorina saxatilis*) – deconfounding the effects of microhabitat and gene flow. Evolution. 1997;51:402–9.

Johannesson K, Johannesson B, Rolán-Alvarez E. Morphological differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail *Littorina saxatilis*. Evolution. 1993;47:1770–87.

Johannesson K, Johannesson B, Lundgren U. Strong natural selection causes microscale allozyme variation in a marine snail. Proc Nat Acad Sci U S A. 1995a;92:2602–6.

Johannesson K, Rolán-Alvarez E, Ekendahl A. Incipient reproductive isolation between two sympatric morphs of the intertidal snail *Littorina saxatilis*. Evolution. 1995b;49:1180–90.

Johannesson K, Havenhand JN, Jonsson PR, Lindegarth M, Sundin A, Hollander J. Male discrimination of female mucous trails permits assortative mating in a marine snail species. Evolution. 2008;62:3178–84.

Johannesson K, Panova M, Kemppainen P, Rolán-Alvarez E, André C, Butlin RK. Repeated evolution of reproductive isolation in a marine snail – unveiling mechanisms of speciation. Philos Trans R Soc Lond B Biol Sci. 2010;365:1735–47.

Johannesson K, Hintz Saltin S, Charrier G, Ring AK, Kvarnemo C, André C, Panova M. Non-random paternity of offspring in a highly promiscuous marine snail suggests postcopulatory sexual selection. Behav Ecol Sociobiol. 2016;70:1357–66.

Kemppainen P, Panova M, Hollander J, Johannesson K. Complete lack of mitochondrial divergence between two species of NE Atlantic marine intertidal gastropods. J Evol Biol. 2009;22:2000–11.

Kulmuni J, Westram AM. Intrinsic incompatibilities evolving as a by-product of divergent ecological selection: considering them in empirical studies on divergence with gene flow. Mol Ecol. 2017;26:3093–103.

Le Pennec G, Butlin RK, Jonsson PR, Larsson AI, Lindborg J, Bergström E, Westram AM, Johannesson K. Adaptation to dislodgement risk on wave-swept rocky shores in the snail *Littorina saxatilis*. PLoS One. 2017;12:e0186901.

Mäkinen T, Panova M, Johannesson K, Tatarenkov A, Appelqvist C, André C. Genetic differentiation on multiple spatial scales in an ecotype-forming marine snail with limited dispersal: *Littorina saxatilis*. Biol J Linn Soc. 2008;94:31–40.

Mikhailova NA, Gracheva YA, Backeljau T, Granovitch AI. A potential species-specific molecular marker suggests interspecific hybridization between sibling species *Littorina arcana* and *L. saxatilis* (Mollusca, Caenogastropoda) in natural populations. Genetica. 2009;137:333–40.

Morjan CL, Rieseberg LH. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Mol Ecol. 2004;13:1341–56.

Murgarella M, Pulu D, Novoa B, Figueras A, Posada D, Canchaya C. A first insight into the genome of the filter-feeder mussel *Mytilus galloprovincialis*. PLoS One. 2016;11(3):e0151561.

Navarro A, Barton NH. Chromosomal speciation and molecular divergence – accelerated evolution in rearranged chromosomes. Science. 2003;300:321–4.

Ng TPT, Williams GA. Size-dependent male mate preference and its association with size-assortative mating in a mangrove snail, *Littoraria ardouiniana*. Ethology. 2014;120:1–8.

Nosil P, Flaxman SM. Conditions for mutation-order speciation. Proc Biol Sci. 2011;278:399–407.

Nosil P, Harmon LJ, Seehausen O. Ecological explanations for (incomplete) speciation. Trends Ecol Evol. 2009;24:145–56.

Olsen JL, Zechman FW, Hoarau G, Coyer JA, Stam WT, Valero M, Aberg P. The phylogeographic architecture of the fucoid seaweed *Ascophyllum nodosum*: an intertidal "marine tree" and survivor of more than one glacial-interglacial cycle. J Biogeogr. 2010;37:842–56.

Otero-Schmitt J, Cruz R, Garcia C, Rolán-Alvarez E. Feeding strategy and habitat choice in *Littorina saxatilis* (Gastropoda:Prosobranchia) and their role in the origin and maintenance of a sympatric polymorphism. Ophelia. 1997;46:205–16.

Panova M, Hollander J, Johannesson K. Site-specific genetic divergence in parallel hybrid zones suggests nonallopatric evolution of reproductive barriers. Mol Ecol. 2006;15:4021–31.

Panova M, Boström J, Hofving T, Areskoug T, Eriksson A, Mehlig B, Mäkinen T, André C, Johannesson K. Extreme female promiscuity in a non-social invertebrate species. PLoS One. 2010;5(3):e9640.

Panova M, Blakeslee AMH, Miller AW, Mäkinen T, Ruiz GM, Johannesson K, André C. Glacial history of the North Atlantic marine snail, *Littorina saxatilis*, inferred from distribution of mitochondrial DNA lineages. PLoS One. 2011;6(3):e17511.

Panova M, Johansson T, Canbäck B, Bentzer J, Alm Rosenblad M, Johannesson K, Tunlid A, André C. Species and gene divergence in *Littorina* snails detected by array comparative genomic hybridization. BMC Genomics. 2014;15:687.

Quesada H, Posada D, Caballero A, Morán P, Rolán-Alvarez E. Phylogenetic evidence for multiple sympatric ecological diversification in a marine snail. Evolution. 2007;61:1600–12.

Rafajlović M, Eriksson A, Rimark A, Saltin SH, Charrier G, Panova M, André C, Johannesson K, Mehlig B. The effect of multiple paternity on genetic diversity of small populations during and after colonisation. PLoS One. 2013;8:e75587.

Rafajlović M, Emanuelsson A, Johannesson K, Butlin RK, Mehlig B. A universal mechanism generating clusters of differentiated loci during divergence-with-migration. Evolution. 2016;70:1609–21.

Ravinet M, Westram A, Johannesson K, Butlin R, André C, Panova M. Shared and non-shared genomic divergence in parallel ecotypes of *Littorina saxatilis* at a local scale. Mol Ecol. 2016;85:287–305.

Ravinet M, Faria R, Butlin RK, Galindo J, Bierne N, Rafajlović M, Noor MAF, Mehlig B, Westram AM. Interpreting the genomic landscape of speciation: a road map for finding barriers to gene flow. J Evol Biol. 2017;30(8):1450–77.

Reid DG. Systematics and evolution of *Littorina*. London: Ray Society; 1996.

Reid DG, Dyal P, Williams ST. A global molecular phylogeny of 147 periwinkle species (Gastropoda, Littorinidae). Zool Scripta. 2012;41:125–36.

Rieseberg LH, Burke JM. The biological reality of species: gene flow, selection, and collective evolution. Taxon. 2001;50:47–67.

Roda F, Liu H, Wilkinson MJ, et al. Convergence and divergence during the adaptation to similar environments by an Australian groundsel. Evolution. 2013;67:2515–29.

Rolán-Alvarez E, Rolán E, Johannesson K. Differentiation in radular and embryonic characters, and further comments on gene flow, between two sympatric morphs of *Littorina saxatilis* (Olivi). Ophelia. 1996;45:1–15.

Rolán-Alvarez E, Johannesson K, Erlandsson J. The maintenance of a cline in the marine snail *Littorina saxatilis*: the role of home site advantage and hybrid fitness. Evolution. 1997;51:1838–47.

Rolán-Alvarez E, Erlandsson J, Johannesson K, Cruz R. Mechanisms of incomplete prezygotic reproductive isolation in an intertidal snail; testing behavioural models in wild populations. J Evol Biol. 1999;12:879–90.

Rolán-Alvarez E, Carballo M, Galindo J, Moran P, Fernández B, Caballero A, Cruz R, Boulding EG, Johannesson K. Nonallopatric and parallel origin of local reproductive barriers between two snail ecotypes. Mol Ecol. 2004;13:3415–24.

Rolán-Alvarez E, Austin CJ, Boulding EG. The contribution of the genus *Littorina* to the field of evolutionary ecology. Oceanogr Mar Biol Ann Rev. 2015;53:157–214.

Roux C, Fraïsse C, Romiguler J, Anciaux GN, Bierne N. Shedding lighton the grey zone of speciation along a continuum of genomic divergence. PLoS Biol. 2017;14:e2000234.

Sadedin S, Hollander J, Panova M, Johannesson K, Gavrilets S. Case studies and mathematical models of ecological speciation 3. Ecotype formation in a Swedish snail. Mol Ecol. 2009;18:4006–23.

Saltin SH, Schade H, Johannesson K. Preference of males for large females causes a partial mating barrier between a large and a small ecotype of *Littorina fabalis* (W.Turton, 1825). J Moll Stud. 2013;79:128–32.

Schluter D, Conte GL. Genetics and ecological speciation. Proc Nat Acad Sci U S A. 2009;106:9955–62.

Seehausen O, Butlin RK, Keller I, Wagner CE, Boughman JW, Hohenlohe P, Peichel CL, et al. Genomics and the origin of species. Nat Rev Genet. 2014;15:176–92.

Soria-Carrasco V, Gompert Z, Comeault AA, Farkas TE, Parchman TL, Johnston JS, Buerkle CA, Feder JL, Bast J, Schwander T, Egan SP, Crespi BJ, Nosil P. Stick insect genomes reveal natural selection's role in parallel speciation. Science. 2014;344:738–42.

Sundell K. Adaptability of two phenotypes of *Littorina saxatilis* (Olivi) to different salinities. J Exp Mar Biol Ecol. 1985;92:115–23.

Tirado T, Saura M, Rolán-Alvarez E, Quesada H. Historical biogeography of the marine snail Littorina *saxatilis* inferred from haplotype and shell morphology evolution in NW Spain. PLoS One. 2016;11:e0161287.

Unckless RL, Orr HA. Dobzhansky-Muller incompatibilities and adaptation to a shared environment. Heredity. 2009;102:214–217f.

Ward RD, Warwick T. Genetic differentiation in the molluscan species *Littorina rudis* and *Littorina arcana* (Prosobranchia: Littorinidae). Biol J Linn Soc. 1980;14:417–28.

Ward RD, Warwick T, Knight AJ. Genetic analysis of ten polymorphic enzyme loci in Littorina saxatilis (Prosobranchia, Mollusca). Heredity. 1986;57:233–41.

Webster SE, Galindo J, Grahame JW, Butlin RK. Habitat choice and speciation. Int J Ecol. 2012;2012:1–12.

Westram AM, Galindo J, Alm Rosenblad M, Grahame JW, Panova M, Butlin RK. Do the same genes underlie parallel phenotypic divergence in different *Littorina saxatilis* populations? Mol Ecol. 2014;23:4603–16.

Westram AM, Panova M, Galindo J, Butlin RK. Targeted resequencing reveals geographical patterns of differentiation for loci implicated in parallel evolution. Mol Ecol. 2016;25:3169–86.

Wilding CS, Butlin RK, Grahame J. Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. J Evol Biol. 2001;14:611–9.

Wolf JBW, Ellegren H. Making sense of genomic islands of differentiation in light of speciation. Nat Rev Genet. 2017;18:87–100.

Wood HM, Grahame JW, Humphray S, Rogers J, Butlin RK. Sequence differentiation in regions identified by a genome scan for local adaptation. Mol Ecol. 2008;17:3123–35.

Yeaman S, Whitlock MC. The genetic architecture of adaptation under migration-selection balance. Evolution. 2011;65:1897–911.

Zhang G, Fang X, Guo X, Li L, Luo R, Xu F, et al. The oyster genome reveals stress adaptation and complexity of shell formation. Nature. 2012;490:49–54.

Ecological Speciation in Corals

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Abstract The ocean is generally a homogenous environment with few geographic barriers that allow populations to connect over hundreds of kilometers, increasing gene flow and slowing down diversification and the formation of species. However, biodiversity in the ocean is vast across thousands of kilometers and even within single individuals (e.g., coral colonies). Species diversity peaks at coral reef ecosystems, which house at least one quarter of the marine biodiversity. Why are these systems so diverse? How do species differentiate despite rampant genetic connectivity? One possibility to explain biodiversity hotspots in the ocean, along with physical barriers, is through ecological factors. Populations can diverge if they specialize ecologically, reducing interbreeding, which can lead to reproductive isolation. We reviewed cases of speciation in coral reefs with emphasis on those driven by ecological factors. We find few studies in coral research using genomic approaches to understand the genetics of reproductive isolation. We propose the cases of the coral *Orbicella* spp. and the octocoral *Eunicea* spp. as ideal examples to study ecological speciation in corals.

Keywords Adaptation · Coral reefs · Coral-algae symbiosis · Corals · Ecological speciation · Genomics

1 Introduction

The study of species formation is not only critical for enhancing marine conservation, but it is also one of the major topics in evolutionary biology (Darwin 1909; Mayr 1942; Coyne and Orr 2004; Nosil and Feder 2012). Species form when

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reproductive isolation (RI) develops, preventing the breeding of groups of organisms and leading to genetically differentiated populations (Mayr 1942). Reproductive isolation is regarded as a fundamental process for species generation. One way to achieve RI is as a by-product of geographical isolation. For example, the rise of the Isthmus of Panama roughly 3 million years ago resulted in profound oceanographic (redirection of currents in the Gulf of Mexico and interoceanic closure) and biological impacts including isolation of populations on either side, preventing gene flow and eventually generating thousands of new sister species on either side of the Isthmus (Lessios 1979; O'Dea et al. 2016). Alternatively, though not mutually exclusive, adaptation to different habitats can result in RI and speciation via ecological factors (Rundle and Nosil 2005; Prada et al. 2008; Prada and Hellberg 2013).

Speciation via geographical barriers has been traditionally emphasized in terrestrial taxa (Coyne and Orr 2004). Physical barriers act as hard boundaries that block gene flow among populations, allowing divergence and the formation of new species. As most populations in terrestrial systems are fragmented across landscapes with little genetic connectivity and restricted gene flow, geographical isolation is often found to be the causative agent for species divergence. In contrast, marine species often disperse across hundreds of kilometers as planktonic larvae, enhancing gene flow among populations and hindering population differentiation and speciation. For marine species in which gene flow persists over large geographical scales, the formation of species may be largely the result of ecologically based divergent selection.

Here we review studies of ecological speciation in marine environments. We highlight those studies that benefited from incorporating modern genomic tools and multidisciplinary work involving ecology, morphology, behavior, experimental, and evolutionary biology. We also favored coral systems given corals' ecological relevance and our own expertise.

2 Biodiversity in the Ocean

Speciation in the sea has been prolific and has resulted in over 243,000 species (WoRMS Editorial Board 2018) with an abundant presence of undescribed and unrecognized cryptic species that could boost biodiversity estimates to at least tenfold (Sala and Knowlton 2006). For example, according to May (1994), 32 of 33 animal phyla occur in the sea, 21 of which are exclusively marine, whereas only 12 phyla occur on land, and only 1 is exclusive to land.

Biodiversity in the sea is not only astonishing based on the number of species but also the uniqueness of body plans, which partly reflects the action of natural selection in these systems. Marine biodiversity is particularly rich in coral reefs, which contain one quarter of all species in the ocean (Reaka-Kudla 2005; Sala and Knowlton 2006). Coral reef ecosystems occur, whereby hermatypic corals grow large colonies that form complex 3D networks of living tissue and a myriad of niches for other species, creating a marine biodiversity hotspot (Birkeland 2015). In the

Caribbean alone, researchers have recorded 12,000 marine species, though this is likely an underestimation considering only a few islands in the Caribbean have been explored and the lack of taxonomic expertise for certain groups (Miloslavich et al. 2010). Coral reefs are ecologically important as corals store carbon in their skeleton and thus act as CO_2 sinks, alleviating carbon dioxide in the atmosphere and regulating other biogeochemical cycles such as sulfur (Raina et al. 2013). In addition, healthy reefs provide both ecosystem services by mitigating beach erosion from storms and hurricanes and economic services including industries in tourism, fisheries, jewelry, aquarium hobbies, and aquaculture (Spalding et al. 2004).

One of the properties of the biodiversity on coral reefs is that it is highly stratified with different kinds of organisms occupying different habitats (Montaggioni and Braithwaite 2009). For example, plate-like corals are often found in deep areas below 25 m at the reef drop off zone. Branching corals need more light and are more resistant to wave action being found in the reef crest and fore reef areas. Massive corals are often found at intermediate habitats. Such segregation of coral species along reef habitats is also reflected at finer scales with a plethora of sister species often occupying different habitats (Knowlton 1993). The co-occurrence of these sister marine species with high dispersal capabilities poses a challenge for evolutionary biologists trying to understand how new species emerge without obvious geographic isolation.

3 Speciation in the Ocean

Speciation has been largely studied on land, where reproductive isolation is often achieved due to physical barriers such as rivers and mountains that isolate populations and generate new species (Coyne and Orr 2004; Mayr 1954; Morris-Pocock et al. 2016; Hayes and Sewlal 2004; Ceccarelli et al. 2016). While speciation via geographical isolation occurs in the ocean (Lessios et al. 2001), the scarcity of physical barriers suggests this mode of isolation does not operate as widely as on land (Palumbi 1994). Contrary to land, many marine organisms engage in external fertilization and have planktonic larvae that can disperse hundreds of kilometers, connecting populations across vast distances (Lessios and Robertson 2006, 2013; Roberts 1997).

The dynamics among populations in the sea differs from that on land in at least two ways: (1) there is higher gene flow among populations, and (2) populations sustain larger number of individuals (i.e., larger effective population sizes). Gene flow and population size influence the rate of speciation. Increased gene flow delays genomic differentiation and speciation. Similarly, larger populations take longer to drift mutations to fixation, further slowing diversification and speciation. Apart from geographical isolation, environmental differentiation often generated by physical variation can influence the formation of species in the sea. Adaptation of populations across these environmental changes such as gradients of light, temperature, and depth can cause ecologically based divergent selection.

4 Ecological Speciation

During ecological speciation, RI is achieved by divergent natural selection acting on ecologically segregated populations even when dispersal is not an impediment to random mating (Rundle and Nosil 2005). In these instances, speciation appears to have occurred due to natural selection acting on genes responsible for ecological traits. Even when gene flow is absent during divergence, ecological speciation can accelerate the process because different alleles should be fixed under different environments under natural selection (Schluter 2009). Similarly, because local adaptation generates alternative states in different environments, when nascent species come into contact, they are less likely to reproduce because both extrinsic and intrinsic factors reduce gene flow (Doebeli 2005; van Doorn et al. 2009). Ecological speciation research has provided evidence that RI can happen rapidly in both plants and animals (Savolainen et al. 2006; Barluenga et al. 2006) producing parallel patterns across taxa and geographical regions (Østbye et al. 2005; Derome and Bernatchez 2006; Quesada et al. 2007; Schluter 2009). Given that the ocean is one of the most stratified systems on earth, ecological divergence may play a fundamental role in promoting speciation in marine taxa with high dispersal potential (Table 1). In fact, ecological segregation is widespread among closely related marine species with genetic differences often detected between habitat-segregated populations with overlapping ranges (Brazeau and Harvell 1994; Carlon and Budd 2002; Levitan et al. 2004; Prada et al. 2008) and adaptation of alternative ecotypes occurring even within meters in species with dispersal potential of hundreds of kilometers (Prada and Hellberg 2014). Segregated marine broadcast spawners often differ in the timing of spawning, which can lead to temporal RI (Knowlton et al. 1997). Thus, habitat segregation has the potential to link ecological and reproductive traits, increasing the likelihood of isolation (van Doorn et al. 2009). This generates assortative mating, which, coupled with habitat specificity, provides conditions where ecological differentiation can drive speciation.

5 Adaptation Across Gradients in the Sea

Variation in the distribution of physical and ecological factors creates environmental niches. Some of the most dissimilar niches occur at opposite ends of temperature gradients across latitudes and depth ranges of light availability and between salinity levels at fresh-to-seawater across estuaries (Table 1). Populations often cope with this environmental variation by adapting to different niches across these gradients, and this divergent selection across such environments creates the condition for ecological speciation.

One of the first described examples of marine speciation driven by ecological factors is that of the sponge *Chondrilla* cf. *nucula* inhabiting mangroves and coral reefs (Duran and Rützler 2006). This species displays a different morphology and

Table 1 Cases of ecological segregation in marine invertebrates

Taxa ID	Common name	Segregation by	Environment	Marker	Reference
Favia fragum	Coral	D	Sea grass beds vs reef	Morphometrics and allozymes	Carlon and Budd (2002)
Neilonella salicensis	Bivalve	D	Deep ocean	Nuclear (28S and calmodulin intron) and mitochondrial (cytochrome c oxidase subunit I)	Glazier and Etter (2014)
Callogorgia	Octocoral	D	Deep ocean	Morphometrics and mitochondrial barcode (cox1 + igr1 + mtMutS) + microsatellites	Quattrini et al. (2013, 2015)
Orbicella	Coral	D	Shallow and mesophotic habitat	Microsatellites	Weil and Knowlton (1994)
Agaricia and Symbiodinium	Coral	D	Shallow and mesophotic habitat	Coral morphometrics and mitochondrial (*atp6*) and ITS2 for *Symbiodinium*	Bongaerts et al. (2013)
Seriatopora hystrix	Coral	D	Shallow and mesophotic habitat	Molecular (mtDNA and ITS2-DGGE) and photo-physiological	Bongaerts et al. (2011)
Eunicella singularis	Octocoral	D	Shallow and mesophotic habitat	Microsatellites and ITS1	Costantini et al. (2016)
Eunicea flexuosa	Octocoral	D	Shallow habitat	Morphometrics, nuclear (18S), and mitochondrial (msh1)	Prada et al. (2008)
Corallium rubrum	Octocoral	D	Mesophotic habitat	Microsatellites	Costantini et al. (2011)
Briareum asbestinum	Octocoral	D	Shallow and mesophotic habitat	Allozymes	Brazeau and Harvell (1994)
Ophiothrix	Bristle stars	D	Intertidal and subtidal (>100 m) temperate waters	Cytochrome oxidase I and 16S rRNA	Taboada and Pérez-Portela (2016)
Plexaura	Octocoral	D	Feeding strategy	Feeding behavior and morphometrics	Lasker et al. (1983)

(continued)

Table 1 (continued)

Taxa ID	Common name	Segregation by	Environment	Marker	Reference
Symbiodinium B1B184 associated with Gorgonia ventalina (coral)	Algae	D	Shallow habitat	Microsatellites	Kirk et al. (2009)
Symbiodinium associated with Seriatopora hystrix (coral)	Algae	D	Shallow habitat	rDNA ITS2	Van Oppen et al. (2011)
Madracis pharensis and Symbiodinium	Coral	D	Shallow and mesophotic habitat	Mitochondrial (mtDNA: nad5) and two nuclear (nDNA: ATPSa and SRP54)	Frade et al. (2010)
Cliona delitrix	Sponge	D	Shallow habitat	Ecology (substratum and habitat preference)	Halperin et al. (2016)
Nacella	Limpet	D and L	Rocky shores	mtDNA COI	González-Wevar et al. (2011)
Celleporella hyalina	Bryozoa	H	Intertidal	Fitness experiments	Hughes (1992)
Actinia tenebrosa	Anemone	H	Intertidal: rock pools and boulder habitats	Allozymes and microsatellite markers	Sherman and Ayre (2008)
Littorina subrotundrata	Snail	H	Salt marsh and rocky intertidal ecotypes	Cytochrome B	Kyle and Boulding (1998)
Chondrilla cf. nucula	Sponge	H	Mangal or coral reef	Morphometrics and mitochondrial (COI)	Duran and Rützler (2006)
Phestilla	Nudibranch	HP	Coral reef, different coral hosts	mtDNA (COI) and rDNA 16S	Faucci et al. (2007)
Synalpheus	Snapping shrimp	HP	Sponge host preference	Ecology (substratum selection and demography), morphometrics, and allozymes	Duffy (1992, 1996)
Ampithoe longimana	Amphipod	HP	Seaweeds	mtCOI and nuclear ITS1	Sotka et al. (2003)

Elysia viridis	Sea slug	HP	Host and feeding preference of temperate macroalgae	Ecology tests	Trowbridge and Todd (2017)
Ampithoidae, *Biancolinidae*, and *Hyalidae*,	Amphipod	HP	Coral, alga, or sponge host	Ecology tests	Poore et al. (2000)
Littorina saxatilis	Snail	I	Rocky intertidal	Morphometrics and allozymes	Johannesson et al. (1993)
Cellana	Limpet	I	Intertidal	mtDNA (12S, 16S, COI) nDNA (ATPSβ, H3)	Bird et al. (2011)
Acrocnida	Bristle stars	I	Intertidal and subtidal temperate waters	Allozymes and mtCOI	Muths et al. (2006)
Petrolisthes	Porcelain crabs	I	Rocky intertidal	Thermal tolerance	Stillman (2002)
Collisella	Limpets	I	Rocky intertidal	Ecology (substratum preference and predation)	Mercurio et al. (1985)
Notoacmea	Limpet	I	Exposed shores or mud-flat segregation	mtCOI and nuclear ITS1	Nakano and Spencer (2007)
Acanthina monodon	Snail	L	Intertidal	Morphometrics (mtCOI was done in a different study and reported no genetic difference)	Sepúlveda and Ibáñez (2012)

The segregation column refers to depth (D), latitudinal gradients (L), habitat (H), host preference (HP), or intertidal height (I). Most studies consider mesophotic environments as habitats beginning at 30 m of depth, but sometimes this varies depending on author's criteria. For more details on mesophotic description, see Laverick et al. (2017)

coloration respective to the environment it inhabits, but, more importantly, populations from the same habitat, even if separated across vast distances, are more genetically alike than populations from different habitats found locally (Duran and Rützler 2006). Similarly, the habitat differentiation of the mobile fish *Halichoeres* spp. between coastal and more oceanic habitats has also been found to be reflected in genetic divergence (Rocha et al. 2005). Ecologically segregated populations will be genetically similar to populations in their same ecological niche even if separated by great distances while being strongly divergent from closer populations that are in different ecological niches.

6 Depth as a Driver of Ecological Speciation in Coral Reefs

An evaluation of sibling species in the sea found that over 50% of the divergences involved depth, therefore sympatric sibling species in the sea are commonly found to prefer depth niches differentially (Knowlton 1993). Depth covaries with light, water motion, sediment transport, and many other physical and chemical factors. Variation in the interaction of these factors produces dissimilar distribution of resources, favoring combinations of traits that result in fitness differences among habitat-segregated populations (Prada et al. 2008; Prada and Hellberg 2013). Along with physiological changes to match the environments at different depths, depth-segregated marine broadcast spawners often differ in the timing of spawning, which can lead to temporal RI (Knowlton et al. 1997). Depth segregation has the potential to link ecological and reproductive traits, increasing the likelihood of speciation (Felsentein 1981; Tomaiuolo et al. 2007; van Doorn et al. 2009).

Two of the best-studied Caribbean systems in which ecological factors seem to have driven speciation across depths are the common *Orbicella* species (formerly known as *Montastraea annularis* complex) and the octocoral *Eunicea flexuosa*. The *Orbicella* genus is one of the major reef-building groups in the Caribbean and includes three species: *O. faveolata*, *O. annularis*, and *O. franksi* (Knowlton et al. 1992). Multiple sources of evidence, including behavior, genetics, and ecology, have shown that each species tends to occupy different habitats (Knowlton et al. 1992; Weil and Knowlton 1994; Lopez et al. 1999; Fukami et al. 2004). In addition, the *Orbicella* species also correspond to distinct ecotypes that segregate by depth (Budd et al. 2012). For example, *O. franksi* prefer deeper areas (>20 m), *O. faveolata* favors intermediate depths, and *O. annularis* is more common in shallower depths (<10 m). They overlap at intermediate depths (Weil and Knowlton 1994; Pandolfi and Budd 2008) and are ecotypically differentiated by coral colony morphology (columnar, massive, or bumpy), which likely provides ecological advantages to each species in its own depth. In fact, genome sequencing provides evidence that the extinction of previous *Orbicella* spp. created a niche gap in which modern *Orbicella* species have thrived, enabling ecological segregation of modern taxa (Prada et al. 2016). Therefore, the columnar morphology of *O. annularis* allows colonies to grow faster and better compete in shallow habitats with high sediment transport. The more

massive form of *O. franksi* allows this coral to increase its area perpendicular to the reception of light that is scarce in deeper environments. Such morphological differences are adaptive and allow the corals to perform better in their native habitats than in nonnative habitats (in the case of *O. annularis*, performance is best in shallow habitats versus deep habitats).

Similar to the *Orbicella* species, *Eunicea flexuosa* shows two genetically distinct, depth-segregated ecotypes that also match morphological differentiation consistent with local adaptation. Although the two distinct morphotypes used to be attributed to phenotypic plasticity, a study that used reciprocal transplantation and molecular markers (nuclear and mitochondrial) found evidence that these morphotypes are not only ecologically but also genetically distinct despite living in sympatry which explains why the morphological characters are consistently fixed for shallow (<5 m) and deep (>17 m) populations (Prada et al. 2008). Moreover, studies have shown that sympatric populations of *Eunicea* segregate by depth and that migration is limited between shallow and deep zones, suggesting that survival is higher for native genotypes from each niche than for foreign recruits (Prada and Hellberg 2013, 2014). Species in this genus take approximately 15 years to reach sexual maturity. By then, immigrant inviability operates in incoming larvae weeding out unfit colonies and selecting for locally adapted ones. In a typical case of ecological speciation, populations of *Eunicea* at different depth zones are fully segregated genetically when living in sympatry, yet populations of each depth specialist maintain high levels of gene flow across the Caribbean. As corals in general delay sexual maturity for years to decades, selection operates for a long time (i.e., long prereproductive selection), resulting in high immigrant filtering efficiency across habitats before reproduction promoting RI (Prada and Hellberg 2013). Both depth-segregated specialists harbor distinct *Symbiodinium* symbiont species that they select from the water column and remain host-specific even after reciprocal transplantation, suggesting algal specificity may be a factor in the ecological segregation of *Eunicea* (Prada et al. 2014).

There are a few cases of segregation by depth across scleractinian corals varying in their degree of speciation from little divergence (population polymorphism) to fully resolved species. *Favia fragum* corals from Panama are thought to be a case of recent speciation. Although there is some overlap at shallower depths (≤1 m), segregation of two *F. fragum* morphotypes is clear, and each morphotype is found at a particular depth (≤1 m vs 3 m) (Carlon and Budd 2002). Polyp morphometrics and allozyme analyses suggest that segregation can be explained by an incipient speciation process with incomplete lineage sorting (divergence-with-gene-flow model) likely due to ecological division and RI since these corals are mostly self-crossing (Carlon and Budd 2002).

In the case of *Seriatopora hystrix* from the Great Barrier Reef, depth segregation is present along the reef slope where ecotypes are exclusive to certain depth ranges. These ecotypes also establish stable symbioses with *Symbiodinium*, suggesting local adaptation to each particular depth niche in both algae and coral (Bongaerts et al. 2011). Similarly to other cases of depth segregation, samples from the same local reef area are much more genetically similar to distant regional samples at the same

depth than to samples within the same area but at a different depth (Van Oppen et al. 2011). Niche diversification based on depth has been reported in the Mediterranean octocoral *Corallium rubrum*, which is separated in two populations within the 20–70 m gradient it inhabits with a population boundary at 40–50 m of depth (Costantini et al. 2011). Another Mediterranean coral, *Eunicella singularis*, has two morphotypes corresponding to a shallow and a deep niche that are in fact isolated genetically (Costantini et al. 2016). In both cases it was hypothesized that the thermocline may prevent deeper larvae from migrating to shallow water populations (Costantini et al. 2011, 2016). Similarly, in Florida and the United States Virgin Islands (USVI), populations of *Porites astreoides* experience low vertical connectivity attributed in some cases (e.g., Dry Tortugas) to mesoscale eddies that result in segregation of shallow and deep populations, whereas gene flow is high between Florida and the USVI (which is almost 2,000 km) despite that these corals release competent larvae that typically settle close to the parental colonies (Serrano et al. 2016). In addition to segregation in the coral host, associated *Symbiodinium* is also segregated by depth (clades A and C inhabit shallow and deep waters, respectively) (Serrano et al. 2016).

Iglesias-Prieto et al. (2004) found vertical distribution in corals depended on the *Symbiodinium* each coral species hosts. Two depth-segregated coral species, *Pocillopora verrucosa* (shallow) and *Pavona gigantea* (deep), harbor a unique algal composition based on ITS2 marker profiling: *Pavona* harbors *Symbiodinium* type C1, and *Pocillopora* harbors *Symbiodinium* type D1. Light-depth segregation in *Symbiodinium* is so strong for these two species that it can alone determine the coral host niche segregation regardless of environmental conditions and therefore influence niche diversification. Genetic evidence supports two depth-associated lineages of the Caribbean coral *Madracis pharensis* that host different algal symbionts. Shallow corals host *Symbiodinium* type B7, whereas deep corals host *Symbiodinium* type B15 (Frade et al. 2010). A similar study of five *Agaricia* coral species found depth segregation in the coral host and host specificity with the algal populations (Bongaerts et al. 2013). And more recently, genome-wide genotyping by RAD sequencing determined that reduced gene flow between depth-segregated *Agaricia fragilis* resulted in genome wide evidence of high selective pressure to depth adaptation despite symbiont type (all *A. fragilis* studied hosted the same algal type) (Bongaerts et al. 2017). Interestingly, however, in the same study *Stephanocoenia intersepta* from the same reef showed no genetic structure between different depths suggesting that each species has unique natural histories and generalizations are hard to support (i.e., deep reef refugia hypothesis) (Bongaerts et al. 2017).

Octocorals are also known to occur at particular depth niches with specific *Symbiodinium* algal symbioses. *Gorgonia ventalina* is an abundant Caribbean species that shows *Symbiodinium* genetic segregation based on depth (Kirk et al. 2009). The presence and maintenance of dinoflagellate algal symbionts is key in determining the ecological niche of a given species (Iglesias-Prieto et al. 2004; Bongaerts et al. 2011; Prada and Hellberg 2014). Genomic and transcriptomic tools have enlightened the ecology of coral-algal symbiosis. For example, in the case of bleaching stress, *Orbicella faveolata* and *Acropora hyacinthus* transcriptomic data

suggest coral physiology remains disturbed for months even after *Symbiodinium* recovery (Pinzón et al. 2015; Thomas and Palumbi 2017). An intriguing possibility is that such physiological stress may be differently handled by corals occupying different niches and containing different symbionts (Parkinson et al. 2016). Another key finding is that transmembrane transport, oxidative stress response, and UV radiation protection genes are enriched in *Symbiodinium* genomes and transcriptomes, which are presumably necessary to maintain symbiosis (González-Pech et al. 2017). It remains to be seen if the evolution of these transmembrane proteins differs between species and populations occupying different habitats with varying light levels such as across depth gradients.

Ecological speciation is not exclusive to shallow environments as species also segregate along the deep ocean as well. Three deep-sea sibling species in the octocoral genus *Callogorgia* also segregate by depth and by the specific environment associated to each depth (mostly explained by temperature, salinity, and calcite saturation) with little overlap, indicating high depth specialization (Quattrini et al. 2013). In particular, genetic evidence from *Callogorgia delta* indicates that these octocorals segregate locally within species and are more responsive to depth than geographical distance supporting the depth-differentiation hypothesis at the species level (Quattrini et al. 2015).

7 Mechanisms of Reproductive Isolation Among Populations Living in Different Habitats

7.1 Spawning Timing

Adaptation to depth results in temporal reproductive isolation. Coral spawning varies across depths with corals in shallow areas perceiving sunset earlier than deeper water colonies, thereby resulting in differential timing of spawning (Knowlton et al. 1997). The best case studied involves the *Orbicella* species (i.e., *O. annularis* mostly on shallow waters, *O. franksi* mostly on deep waters, and *O. faveolata* in both shallow and deep waters). *O. franksi* spawns approximately 2 h after sunset, whereas *O. annularis* and *O. faveolata* spawn 3:40 h and 4:00 h after sunset, respectively (Levitan et al. 2004, 2011). This 2-h window is ample to avoid cross-fertilization between *O. franksi* and *O. annularis* as gametes dilute and age quickly in the water column; and the overlap between *O. annularis* and *O. faveolata* does produce successful crosses at least in the laboratory (Levitan et al. 2004, 2011). In *Orbicella*, adaptation to different depths causes the development of RI due to timely species-specific gamete release events (Weil and Knowlton 1994; Levitan et al. 2011). Spawning times are sufficiently different to prevent hybridization even when corals are found in sympatry, yet conspecifics will spawn at their corresponding time. There is correlation between genotype and timing of spawning in *Orbicella* corals (Levitan et al. 2011). Furthermore, depth isolated groups from the

same species will spawn at comparable times indicating a strong species-specific spawning behavior as seen in *O. franksi*, *O. faveolata*, as well as other corals such as *M. cavernosa* and *Diploria strigosa* (Vize 2006; Villinski 2003).

The underlying genomic architecture of spawning behavior is partially understood. Heritable genomic components responsible for spawning behavior are thought to be associated with circadian clock networks that are triggered differently during spawning time. Spawning in corals is photoregulated and possibly under the influence of circadian rhythm genes (Kaniewska et al. 2015). Circadian rhythm gene networks are composed of highly conserved proteins in metazoans (Reitzel and Behrendt 2010), yet they are known to play a role in RI between species. Because most proteins involved in biorhythms detected in corals are transcription factors (Levy et al. 2007; Shoguchi et al. 2013), it is likely that timing of spawning and divergence in spawning time among populations and species are controlled at the transcriptional level. The *O. faveolata* genome has revealed the presence of approximately 18 circadian rhythm protein families that are likely involved in controlling spawning time in corals.

Some of the genes implicated in differential timing of spawning are responsive to blue light from lunar irradiance (Gorbunov and Falkowski 2002), and evidence from *Acropora millepora* corals supports that at least two blue-light-sensing photoreceptor genes (cryptochromes *cry1* and *cry2*) are responsive to the moonlight phases in this species (Levy et al. 2007). Studies show that gene expression measured using EST of *cry2* was increased in full moon nights as opposed to new moon nights indicating this gene may be operating the circadian clock thereby participating in the regulation of spawning timing, although the involvement of other genes (like opsins) involved cannot be ruled out (Levy et al. 2007). There is not a clear understanding of what triggers spawn timing behavior in corals. It may be linked to a direct response to a light cues such as darkness (i.e., if the cue is shifted, the behavior shifts), or it could be operating under an entrained biological clock (i.e., if the cue is shifted or removed, the behavior continues in a rhythmic manner for some time). Most likely, at least in *Orbicella* spp., sunset is the trigger that "starts the countdown" to spawning timing. Current studies of the transcriptome network that operates the temporal isolation behavior in *Orbicella franksi* and *Orbicella annularis* indicate a strong species-specific difference in the genes differentially expressed though these genes underlie similar functions (González et al. in press).

7.2 Sperm-Egg Recognition Systems

In addition to differential timing of spawning, corals reproductively isolate via chemical variations in the proteins involved with sperm-egg interactions, which mediate whether fertilization is possible. After spawning and before fertilization, gametes must find and recognize each other as compatible. Gamete recognition and compatibility is crucial for successful reproduction. The sperm and egg of compatible individuals chemically recognize each other via the interaction of proteins on

their surfaces (Vacquier 1998). These reproductive proteins ultimately permit fertilization and thus ensure RI in most marine broadcast spawners. Proteins responsible for gamete interactions are best known in sea urchins, abalone, and turban snail species, although many eukaryote taxa are known to have reproductive proteins (Pujolar and Pogson 2011; Palmer et al. 2013; Hellberg et al. 2012; Lima and McCartney 2013; Clark et al. 2006). Reproductive proteins are known to be among the fastest-evolving proteins (Metz et al. 1998; Swanson and Vacquier 2002). In the case of rapid evolution of reproductive proteins, and especially those involved in gamete recognition, adaptive evolution has been attributed to a series of inter- and intraspecific fertilization conflicts that seem to constantly favor rapid protein change, especially in external fertilizers (Vacquier and Swanson 2011).

One hypothesis for the evolution of sperm-egg proteins in marine organisms is reinforcement which prevents prezygotic contact in sympatry by controlling gamete recognition such that eggs select for conspecific sperm (known as conspecific sperm precedence) or assortative mating (Marshall et al. 2002; Fogarty et al. 2012; Palumbi 1999). This is the case of *Echinometra oblonga* and *Echinometra* sp. C, which may interbreed in no-choice crosses but that do not hybridize naturally. The eggs of these species also select for conspecific sperm (Geyer and Palumbi 2005). These proteins tend to be rapidly evolving and are attributed the ability to explain rapid speciation in marine systems even in sympatry (Geyer and Palumbi 2005; Palumbi 2009). In cases that gamete recognition fails to prevent all hybridization, ecological factors such as habitat or depth segregation and temporal and/or gametic isolation may aid in maintaining prezygotic isolation (Lessios 2007). Morphological features in gametes (sperm shape, egg structure and size), motility limitations, and even chemical cues (pheromones) may also operate as prezygotic barriers in broadcast marine spawners (Wolstenholme 2004; Levitan 2006; Manier and Palumbi 2008; Marks et al. 2008).

An additional hypothesis for the evolution of sperm-egg proteins is sexual conflict. Intraspecific crossings are limited to the fertilization of an egg with a single sperm, since polyspermy (the fertilization of one egg by more than one sperm) leads to embryo death. As a result, sexual conflict arises between eggs and sperm, such that eggs have mechanisms to avoid polyspermy, while sperm competition results in mechanisms to overcome the egg barriers. This is a sperm-density-dependent scenario as rare alleles have higher fertilization rates when sperm density is high, whereas more common alleles have higher fertilization rates when sperm density is low (Levitan and Ferrell 2006). In some organisms like mammals, birds, and echinoderms, eggs are able to block polyspermy after one sperm comes in successful contact with the egg (reviewed in Karr et al. 2009). Other species modify the egg receptors to reduce the chances of insemination by multiple sperm, while sperm receptors are constantly being modified in order to fertilize eggs at all costs, a way of sexual conflict (reviewed in Levitan 2010). Sexual selection can also operate through cryptic female choice, which occurs when eggs prefer certain sperm surface alleles resulting in higher fertilization rates for those allele carriers (Eberhard 1996). Fertilization is highly dependent on density and genotype frequency of both sperm and eggs; therefore to understand the evolution of reproductive isolation based on gamete recognition proteins, studies are more fruitful when observations are taken in the context of the organism's ecology (Levitan and Ferrell 2006; Palumbi 2009).

In summary, in external fertilizers like sea urchins, snails, and other invertebrates, gamete recognition proteins play a key role regulating egg-sperm interactions, reproductively isolating taxa and, given their fast evolution, facilitating speciation. It is unknown if gamete recognition proteins are present in corals, but low fertilization rates in self-fertilization trials (Szmant et al. 1997) and interspecific crosses suggest they may occur and mediate fertilization (Knowlton et al. 1997). However, it is known that asymmetric conspecific sperm precedence exists in *Orbicella* such that the early spawner *O. franksi* shows strong preference toward sperm of its species, whereas the late spawner *O. annularis* does not show strong preference in choice experiments with both species (Fogarty et al. 2012). The ecology of these species should be taken into account considering that by the time *O. annularis* spawns, leftover *O. franksi* sperm may just be too diluted and old to naturally fertilize fresh *O. annularis* eggs. When the spawning times overlap in the case of *O. annularis* and *O. faveolata*, gametes are incompatible as shown by unsuccessful laboratory cross experiments, hence preventing hybridization even when sibling congenerics are found in sympatry (Levitan et al. 2004; Szmant et al. 1997).

8 Genomics of Coral Speciation

Comparative genomic research is now feasible due to the evolution of sequencing platforms and the growing myriad of respective sophisticated analysis tools. Areas of interest within the scope of model systems have devoted attention to genome-wide association studies (GWAS). In the case of cnidarians, and particularly corals, some studies now incorporate these new technologies. Genome-wide genotyping has been used to assess fine population genetics and diversity in a physical range. Genome-wide data suggest *Acropora palmata* populations seem to segregate by geography (Devlin-Durante and Baums 2017), yet *Orbicella* species segregate by depth. This technique has also shown the lack of genetic difference in *Acropora digitifera* from Japanese reefs (Shinzato et al. 2015).

The life histories of *Eunicea* and *Orbicella* species present a great natural experiment to study how prezygotic barriers operate in long-lived broadcast spawning corals. The highly continuous genome of *Orbicella faveolata* allows the study of evolution of sperm-egg recognition proteins in corals (Prada et al. 2016). Our preliminary analysis in *Orbicella* corals indicates that substantial sequence divergence exists across candidate reproductive proteins. Figure 1a illustrates that *CatsperD*, a sperm motility protein (Chung et al. 2011), is highly dissimilar between *O. faveolata* and *O. annularis*. We hypothesize *CatsperD* may contribute to prezygotic barriers since sperm need to swim to reach the egg and different motilities elicit different mechanical responses in the egg layers (Levitan 2000). The second molecule with substantial differences between *Orbicella* species is the receptor for egg jelly protein (REJ), which is a known sperm-egg-binding protein of the acrosomal reaction in sea urchins (Moy et al. 1996; Karr et al. 2009). These candidate proteins may be partially responsible for RI in these species.

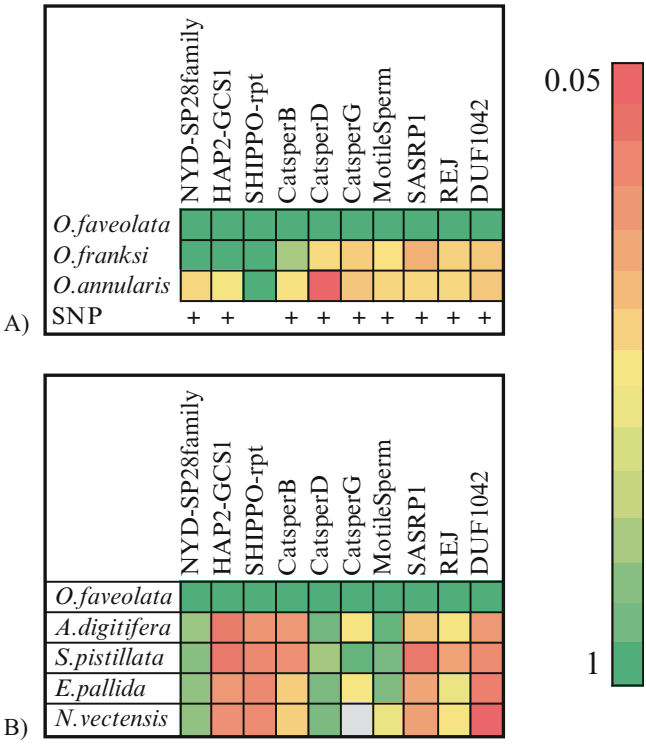

Fig. 1 We retrieved ortholog protein sequences from protein models from the genomes of four symbiotic cnidarians (*O. faveolata*, *Acropora digitifera*, *Stylophora pistillata*, and *Exaiptasia pallida*) and one asymbiotic cnidarian (*Nematostella vectensis*) using blast bidirectional best hit (BBHs) (Altschul et al. 1990). We did protein alignments and curation with ClustalW (Thompson et al. 1994) and Gblocks (Castresana 2000). We built protein distance matrices using Hamming dissimilarities algorithm implemented in Ugene (Okonechnikov et al. 2012). The heatmaps of the protein distances between different reproductive proteins in five Cnidarians are depicted. Green colors represent closer distances (fully conserved proteins equal to 1), while red colors represent more distant relationships (equals a value of 0.05). Gray indicates sequence absence. (**a**) Comparison among sister *Orbicella* species. (**b**) Comparison of *O. faveolata*, *Acropora digitifera*, *Stylophora pistillata*, and *Exaiptasia pallida genomes* and *Nematostella vectensis*

9 Conclusion

Environmental gradients often drive genetic segregation in marine populations, and ecological speciation is common in the sea. One of the main examples of ecological speciation in the ocean is depth segregation on coral reefs. Organisms that harbor photosynthetic symbionts, such as scleractinian corals and octocorals, are bound to physiological requirements of both host and algal symbionts. These requirements are often quite distinct due to restrictions of light penetration to the benthos, ultimately leading to reproductive isolation among populations along this depth gradient. In species with delayed reproduction such as corals, selection acts for years to decades

and effectively removes unfit individuals. Adaptation to depth in these systems is tied to reproductive isolation as light cues drive gamete release timing providing temporal isolation. The rapid evolution of sperm-egg recognition proteins provides an additional prezygotic isolating barrier to maintain and generate biodiversity in the sea. Genomic tools are enhancing our understanding of genetic variants associated with local adaptation as well as elucidating the molecular mechanisms driving reproductive isolation and speciation in the sea. The use of multidisciplinary research that combines genomic approaches with field biology promises to close gaps in our understanding of ecological genomics and marine speciation.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature. 2006;439(7077):719–23. https://doi.org/10.1038/nature04325.

Bird CE, Holland BS, Bowen BW, Toonen RJ. Diversification of sympatric broadcast-spawning limpets (Cellana spp.) within the Hawaiian archipelago. Mol Ecol. 2011;20(10):2128–41. https://doi.org/10.1111/j.1365-294X.2011.05081.x.

Birkeland C, editor. Coral reefs in the anthropocene. Life and death of coral reefs. Dordrecht: Springer; 2015.

Bongaerts P, Riginos C, Hay KB, van Oppen MJH, Hoegh-Guldberg O, Dove S. Adaptive divergence in a scleractinian coral: physiological adaptation of Seriatopora hystrix to shallow and deep reef habitats. BMC Evol Biol. 2011;11:303.

Bongaerts P, Frade PR, Ogier JJ, Hay KB, van Bleijswijk J, Englebert N, et al. Sharing the slope: depth partitioning of agariciid corals and associated symbiodinium across shallow and mesophotic habitats (2-60 m) on a Caribbean reef. BMC Evol Biol. 2013;13(1):205. https://doi.org/10.1186/1471-2148-13-205.

Bongaerts P, Riginos C, Brunner R, Englebert N, Smith SR, Hoegh-guldberg O. Deep reefs are not universal refuges: reseeding potential varies among coral species. Sci Adv. 2017;3(2):e1602373.

Brazeau DA, Harvell CD. Genetic structure of local populations and divergence between growth forms in a clonal invertebrate, the Caribbean octocoral Briareum asbestinum. Mar Biol. 1994;119(1):53–60. https://doi.org/10.1007/BF00350106.

Budd AF, Fukami H, Smith ND, Knowlton N. Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). Zool J Linnean Soc. 2012;166(3):465–529. https://doi.org/10.1111/j.1096-3642.2012.00855.x.

Carlon DB, Budd AF. Incipient speciation across a depth gradient in a scleractinian coral? Evolution. 2002;56(11):2227–42.

Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol. 2000;17(4):540–52.

Ceccarelli FS, Ojanguren-Affilastro AA, Ramírez MJ, Ochoa JA, Mattoni CI, Prendini L. Andean uplift drives diversification of the bothriurid scorpion genus Brachistosternus. J Biogeogr. 2016;43:1942–54. https://doi.org/10.1111/jbi.12760.

Chung J-J, Navarro B, Krapivinsky G, Krapivinsky L, Clapham DE. A novel gene required for male fertility and functional CATSPER channel formation in spermatozoa. Nat Commun. 2011;2:153. https://doi.org/10.1038/ncomms1153.

Clark NL, Aagaard JE, Swanson WJ. Evolution of reproductive proteins from animals and plants. Reproduction. 2006;131(1):11–22. https://doi.org/10.1530/rep.1.00357.

Costantini F, Rossi S, Pintus E, Cerrano C, Gili JM, Abbiati M. Low connectivity and declining genetic variability along a depth gradient in Corallium rubrum populations. Coral Reefs. 2011;30(4):991–1003. https://doi.org/10.1007/s00338-011-0771-1.

Costantini F, Gori A, Lopez-González P, Bramanti L, Rossi S, Gili JM, et al. Limited genetic connectivity between gorgonian morphotypes along a depth gradient. PLoS One. 2016;11(8):1–20. https://doi.org/10.1371/journal.pone.0160678.

Coyne JA, Orr HA. Speciation. Sunderland: Sinauer Associates; 2004.

Darwin C. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. London: Cassell; 1909.

Derome N, Bernatchez L. The transcriptomics of ecological convergence between 2 limnetic coregonine fishes (Salmonidae). Mol Biol Evol. 2006;23(12):2370–8. https://doi.org/10.1093/molbev/msl110.

Devlin-Durante MK, Baums IB. Genome-wide survey of single-nucleotide polymorphisms reveals fine-scale population structure and signs of selection in the threatened Caribbean Elkhorn coral, *Acropora palmata*. Peer J. 2017;5:e4077. https://doi.org/10.7717/peerj.4077.

Doebeli M. Adaptive speciation when assortative mating is based on female preference for male marker traits. J Evol Biol. 2005;18(6):1587–600. https://doi.org/10.1111/j.1420-9101.2005.00897.x.

Duffy JE. Host use patterns and demography in a guild of tropical sponge-dwelling shrimps. Mar Ecol Prog Ser. 1992;90:127–38. https://doi.org/10.3354/meps090127.

Duffy JE. Species boundaries, specialization, and the radiation of sponge-dwelling alpheid shrimp. Biol J Linn Soc. 1996;58(3):307–24. https://doi.org/10.1006/bijl.1996.0038.

Duran S, Rützler K. Ecological speciation in a Caribbean marine sponge. Mol Phylogenet Evol. 2006;40(1):292–7. https://doi.org/10.1016/j.ympev.2006.02.018.

Eberhard W. Female control: sexual selection by cryptic female choice. Princeton: Princeton University Press; 1996. p. 501.

Faucci A, Toonen RJ, Hadfield MG. Host shift and speciation in a coral-feeding nudibranch. Proc Biol Sci. 2007;274(1606):111–9. https://doi.org/10.1098/rspb.2006.3685.

Felsentein J. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution. 1981;35(1):124–38.

Fogarty ND, Lowenberg M, MN Ojima NK, Levitan DR. Asymmetric conspecific sperm precedence in relation to spawning times in the Montastraea annularis species complex (Cnidaria: Scleractinia). J Evol Biol. 2012;25:1–8. https://doi.org/10.1111/j.1420-9101.2012.02625.x.

Frade PR, Reyes-Nivia MC, Faria J, Kaandorp JA, Luttikhuizen PC, Bak RPM. Semi-permeable species boundaries in the coral genus Madracis: introgression in a brooding coral system. Mol Phylogenet Evol. 2010;57(3):1072–90. https://doi.org/10.1016/j.ympev.2010.09.010.

Fukami H, Budd AF, Levitan DR, Jara J, Kersanach R, Knowlton N. Geographic differences in species boundaries among members of the Montastraea annularis complex based on molecular and morphological markers. Evolution. 2004;58(2):324–37.

Geyer LB, Palumbi SR. Conspecific sperm precedence in two species of tropical sea urchins. Evolution. 2005;59(1):97–105. https://doi.org/10.1554/04-202.

Glazier AE, Etter RJ. Cryptic speciation along a bathymetric gradient. Biol J Linn Soc. 2014;113(4):897–913. https://doi.org/10.1111/bij.12389.

González AM, Sebastian A, Prada C, Levitan D, Knowlton N, Albert I, Levy O, Medina M. Genomics of temporal isolation in Orbicella franksi and Orbicella annularis. in press.

González-Pech RA, Ragan MA, Chan CX. Signatures of adaptation and symbiosis in genomes and transcriptomes of symbiodinium. Sci Rep. 2017;7(1):1–10. https://doi.org/10.1038/s41598-017-15029-w.

González-Wevar CA, Nakano T, Cañete JI, Poulin E. Concerted genetic, morphological and ecological diversification in Nacella limpets in the Magellanic Province. Mol Ecol. 2011;20(9):1936–51. https://doi.org/10.1111/j.1365-294X.2011.05065.x.

Gorbunov MY, Falkowski PG. Photoreceptors in the cnidarian hosts allow symbiotic corals to sense blue moonlight. Limnol Oceanogr. 2002;47(1):309–15. https://doi.org/10.4319/lo.2002.47.1.0309.

Halperin AA, Chaves-Fonnegra A, Gilliam DS. Coral-excavating sponge Cliona delitrix: current trends of space occupation on high latitude coral reefs. Hydrobiologia. 2016;790(1):1–12. https://doi.org/10.1007/s10750-016-3042-x.

Hayes FE, Sewlal J-a N. The Amazon River as a dispersal barrier to passerine birds: effects of river width, habitat and taxonomy. J Biogeogr. 2004;31(11):1809–18.

Hellberg ME, Dennis AB, Arbour-Reily P, Aagaard JE, Swanson WJ. The Tegula tango: a coevolutionary dance of interacting, positively selected sperm and egg proteins. Evolution. 2012;66(6):1681–94. https://doi.org/10.1111/j.1558-5646.2011.01530.x.

Hughes DJ. Genotype-environment interactions and relative clonal fitness in a marine bryozoan. J Anim Ecol. 1992;61(2):291–306.

Iglesias-Prieto R, Beltrán VH, LaJeunesse TC, Reyes-Bonilla H, Thomé PE. Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern pacific. Proc Biol Sci/R Soc. 2004;271(1549):1757–63. https://doi.org/10.1098/rspb.2004.2757.

Johannesson K, Johannesson B, Rolan-Alvarez E. Morphological differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail Littorina saxatilis. Evolution. 1993;47(6):1770–87.

Kaniewska P, Alon S, Karako-Lampert S, Hoegh-Guldberg O, Levy O. Signaling cascades and the importance of moonlight in coral broadcast mass spawning. elife. 2015;4:1–14. https://doi.org/10.7554/eLife.09991.

Karr TL, Swanson WJ, Snook RR. The evolutionary significance of variation in sperm-egg interactions. In: Birkhead T, Hosken D, Pitnick S, editors. Sperm biology. 1st ed. Amsterdam: Elsevier; 2009. p. 305–65. https://doi.org/10.1016/B978-0-12-372568-4.00008-2.

Kirk NL, Andras JP, Drew Harvell C, Santos SR, Coffroth MA. Population structure of symbiodinium sp. associated with the common sea fan, Gorgonia ventalina, in the Florida Keys across distance, depth, and time. Mar Biol. 2009;156(8):1609–23. https://doi.org/10.1007/s00227-009-1196-z.

Knowlton N. Sibling species in the sea. Annu Rev Ecol Syst. 1993;24(1993):189–216.

Knowlton N, Weil E, Weigt LA, Guzmán HM. Sibling species in Montastraea annularis, coral bleaching, and the coral climate record. Science (New York, NY). 1992;255(5042):330–3. https://doi.org/10.1126/science.255.5042.330.

Knowlton N, Mate JL, Guzman HM, Rowan R, Jara J. Direct evidence for reproductive isolation among the three species of the Montastraea annularis complex in central America (Panama and Honduras). Mar Biol. 1997;127:705–11.

Kyle CJ, Boulding EG. Molecular genetic evidence for parallel evolution in a marine gastropod, Littorina subrotundata. Proc R Soc Lond B. 1998;265:303–8. https://doi.org/10.1098/rspb.1998.0296.

Lasker HR, Gottfried MD, Coffroth MA. Effects of depth on the feeding capabilities of two octocorals. Mar Biol. 1983;73(1):73–8. https://doi.org/10.1007/BF00396287.

Laverick JH, Andradi-brown DA, Rogers AD. Using light-dependent scleractinia to define the upper boundary of mesophotic coral ecosystems on the reefs of Utila, Honduras. PLoS One. 2017;12(8):e0183075.

Lessios HA. Use of Panamanian sea urchins to test the molecular clock. Nature. 1979;280(5723):599. https://doi.org/10.1038/280599a0.

Lessios H. Reproductive isolation between species of sea urchins. Bull Mar Sci. 2007;81(2):191–208.

Lessios HA, Robertson DR. Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. Proc Biol Sci/R Soc. 2006;273(1598):2201–8. https://doi.org/10.1098/rspb.2006.3543.

Lessios HA, Robertson DR. Speciation on a round planet: phylogeography of the goatfish genus Mulloidichthys. J Biogeogr. 2013;40(12):2373–84. https://doi.org/10.1111/jbi.12176.

Lessios H, Kessing BD, Pearse JS. Population structure and speciation in tropical seas: global phylogeography of the sea urchin Diadema. Evolution. 2001;55(5):955–75. https://doi.org/10.1111/j.0014-3820.2001.tb00613.x.

Levitan DR. Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin Lytechinus variegatus. Proc R Soc B Biol Sci. 2000;267(1443):531–4. https://doi.org/10.1098/rspb.2000.1032.

Levitan DR. The relationship between egg size and fertilization success in broadcast-spawning marine invertebrates. Integr Comp Biol. 2006;46(3):298–311. https://doi.org/10.1093/icb/icj025.

Levitan DR. Sexual selection in external fertilizers. In: Evolutionary behavioral ecology. New York: Oxford University Press; 2010. p. 365–79.

Levitan DR, Ferrell DL. Selection on gamete recognition proteins depends on sex, density, and genotype frequency. Science. 2006;312(5771):267–9.

Levitan DR, Fukami H, Jara J, Kline D, McGovern TM, McGhee KE, et al. Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the Montastraea annularis species complex. Evolution. 2004;58(2):308–23.

Levitan DR, Fogarty N, Jara J, Lotterhos KE, Knowlton N. Genetic, spatial, and temporal components of precise spawning synchrony in reef building corals of the montastraea annularis species complex. Evolution. 2011;65(5):1254–70.

Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, Miller DJ, et al. Light-responsive cryptochromes from a simple multicellular animal, the coral Acropora millepora. Science. 2007;318(5849):467–70.

Lima TG, McCartney MA. Adaptive evolution of M3 lysin-A candidate gamete recognition protein in the Mytilus edulis species complex. Mol Biol Evol. 2013;30(12):2688–98. https://doi.org/10.1093/molbev/mst165.

Lopez JV, Kersanach R, Rehner SA, Knowlton N. Molecular determination of species boundaries in corals: genetic analysis of the Montastraea annularis complex using amplified fragment length polymorphisms and a microsatellite marker. Biol Bull. 1999;196(1):80–93.

Manier MK, Palumbi SR. Intraspecific divergence in sperm morphology of the green sea urchin, Strongylocentrotus droebachiensis: implications for selection in broadcast spawners. BMC Evol Biol. 2008;8(1):283. https://doi.org/10.1186/1471-2148-8-283.

Marks JA, Biermann CH, Eanes WF, Kryvi H, Marks JA, Biermann CH, et al. Divergence between Pacific and Atlantic oceans linked references are available on JSTOR for this article: Sperm polymorphism within the sea urchin Strongylocentrotus droebachiensis: divergence between Pacific and Atlantic oceans. Biol Bull. 2008;215(2):115–25.

Marshall JL, Arnold ML, Howard DJ. Reinforcement: the road not taken. Trends Ecol Evol. 2002;17(12):558–63.

May RM. Biological diversity: differences between land and sea. Philos Trans R Soc Lond B. 1994;343(1303):105–11.

Mayr E. Systematics and the origin of species from the viewpoint of a zoologist. New York: Columbia University Press; 1942.

Mayr E. Geographic speciation in tropical echinoids. Evolution. 1954;8(1):1–18.

Mercurio K, Palmer A, Lowell R. Predator-mediated microhabitat partitioning by two species of visually cryptic, intertidal limpets. Ecology. 1985;66(5):1417–25.

Metz EC, Robles-Sikisaka R, Vacquier VD. Nonsynonymous substitution in abalone sperm fertilization genes exceeds substitution in introns and mitochondrial DNA. Proc Natl Acad Sci U S A. 1998;95(18):10676–81. https://doi.org/10.1073/pnas.95.18.10676.

Miloslavich P, Díaz JM, Klein E, Alvarado JJ, Díaz C, Gobin J, et al. Marine biodiversity in the Caribbean: regional estimates and distribution patterns. PLoS One. 2010;5(8):e11916. https://doi.org/10.1371/journal.pone.0011916.

Montaggioni L, Braithwaite CJR. Chapter three structure, zonation and dynamic patterns of coral reef communities. Dev Mar Geol. 2009;5:67–122. https://doi.org/10.1016/S1572-5480(09)05003-9.

Morris-Pocock JA, Anderson DJ, Friesen VL. Biogeographical barriers to dispersal and rare gene flow shape population genetic structure in red-footed boobies (Sula Sula). J Biogeogr. 2016;43(11):2125–35. https://doi.org/10.1111/jbi.12780.

Moy GW, Mendoza LM, Schulz JR, Swanson WJ, Glabe CG, Vacquier VD. The sea urchin sperm receptor for egg jelly is a modular protein with extensive homology to the human polycystic kidney disease protein, PKD1. J Cell Biol. 1996;133(4):809–17.

Muths D, Davoult D, Gentil F, Jollivet D. Incomplete cryptic speciation between intertidal and subtidal morphs of Acrocnida brachiata (Echinodermata: Ophiuroidea) in the Northeast Atlantic. Mol Ecol. 2006;15(11):3303–18. https://doi.org/10.1111/j.1365-294X.2006.03000.x.

Nakano T, Spencer HG. Simultaneous polyphenism and cryptic species in an intertidal limpet from New Zealand. Mol Phylogenet Evol. 2007;45(2):470–9. https://doi.org/10.1016/j.ympev.2007.07.020.

Nosil P, Feder JL. Genomic divergence during speciation: causes and consequences. Philos Trans R Soc Lond Ser B Biol Sci. 2012;367(1587):332–42. https://doi.org/10.1098/rstb.2011.0263.

O'Dea A, Lessios HA, Coates AG, Eytan RI, Restrepo-Moreno SA, Cione AL, et al. Formation of the isthmus of Panama. Sci Adv. 2016;2(8):1–12. https://doi.org/10.1126/sciadv.1600883.

Okonechnikov K, Golosova O, Fursov M, Team U. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics. 2012;28(8):1166–7.

Østbye K, Næsje TF, Bernatchez L, Sandlund OT, Hindar K. Morphological divergence and origin of sympatric populations of European whitefish (Coregonus Lavaretus L.) in Lake Femund, Norway. J Evol Biol. 2005;18(3):683–702. https://doi.org/10.1111/j.1420-9101.2004.00844.x.

Palmer MR, McDowall MH, Stewart L, Ouaddi A, MacCoss MJ, Swanson WJ. Mass spectrometry and next-generation sequencing reveal an abundant and rapidly evolving abalone sperm protein. Mol Reprod Dev. 2013;80(6):460–5. https://doi.org/10.1002/mrd.22182.

Palumbi SR. Genetic divergence, reproductive isolation and marine speciation. Annu Rev Ecol Syst. 1994;25:547–72.

Palumbi SR. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. Proc Natl Acad Sci U S A. 1999;96(22):12632–7. https://doi.org/10.1073/pnas.96.22.12632.

Palumbi SR. Speciation and the evolution of gamete recognition genes: pattern and process. Heredity. 2009;102(1):66–76. https://doi.org/10.1038/hdy.2008.104.

Pandolfi JM, Budd AF. Morphology and ecological zonation of Caribbean reef corals: the Montastraea 'annularis' species complex. Mar Ecol Prog Ser. 2008;369:89–102. https://doi.org/10.3354/meps07570.

Parkinson JE, Baumgarten S, Michell CT, Baums IB, LaJeunesse TC, Voolstra CR. Gene expression variation resolves species and individual strains among coral-associated dinoflagellates within the genus Symbiodinium. Genome Biol Evol. 2016;8(3):665–80. https://doi.org/10.1093/gbe/evw019.

Pinzón JH, Kamel B, Burge CA, Drew Harvell C, Medina M, Weil E, et al. Whole transcriptome analysis reveals changes in expression of immune-related genes during and after bleaching in a reef-building coral. R Soc Open Sci. 2015;2(4):140214. https://doi.org/10.1098/rsos.140214.

Poore AGB, Watson MJ, de Nys R, Lowry JK, Steinberg PD. Patterns of host use among alga- and sponge- associated amphipods. Mar Ecol Prog Ser. 2000;208:183–96. https://doi.org/10.3354/meps208183.

Prada C, Hellberg ME. Long prereproductive selection and divergence by depth in a Caribbean candelabrum coral. Proc Natl Acad Sci U S A. 2013;110(10):3961–6. https://doi.org/10.1073/pnas.1208931110.

Prada C, Hellberg ME. Strong natural selection on juveniles maintains a narrow adult hybrid zone in a broadcast spawner. Am Nat. 2014;184(6):702–13. https://doi.org/10.1086/678403.

Prada C, Schizas NV, Yoshioka PM. Phenotypic plasticity or speciation? A case from a clonal marine organism. BMC Evol Biol. 2008;8(1961):47. https://doi.org/10.1186/1471-2148-8-47.

Prada C, McIlroy SE, Beltrán DM, Valint DJ, Ford SA, Hellberg ME, et al. Cryptic diversity hides host and habitat specialization in a gorgonian-algal symbiosis. Mol Ecol. 2014;23(13):3330–40. https://doi.org/10.1111/mec.12808.

Prada C, Hanna B, Budd AF, Woodley CM, Schmutz J, Grimwood J, et al. Empty niches after extinctions increase population sizes of modern corals. Curr Biol. 2016;26(23):3190–4. https://doi.org/10.1016/j.cub.2016.09.039.

Pujolar JM, Pogson GH. Positive Darwinian selection in gamete recognition proteins of Strongylocentrotus sea urchins. Mol Ecol. 2011;20:4968–82. https://doi.org/10.1111/j.1365-294X.2011.05336.x.

Quattrini AM, Georgian SE, Byrnes L, Stevens A, Falco R, Cordes EE. Niche divergence by deep-sea octocorals in the genus Callogorgia across the continental slope of the Gulf of Mexico. Mol Ecol. 2013;22(15):4123–40. https://doi.org/10.1111/mec.12370.

Quattrini AM, Baums IB, Shank TM, Morrison C, Cordes EE. Testing the depth-differentiation hypothesis in a deepwater octocoral. Proc R Soc B Biol Sci. 2015;282(1807):20150008. https://doi.org/10.1098/rspb.2015.0008.

Quesada H, Posada D, Caballero A, Morán P, Rolán-Alvarez E. Phylogenetic evidence for multiple sympatric ecological diversification in a marine snail. Evolution. 2007;61(7):1600–12. https://doi.org/10.1111/j.1558-5646.2007.00135.x.

Raina J-B, Tapiolas DM, Foret S, Lutz A, Abrego D, Ceh J, et al. DMSP biosynthesis by an animal and its role in coral thermal stress response. Nature. 2013;502:677–80. https://doi.org/10.1038/nature12677.

Reaka-Kudla ML. Biodiversity of Caribbean coral reefs. In: Caribbean marine biodiversity. Lancaster: Des Tech; 2005. p. 259–76.

Reitzel AM, Behrendt L, Tarrant AM. Light entrained rhythmic gene expression in the sea anemone Nematostella vectensis: the evolution of the animal circadian clock. PLoS One. 2010;5(9):1–9. https://doi.org/10.1371/journal.pone.0012805.

Roberts CM. Connectivity and management of Caribbean coral reefs. Science. 1997;278(5342):1454–7.

Rocha LA, Robertson DR, Roman J, Bowen BW. Ecological speciation in tropical reef fishes. Proc R Soc B. 2005;272:573–9. https://doi.org/10.1098/2004.3005.

Rundle HD, Nosil P. Ecological speciation. Ecol Lett. 2005;8(3):336–52. https://doi.org/10.1111/j.1461-0248.2004.00715.x.

Sala E, Knowlton N. Global marine biodiversity trends. Annu Rev Environ Resour. 2006;31(1):93–122. https://doi.org/10.1146/annurev.energy.31.020105.100235.

Savolainen V, Anstett MC, Lexer C, Hutton I, Clarkson JJ, Norup MV, et al. Sympatric speciation in palms on an oceanic island. Nature. 2006;441(7090):210–3. https://doi.org/10.1038/nature04566.

Schluter D. Evidence for ecological speciation and its alternative. Science (New York, NY). 2009;323(5915):737–41. https://doi.org/10.1126/science.1160006.

Sepúlveda RD, Ibáñez CM. Clinal variation in the shell morphology of intertidal snail Acanthina monodon in the Southeastern Pacific Ocean. Mar Biol Res. 2012;8:363–72. https://doi.org/10.1080/17451000.2011.634813.

Serrano XM, Baums IB, Smith TB, Jones RJ, Shearer TL, Baker AC. Long distance dispersal and vertical gene flow in the Caribbean brooding coral Porites astreoides. Sci Rep. 2016;6:21619. https://doi.org/10.1038/srep21619.

Sherman CDH, Ayre DJ. Fine-scale adaptation in a clonal sea anemone. Evolution. 2008;62(6):1373–80. https://doi.org/10.1111/j.1558-5646.2008.00375.x.

Shinzato C, Mungpakdee S, Arakaki N, Satoh N. Genome-wide SNP analysis explains coral diversity and recovery in the Ryukyu archipelago. Sci Rep. 2015;5:1–8. https://doi.org/10.1038/srep18211.

Shoguchi E, Tanaka M, Shinzato C, Kawashima T, Satoh N. A genome-wide survey of photoreceptor and circadian genes in the coral, Acropora digitifera. Gene. 2013;515(2):426–31. https://doi.org/10.1016/j.gene.2012.12.038.

Sotka EE, Wares JP, Hay ME. Geographic and genetic variation in feeding preference for chemically defended seaweeds. Evolution. 2003;57(10):2262–76.

Spalding M, Kramer P, Green E, Greenhalgh S, Nobles H, Kool J. Reefs at risk in the Caribbean. Washington: World Resources Institute; 2004.

Stillman JH. Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus Petrolisthes. Integr Comp Biol. 2002;42(4):790–6. https://doi.org/10.1093/icb/42.4.790.

Swanson WJ, Vacquier VD. The rapid evolution of reproductive proteins. Genetics. 2002;3:137–44. https://doi.org/10.1038/nrg/733.

Szmant AM, Weil E, Miller MW, Colon DE. Hybridization within the species complex of the scleractinan coral Montastraea annularis. Mar Biol. 1997;129:561–72.

Taboada S, Pérez-Portela R. Contrasted phylogeographic patterns on mitochondrial DNA of shallow and deep brittle stars across the Atlantic-Mediterranean area. Sci Rep. 2016;6 (32425):1–13. https://doi.org/10.1038/srep32425.

Thomas L, Palumbi SR. The genomics of recovery from coral bleaching. Proc R Soc B Biol Sci. 2017;284(1865):20171790. https://doi.org/10.1098/rspb.2017.1790.

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673–80.

Tomaiuolo M, Hansen TF, Levitan DR. A theoretical investigation of sympatric evolution of temporal reproductive isolation as illustrated by marine broadcast spawners. Evolution. 2007;61(11):2584–95. https://doi.org/10.1111/j.1558-5646.2007.00218.x.

Trowbridge CD, Todd CD. Host-plant change in marine specialist herbivores: ascoglossan sea slugs on introduced macroalgae. Ecol Monogr. 2017;71(2):219–43.

Vacquier VD. Evolution of gamete recognition proteins. Science (New York, NY). 1998;281(5385):1995–8.

Vacquier VD, Swanson WJ. Selection in the rapid evolution of gamete recognition proteins in marine invertebrates. Cold Spring Harb Perspect Biol. 2011;3(11):a002931. https://doi.org/10.1101/cshperspect.a002931.

van Doorn SG, Edelaar P, Weissing FJ. On the origin of species by natural and sexual selection. Science. 2009;326(5960):1704–7.

Van Oppen MJH, Bongaerts P, Underwood JN, Peplow LM, Cooper TF. The role of deep reefs in shallow reef recovery: an assessment of vertical connectivity in a brooding coral from west and East Australia. Mol Ecol. 2011;20(8):1647–60. https://doi.org/10.1111/j.1365-294X.2011.05050.x.

Villinski JT. Depth-independent reproductive characteristics for the Caribbean reef-building coral Montastraea faveolata. Mar Biol. 2003;142:1043–53. https://doi.org/10.1007/s00227-002-0997-0.

Vize PD. Deepwater broadcast spawning by Montastraea cavernosa, Montastraea franksi, and Diploria strigosa at the flower garden banks, Gulf of Mexico. Coral Reefs. 2006;25(1):169–71. https://doi.org/10.1007/s00338-005-0082-5.

Weil E, Knowlton N. A multi-character analysis of the Caribbean coral Montastraea annularis (Ellis and Solander, 1786) and its two sibling species, M. Faveolata (Ellis and Solander, 1786). Bull Mar Sci. 1994;55(1):151–75.

Wolstenholme JK. Temporal reproductive isolation and gametic compatibility are evolutionary mechanisms in the Acropora Humilis species group (Cnidaria; Scleractinia). Mar Biol. 2004;144:567–82. https://doi.org/10.1007/s00227-003-1209-2.

WoRMS Editorial Board. World Register of Marine Species. 2018. Available from http://www.marinespecies.org at VLIZ. Accessed 2018-07-06. https://doi.org/10.14284/170.

Environmental Epigenomics and Its Applications in Marine Organisms

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Abstract Although epigenetics is still a relatively new discipline, its development during the last 10 years has revolutionized the current understanding of genome structure and function. The present chapter provides an insight on the exciting field of environmental epigenetics (i.e., the cause-effect relationships between environmental signals and epigenetic modifications altering phenotypes) and its potential applications for different types of studies in the marine environment. In the first part of this chapter, this work focuses on defining epigenetics, the different mechanisms involved in the epigenetic regulation of gene expression, as well as their potential role during the evolution of life on Earth. In the second part, this chapter moves into the potential applications of epigenetics in marine organisms, using current research projects on model species ranging from marine invertebrates to large marine megafauna as references. Overall, the present contribution underscores the importance of environmental epigenetic studies in marine organisms to better understand how organisms respond to their surrounding environment,

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fostering the development of a new generation of biomarkers enhancing restoration, conservation, and management efforts.

Keywords Biomonitoring · Chromatin · DNA methylation · Epigenetics · Mechanisms · ncRNAs · Population parameters · Restoration

1 Introduction

1.1 What Is Epigenetics?

The word "epigenetics" was originally coined by Conrad Waddington in 1942, referring to how genotypes give rise to phenotypes during development (Waddington 1942). Since then, the definition of epigenetics has been reshaped multiple times in order to keep up with the advances in biological knowledge. In this book chapter, epigenetics will be referred to as "The study of phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression that are not dependent on changes to DNA sequence" (Deans and Maggert 2015). In this context, heritability is defined as involving both mitotic and meiotic inheritance, and thus, epigenetic mechanisms need not be confined to processes that are inherited across generations (Metzger and Schulte 2016).

One of the most important challenges associated with the differentiation of the eukaryotic cell was organizing an extremely large genome within the reduced space of the cell nucleus (e.g., human diploid DNA is approximately 2 m long and needs to be packed within a cell nucleus of 6 μm of diameter). Such a high degree of condensation is achieved through the association of DNA with chromosomal proteins, forming a structure known as chromatin (van Holde 1989). The structural determinants of chromatin are extremely conserved across eukaryotes, underscoring their critical roles (Malik and Henikoff 2003; Ammar et al. 2012). However, chromatin also plays a functional role by regulating access to DNA in a well-coordinated and tightly regulated manner. Thus, this polymer can be defined as a highly dynamic structure where numerous proteins, transcription factors (TF), chemical marks (e.g., DNA methylation and histone posttranslational modifications), and other molecules (e.g., noncoding RNAs) work together to modify the architecture and accessibility to the DNA and, ultimately, regulate gene expression (Luger et al. 2012; Magistri et al. 2012; Table 1 and Fig. 1).

Chromatin provides a framework for the study of epigenetics, with this constituting an exciting frontier to understand how the environment influences the regulation of DNA function and the resulting phenotypic variation (i.e., phenotypic plasticity) observed in living organisms (Cortessis et al. 2012; Bollati and Baccarelli 2010; Suarez-Ulloa et al. 2015). The cause-effect relationships between environmental changes and epigenetic variation constitute the basis for environmental epigenetic studies (Feil and Fraga 2012). This discipline provides a powerful approach to study environmental responses in different ecosystems, notably in

Table 1 Main epigenetic mechanisms shaping the regulatory landscape of eukaryotic cells in response to environmental signals

Epigenetic mechanism	Definition	References
DNA methylation	Covalent incorporation of a methyl group to a DNA base. In metazoans, this often occurs on the 5 carbon of a cytosine	Jones (2012), Okano et al. (1999), Tahiliani et al. (2009), Li and Zhang (2014)
Histone posttranslational modifications and incorporation of histone variants	DNA is wrapped around nucleosomes formed by protein octamers of core histones (H2A, H2B, H3 and H4). Histones are subject to posttranslational modifications (PTMs) altering DNA-nucleosome interactions. Chromatin structure can be also altered by incorporation of histone variants replacing their canonical counterparts. Overall, histones regulate the access to the DNA by modifying chromatin structure	Ausio (2006), Kouzarides (2007), Henikoff and Ahmad (2005), Bannister and Kouzarides (2011)
Noncoding RNAs	RNAs transcribed from DNA but not translated into proteins, these RNAs generally function in controlling gene expression. There are many types of noncoding RNA (ncRNA) such as miRNA, siRNA, piRNA, and lncRNA. RNAs can be methylated, as part of the epitranscriptome	Palazzo and Lee (2015), Vidigal and Ventura (2015), Zhang et al. (2014), Carthew and Sontheimer (2009)

marine ecosystems subject to the harmful effects of global climate change (i.e., changes in water temperature, pH, salinity, and anthropogenic pollutants; Harley et al. 2006). Yet, while there is evidence supporting an epigenetic basis for the acquisition and transgenerational inheritance of acclimatized phenotypes in response to environmental changes, the mechanisms underlying such responses remain unclear (Vignet et al. 2015; Marsh and Pasqualone 2014; Greco et al. 2013; Navarro-Martín et al. 2011; Vandegehuchte et al. 2009). More precisely, our knowledge about how these mechanisms occur in response to specific stressors during different developmental stages, as well as how they interconnect and set the foundations for longer-term adaptation processes, is still very limited.

The present contribution will discuss foundational works in the field of environmental epigenetics, along with actual research defining the current understanding of epigenetics and the potential application of epigenetic studies in the marine environment. The first section of this work will introduce the main epigenetic mechanisms, how they can be influenced by the environment, as well as their potential for inheritance within and across generations. Subsequently, Sect. 2 will highlight specific fields of application for epigenetics with particular emphasis on the marine environment.

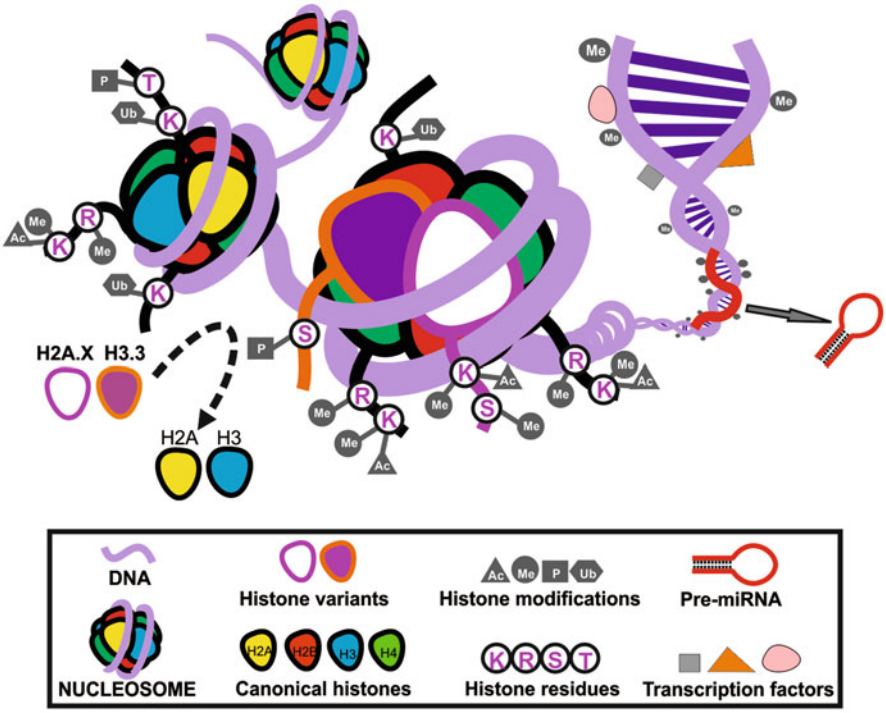

Fig. 1 Chromatin structure as a framework for epigenetic mechanisms. Various mechanisms have the potential to encode epigenetic information and regulate gene expression including DNA methylation (Me), the replacement of canonical histones by specialized histone variants in nucleosomes (e.g., H2A and H3 by H2A.X and H3.3, respectively), posttranslational modifications of histone residues (e.g., *Ac* acetylation, *Me* methylation, *P* phosphorylation, *Ub* ubiquitination), noncoding RNAs (e.g., miRNAs) or binding of transcription factors to the DNA

1.2 Main Epigenetic Mechanisms

1.2.1 DNA Methylation

DNA methylation is arguably the most studied epigenetic mark, involving the covalent incorporation of methyl groups to DNA bases (Table 1). DNA methylation marks have been described in genomes of organisms belonging to all domains of life, especially in the metazoan lineage within eukaryotes. The most common form of DNA methylation occurs at the fifth carbon of a cytosine, typically in the context of CpG dinucleotides, establishing a 5-methylcytosine (5mC) residue (Jones 2012). The reaction resulting in the addition of a methyl group to the carbon 5 of a cytosine is catalyzed by DNA methyltransferase (DNMT) enzymes (Okano et al. 1999). In mammals, DNMT3A and DNMT3B establish de novo DNA methylation patterns during embryonic development. Meanwhile, the enzyme DNMT1 binds to hemimethylated DNA and maintains those methylation marks after each cell

division, taking advantage of the symmetry of the CpG motif. DNA methylation is thus propagated unless removed by active [e.g., by ten-eleven translocation (TET) proteins (Tahiliani et al. 2009)] or passive mechanisms [e.g., lack of DNMT1 activity (Li and Zhang 2014)].

Genome Distribution and Regulatory Role of DNA Methylation

Despite its ancient origin and widespread occurrence, there is considerable variation in the 5mC distribution patterns and functions among taxa. Accordingly, even important model species show no detectable (e.g., *Caenorhabditis elegans* and *Saccharomyces cerevisiae*) or very low levels (e.g., *Drosophila melanogaster* displays low methylation levels detected only at early stages of development) of 5mC (Capuano et al. 2014; Bird 2002). Within metazoans, the genomes of vertebrate organisms are generally heavily methylated, with most CpGs exhibiting methylation marks, with the exception of those located at CpG islands (CGIs) which remain mostly unmethylated (Suzuki and Bird 2008). In contrast with this global distribution, most invertebrate genomes exhibit a mosaic pattern of 5mC distribution, with long stretches of highly methylated DNA interspersed with unmethylated regions (Tweedie et al. 1997; Feng et al. 2010). Interestingly, DNA methylation occurs mainly in gene bodies in invertebrates (including exons and introns of protein-coding regions, Suzuki et al. 2007; Zemach et al. 2010), while vertebrate genomes are commonly methylated also in intergenic regions, promoters, and transposable elements (Feng et al. 2010).

The functional effect of DNA methylation is highly dependent on the genomic context. Accordingly, high levels of methylation in proximal upstream promoters and enhancers are usually linked to transcriptional repression, through association with methyl-binding domain (MBD) proteins or through inhibition of transcription factor binding (Klose and Bird 2006; Deaton and Bird 2011). In contrast, gene body methylation is highly correlated with actively transcribed genes, reduction of transcriptional noise, and regulation of alternative splicing (Jones 2012; Huh et al. 2013; Shukla et al. 2011). Thanks to its regulatory role, DNA methylation is involved in critical biological processes such as cell differentiation and embryonic development (Smith and Meissner 2013). Furthermore, DNA methylation is also necessary to maintain genome integrity and even for defense purposes, as it is involved in the silencing of transposable elements in some species, as well as X-chromosome inactivation and genomic imprinting in mammals (Suzuki and Bird 2008; Jones 2012).

DNA Methylation Responses in Marine Environments

Although most of the current knowledge concerning DNA methylation derives from studies in mammalian model organisms, similar distribution patterns and transcriptional regulatory roles for this epigenetic mark have been shown in other chordates

(Peat et al. 2017; Metzger and Schulte 2016). Accordingly, the recent publication of the DNA methylome of the elephant shark *Callorhinchus milii* shows a global distribution of DNA methylation marks and a correlation with gene expression similar to that described in other vertebrates (Peat et al. 2017). Although functional information in non-model invertebrates is still scarce, several reports focused on marine species have contributed to filling this gap, including studies in the Pacific oyster (*Crassostrea gigas*) evidencing a correlation between gene body DNA methylation and high levels of gene expression (Gavery and Roberts 2013), as well as roles for DNA methylation in the regulation of alternative splicing (Gavery and Roberts 2013; Song et al. 2017) and embryonic development (Riviere et al. 2017).

Dynamic changes in DNA methylation are dependent on intrinsic genetic factors but also environmental factors (Feil and Fraga 2012; Fraga et al. 2005). Indeed, there is increasing evidence suggesting that changes in 5mC states can be triggered by changes in environmental conditions, contributing to phenotypic plasticity in organisms during subsequent responses (Kelly et al. 2012; Foo and Byrne 2016). Several works in marine animals further illustrate the links between DNA methylation and environmental changes. For instance, Marsh and Pasqualone reported that Antarctic polychaete embryos raised at different temperatures showed striking differences in DNA methylation patterns upon reaching adulthood, with increased DNA methylation levels on those raised at higher temperatures (Marsh and Pasqualone 2014). In a global climate change context, a study simulating ocean acidification conditions reported increased levels of global DNA methylation in the scleractinian coral *Pocillopora damicornis* after exposure to high pCO_2 conditions (Putnam et al. 2016). Similarly, DNA methylation changes have also been observed in the eastern oyster, *Crassostrea virginica*, in response to toxin-producing harmful algal blooms (González-Romero et al. 2017), although in this case DNA methylation decreased during exposure to increased levels of toxins. Interestingly, another recent report described lower levels of DNA methylation during the initial expansion phase of the invasive pygmy mussel *Xenostrobus securis*, potentially increasing phenotypic plasticity facilitating settling in the new environment (Ardura et al. 2017).

Among fishes, freshwater species such as the model zebrafish have dominated the literature (Metzger and Schulte 2016; Gavery and Roberts 2017). However, there are an increasing number of studies in marine species analyzing changes in DNA methylation in response to different conditions. For instance, DNA methylation in response to thermal variation has been studied in species such as the European sea bass, *Dicentrarchus labrax* (Anastasiadi et al. 2017); the Atlantic salmon, *Salmo salar* (Burgerhout et al. 2017); the Atlantic cod, *Gadus morhua* (Skjærven et al. 2014); the Senegalese sole, *Solea senegalensis* (Campos et al. 2013); and the tongue sole, *Cynoglossus semilaevis* (Shao et al. 2014). Of note here is the work by Varriale and Bernardi (2006) analyzing DNA methylation levels in 75 species of fish living at different latitudes and reporting higher 5mC levels in those living at lower temperatures (Varriale and Bernardi 2006). Changes in DNA methylation have also been observed in response to salinity variation in the tongue sole, *Cynoglossus semilaevis* (Li et al. 2017); cadmium exposure in the European eel, *Anguilla anguilla* (Pierron et al. 2014); hexabromocyclododecane and 17β-estradiol exposure in the three-

spined stickleback, *Gasterosteus aculeatus* (Aniagu et al. 2008); tributyltin and triphenyltin exposure in the sea ruffe, *Sebastiscus marmoratus* (Wang et al. 2009); and even environmental-caused tumorigenesis in the common dab, *Limanda limanda* (Mirbahai et al. 2011). In addition, changes in DNA methylation during development were also studied in the Atlantic salmon, *Salmo salar* [early maturation stages (Morán and Pérez-Figueroa 2011)]; the sea lamprey, *Petromyzon marinus* [metamorphosis (Covelo-Soto et al. 2015; Metzger and Schulte 2016)]; or the European eel, *Anguilla anguilla* [metamorphosis (Trautner et al. 2017)]. Overall, the number of publications applying DNA methylation analyses in marine species has increased dramatically in recent years. However, its use in other areas such as population studies remains largely unexplored. In this sense, DNA methylation could be used for species differentiation, behavior analysis, or estimation of demographic parameters such as age or sex of specific individuals in a population. The state of the art of these and other potential applications will be discussed in subsequent sections.

1.2.2 Histones, Histone Variants, and Histone Posttranslational Modifications

The chromatin fiber is constituted by fundamental subunits known as nucleosomes, each consisting of an octamer of architectural chromosomal proteins known as histones associated with DNA. Two copies of each core histone (H2A, H2B, H3, and H4) interact to form the nucleosome core particle (NCP), around which two left-handed super helical turns of DNA are wrapped (van Holde 1989, Table 1). Adjacent nucleosomes are joined together by short irregular stretches of linker DNA that interact with linker H1 histones, resulting in an additional folding of the chromatin fiber. Histones are small basic proteins with high affinity for the acidic DNA. They contain two structurally differentiated regions: a globular domain facilitating histone-histone interactions during nucleosome assembly and two unstructured tails (N- and C-terminal) that protrude from the nucleosome particle (Luger et al. 1997). Importantly, histones are not mere structural components of the chromatin but also critical determinants of its functionality (Allis et al. 2015). Indeed, histones can profoundly affect chromatin structure and its functional state by changing its local environment. This can be achieved in several ways. On the one hand, histones can be posttranslationally modified (PTM) at specific residues (Bannister and Kouzarides 2011) altering their electrostatic properties and, consequently, their affinity for DNA and other proteins. On the other hand, the replacement of canonical histones with specialized histone variants affects even to a greater extent the structure of the chromatin (Ausio 2006). Overall, the combination of histone variability and the different PTMs generate an enormous diversity in the nucleosome composition, creating a great variety of chromatin environments and transcriptional states (Henikoff and Ahmad 2005; Kouzarides 2007).

Histone- and Chromatin-Mediated Environmental Responses

In recent years, evidence has accumulated supporting the role of histone variants and histone modifications in environmental responses (Talbert and Henikoff 2014; Kasinsky et al. 2011). For instance, some environmental stressors can affect the DNA causing double-strand breaks (DSB). Upon damage, histone variant H2A.X undergoes rapid phosphorylation constituting a focus surrounding the damaged area. Along with modifications in other variants such as H2A.Z, macroH2A, or H3.3, these events constitute the earliest responses activating DNA repair pathways in the cell (Talbert and Henikoff 2014; Li et al. 2005). Histone H2A.Z has also been associated with responses to other environmental cues such as temperature (Kumar and Wigge 2010) or seasonal changes (Simonet et al. 2013) by regulating the expression of environmentally responsive genes (Adam et al. 2001; Coleman-Derr and Zilberman 2012; Wan et al. 2009). Additionally, PTMs such as acetylation (Wan et al. 2009) or ubiquitination (Simonet et al. 2013) targeting H2A.Z are involved in these responses. The histone variant macroH2A has also been involved in the seasonal acclimatization of the carp fish through the transcriptional regulation of the ribosomal cistron (Araya et al. 2010). In addition, although not an epigenetic feature in sensu stricto, histones also display an effective antimicrobial activity, and the extracellular release of histones is a widespread mechanism involved in the defense against pathogens that has been described in several marine organisms (Patat et al. 2004; Smith et al. 2010; Poirier et al. 2014; Sathyan et al. 2012; Destoumieux-Garzón et al. 2016).

Despite their critical roles during environmental epigenetic responses, detailed studies addressing the role of chromatin structural components are still lacking in non-model marine organisms. This is essentially due to the lack of knowledge about their chromatin structure, as well as to the absence of specific antibodies enabling the dynamic study of these proteins genome-wide. During the last decade, however, several studies have advanced in the description of chromatin components in marine animals, especially bivalve molluscs [reviewed in (González-Romero et al. 2012a, b; Suarez-Ulloa et al. 2015)], evidencing a high degree of conservation (Rivera-Casas et al. 2016a, b; González-Romero et al. 2012a, b) but also intriguing divergence in some cases (Rivera-Casas et al. 2016a, b). Detailed guidelines for the study of chromatin-associated proteins in bivalves have been recently published based on these studies (Rivera-Casas et al. 2017) paving the way to expand this type of analysis. In addition, studies in bivalve molluscs have shown the involvement of histone variants and PTMs in environmental-triggered responses. Accordingly, it has been recently reported that histone variant H2A.X is rapidly phosphorylated in the eastern oyster *C. virginica* during responses to harmful algal blooms and toxin exposure (González-Romero et al. 2017) and that histone methylation is influenced by changes in temperature during the development of the Pacific oyster *C. gigas* (Fellous et al. 2015). Overall, although the number of studies in marine organisms

analyzing the role of histone variants and histone modifications in environmental responses is still very scarce, the studies above evidence the need to put more effort in the characterization of the protein component of the chromatin.

1.2.3 RNA-Mediated Regulation of Gene Expression

Many eukaryotic genomes are characterized as concentrating protein-coding DNA regions within a very limited space of the overall genome (e.g., 1–2% in humans), compared to the proportion of DNA formerly considered as "genomic junk." However, it is now known that most of the DNA in eukaryotic genomes is indeed transcribed and potentially functional, producing different types of noncoding RNAs (ncRNA) which can play fundamental roles in the cell (Palazzo and Lee 2015), including structural functions and regulation of gene expression (Vidigal and Ventura 2015; Zhang et al. 2014). Regulatory activity might take place either through direct mechanisms (e.g., mRNA interference, splicing, or degradation) or through the modulation of other epigenetic mechanisms, notably DNA methylation and histone modifications (Carthew and Sontheimer 2009). In fact, one of the best-known roles of ncRNA in epigenetics is the regulation of genetic imprinting, where genes are selectively silenced by heavy DNA methylation depending on their maternal or paternal origin.

The RNA-mediated regulation of gene expression is considered to have evolved convergently in animals, plants, and protists independently, based on the high level of evolutionary conservation and the remarkable responsiveness to environmental stress found in regulatory ncRNA molecules across taxa (Zhang et al. 2011). The different types of ncRNA can be broadly classified based on their size into short ncRNA [sncRNA <30 nucleotides (nt)] and long ncRNA (lncRNA >200 nt). The former group comprises three major classes, short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), and microRNAs (miRNAs), all of them with specific functions in epigenetic mechanisms (Holoch and Moazed 2015). The present section focuses particularly on miRNAs, the most widely studied type of small ncRNA, which are approximately 22 nt long and can block the translation of mRNA by hybridizing with imperfect complementary sequences at 3′-UTR of their targets. Optimized sequencing methods allow for the characterization of these short noncoding RNA transcripts in any organism, facilitated by the high level of conservation they display within metazoans. Moreover, predictive models have been developed to identify potential targets among protein-coding transcripts (mRNA), streamlining the characterization of regulatory networks. Interestingly, despite the relatively high evolutionary conservation of miRNA sequences, a high rate of gene turnover and different regulatory mechanisms have been proposed for cnidarians in contrast to bilaterian animals (Moran et al. 2014). These observations resemble the relevant differences existing between miRNA in animals vs. plants, where miRNA regulates the expression of target mRNA through cleavage and plays a role in directing DNA methylation events (Wu et al. 2010).

Epigenetic Role of miRNAs During Environmental Responses

Interestingly, recent findings suggest that miRNAs might be instrumental in cellular intercommunication, since these molecules can be observed in extracellular fluids inside vesicles or as part of protein complexes (Zhang et al. 2015). Thus, these findings lead to another critical question: Do miRNAs participate in the transgenerational epigenetic inheritance by carrying environmental information from somatic cells to the germ line? (Zhang et al. 2015; Cossetti et al. 2014). Similarly, the possibility of a miRNA-based communication between microbiota and host organism adds further interest to this specific type of ncRNA. While most studies have been focused in model vertebrates, miRNAs have been also identified in several non-model organisms, including marine vertebrates and invertebrates. Accordingly, miRNA transcriptomes have been identified and characterized in fish (Li et al. 2016), in marine mammals (Segawa et al. 2016), and also in invertebrates such as molluscs (Xu et al. 2014; Picone et al. 2017; Jiao et al. 2014), cnidarians (Gajigan and Conaco 2017; Liew et al. 2014), and sponges (Liew et al. 2016).

Similar to the case of other epigenetic mechanisms, the role of miRNAs during environmental responses is starting to be deciphered, supporting their value to study acclimatory responses under rapidly changing environments and their biomarker potential. For instance, specific miRNAs have been shown to participate in fish responses to hypoxia (Lau et al. 2014) and thermal stress (Bizuayehu et al. 2015). In addition, the role of environmentally responsive miRNAs has been linked to crucial physiological processes including reproduction in fishes (Juanchich et al. 2013; Tse et al. 2016). The key regulatory role of miRNAs during environmental responses has been demonstrated in marine invertebrates (Huo et al. 2017; Zhao et al. 2016a, b), as well as in marine microorganisms (Gierga et al. 2012). In particular, the potential of miRNA to be transferred extracellularly via vesicles or protein complexes makes them particularly promising to understand host-microbe interactions like in the case of coral-dinoflagellate symbiosis, where the latter have been shown to produce miRNAs complementary to mRNAs in the coral host (Lin et al. 2015).

1.2.4 Epigenetic Regulatory Networks

The regulation of eukaryotic gene expression is possible thanks to the complex coordinated action of different genetic and epigenetic mechanisms. Although still largely unknown, recent reports have identified the interplay among some types of ncRNA (e.g., siRNA or lncRNA) as critical mediators of DNA methylation and histone modifications. For instance, lncRNAs have been repeatedly reported to initiate DNA methylation events through the interaction with DNA methyltransferases (Zhao et al. 2016a, b). However, both long and short noncoding RNAs other than miRNAs (e.g., lncRNA, siRNA, piRNA) have been also associated with methylation of histone tails through different mechanisms, leading to different chromatin states including heterochromatinization (i.e., global silencing of genes by increased chromatin

compaction; Joh et al. 2014). On the other hand, it has been reported that DNA methylation marks at promoters of metazoan miRNA genes exert an indirect, although critical, effect in gene regulation by modulating the expression of this interference mechanism, illustrating the complexity of this multilevel epigenetic regulatory network (Parodi et al. 2016).

DNA methylation in promoters is usually excluded from regions containing nucleosomes with transcriptionally active marks such as H3 methylation (H3K4me2 and H3K4me3) or presence of H2A.Z (Gu et al. 2015; Zilberman et al. 2008). In this sense, work in mammals has shown that methylation of H3K4 strongly inhibits the initiation of the de novo methylation process (Ooi et al. 2007). Overall, the modeling of these regulatory networks has been attempted using a systems biology approach and computational methods in biomedical research (Artyomov et al. 2010; Chen and Li 2016). Despite the general lack of these kinds of studies using marine organisms, these studies convey the promise of more specific biomarkers for disease or environmental stress obtained through the combination of heterogeneous epigenetic data that can inform about the health of the organism or populations. Moreover, they have the potential to be used as a proxy to inform about subtle changes in the environment working as bioindicators of the quality of ocean waters.

1.3 Inheritance of Epigenetic Modifications

A critical aspect to consider when analyzing the contribution of epigenetic mechanisms to phenotypic plasticity is their inheritance and whether this is referring to mitotic cellular divisions or the meiotic transmission to further generations. While some epigenetic marks can persist in a cell for decades [e.g., DNA methylation-mediated gene silencing (Klose and Bird 2006)] or be transmitted transgenerationally [e.g., DNA methylation marks (Kuhlmann et al. 2014), small RNAs (Chen and Li 2016)], other epigenetic modifications can rapidly change between modified and unmodified states depending on environmental cues [e.g., histone acetylation (Turner 2000)]. The highly dynamic nature observed in epigenetic modifications has motivated the differentiation of two types of approaches for their study: intragenerational epigenetics (contributing to intragenerational plasticity, IGP; see Fig. 2) and transgenerational epigenetics (contributing to transgenerational plasticity, TGP; see Fig. 2; Burggren 2016). Accordingly, the first is primarily focused on the mechanistic basis underlying gene expression changes produced by epigenetic marks and its persistence in an individual (e.g., epigenetics of diseases). On the other hand, transgenerational epigenetics is interested in the persistence of particular epigenetic marks across generations, more precisely, the transmission of epigenetic marks beyond the F2 generation, ruling out a direct environmental effect in primordial germ cells (Feil and Fraga 2012). Thus, transgenerational epigenetics constitutes a very innovative and powerful tool to study adaptation and population modeling (Etchegaray and Mostoslavsky 2016). Yet, despite the evident appeal of the transgenerational approach, our understanding of the basic mechanisms by which

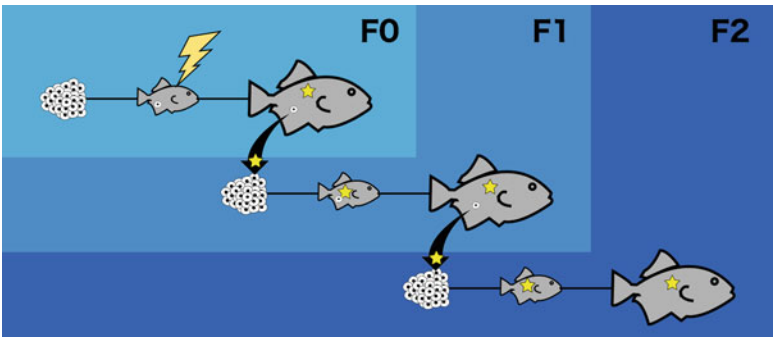

Fig. 2 Intragenerational plasticity (IGP) and transgenerational plasticity (TGP) phenomena triggered by environmental signals. Environmental signals may influence the phenotype of an organism through epigenetic modifications regulating gene expression, even at later stages in life long after exposure (considering F0 only). The phenotypic outcome of these environmentally induced epigenetic modifications may be acclimatory or deleterious (e.g., disease) and can be referred to as intragenerational plasticity (IGP). This environmental information can be transmitted to subsequent generations (F1–onward) by means of stable epigenetic marks in the germ line. In order to qualify as transgenerational plasticity (TGP), this transmission must occur until at least F2 in the case of non-eutherian fish, or until at least F3 in the case of viviparous species. This is because exposure of the gestating female (F0) that modify the epigenome could result in simultaneous direct exposure of the developing embryo (F1) and the developing germ line of the embryo (F2) (Mirbahai and Chipman 2014)

epigenetic marks persist, modulate expression, and survive reprogramming events in the zygote is still very limited and can lead us to erroneous assumptions about their role in inherited phenotypes.

1.3.1 Epigenetic Reprogramming

Epigenetic reprogramming events have been best characterized in the germ line and during early stages of mammalian embryogenesis. Such events represent major barriers for the transmission of epigenetic marks to the next generation, since the majority of DNA methylation marks and practically all of histones and their PTMs are removed from chromatin and replaced by protamines during male gametogenesis (Eirín-López and Ausió 2009), only to be restored afterward during cell differentiation (Morgan et al. 2005). Studies of DNA methylation during zebrafish development revealed similar patterns to those observed in mammals, supporting and expanding these observations to other vertebrates (Riviere et al. 2013). Oppositely, extensive epigenetic reprogramming events have not been observed in plants nor in most invertebrate species (with the exception of social insects). These observations have led to hypothesize that transgenerational inheritance of DNA methylation marks would be more plausible in these taxonomic groups (Sano and Kim 2013; Hauser et al. 2011).

An interesting perspective on the application of epigenetics in ecology and evolution of marine organisms was put forward by Verhoeven et al. (2016), emphasizing the relevance of transgenerational epigenetics for the development of such fields. There, DNA methylation was designated as the only mechanism able to carry epigenetic information transgenerationally, dismissing the already confirmed roles of small RNAs (Chen and Li 2016) and chromatin modifications (e.g., Siklenka et al. 2015). It is important to point out that such dismissal could be motivated by a lack of information or precise knowledge about the contribution of these mechanisms to this process. Indeed, the transgenerational transmission of epigenetic information does not need to occur by the direct transmission of a specific mark (generally reset at some stage), but instead it could involve the translation of such information across diverse epigenetic marks. It is therefore fundamental to continue to investigate the relationships between different epigenetic mechanisms over the base of specific marks and elicited changes in gene expression patterns as well as the environmental factors triggering those epigenetic marks in the first place (Cortessis et al. 2012). By doing so, it would be possible to move toward identifying the nature and inheritance of these modifications and their implication in adaptive responses (Tricker 2015).

1.4 Epigenetic Determinants of Evolutionary Change

Understanding the contribution of epigenetic mechanisms to organismal acclimatization and adaptation under rapidly changing environments constitutes one of the current greatest challenges in modern biology. The potentially heritable nature of epigenetic modifications (and their subsequent contribution to the inheritance of environmentally acquired phenotypes) is revolutionizing the current understanding of the mechanisms underlying evolutionary change. Indeed, through epigenetic modifications, it is possible to provide a mechanistic basis for well-known evolutionary phenomena including phenotypic plasticity (PP) (Rando and Verstrepen 2007). In addition, epigenetic diversity may act as a compensatory mechanism in populations where genetic diversity is low, increasing phenotypic variability. This mechanism has been shown to be critical for the establishment and success of invasive species in new environments as has been seen in marine invertebrates (Ardura et al. 2017; Pu and Zhan 2017). More importantly, epigenetic modifications could facilitate extremely rapid and acclimatized phenotypic responses to global climate change in much shorter time scales than those required for the fixation of genetic variants providing increased fitness (Rando and Verstrepen 2007). Given the rapid pace of global climate change and its critical impact on marine environments, the characterization of the role played by epigenetic mechanisms during acclimatization and adaptation will help develop better population assessment and management strategies.

The contribution of epigenetic modifications to rapid acclimatization and adaptation may be even closer to classical mutation-selection theories than previously

thought. This is best illustrated by studies finding high mutation rates (up to tenfold compared with non-methylated DNA) at hypermethylated CpG sites. Although this observation has been linked to altered cancer states, it could also contribute to adaptation and evolution (Guerrero-Bosagna et al. 2005). Therefore, epigenetics provides an attractive framework to explain mechanisms of rapid evolution, offering great potential for conservation efforts. These ideas have been considered by coral researchers proposing an innovative concept coined as "assisted evolution" (van Oppen et al. 2017). This approach suggests the implementation of selective breeding and preconditioning treatments artificially increase tolerance of organisms to environmental stress through the manipulation of environmental conditions. This notion is supported by the concept of priming hormesis (the environmental "priming" of certain physiological processes can improve their functioning later in life) as a nonlinear dose-response relationship where beneficial consequences of low-level exposure to environmental stress or pollution may increase the organism's tolerance to higher levels of such pollution or stress later in life (Costantini 2014).

2 Environmental Epigenetic Applications in Marine Ecosystems: Current and Future Perspectives

The present section summarizes some of the most relevant research directions illustrating the current relevance of environmental epigenetic studies in marine organisms. For that purpose, examples encompassing a broad range of taxa have been chosen. Since epigenetics research is still in its infancy in non-model organisms, especially in the marine environment, many of the studies discussed below are still efforts in progress.

2.1 Epigenetic Assessment of Health and Stress in Marine Organisms

Genetic disorders can be identified, and in many instances treated, through analyses revealing alterations in the DNA sequence. However, these analyses have intrinsic limitations at the time of revealing direct changes in gene function motivated by heterogeneous environmental conditions. Environmental epigenetic analyses fill that gap (Bollati and Baccarelli 2010), providing a framework for developing sensible epigenetic biomarkers. Such approach has been pioneered in human health sciences for a while now, notably linked to cancer biology (Sharma et al. 2010). Overall, the combination of genetic and epigenetic analyses is ushering basic research and applied therapies into the age of personalized medicine, incorporating genetic and environmental diversity into current studies. Importantly for ecological and toxicological research, the information currently being generated in model

organisms can be readily expanded to different taxa across different environments, thanks to the evolutionary conservation of the fundamental components of epigenetic machinery (Feng et al. 2010; Lee et al. 2010; Goll and Bestor 2005; Lowdon et al. 2016).

2.1.1 Epigenetic Biomarkers of Disease

Epigenetic modifications are becoming a popular new source of health biomarkers in humans. These biomarkers have been linked to ongoing conditions, such as in the case of cancer-specific hypermethylation of CpG islands (Herman and Baylin 2003). In addition, epigenetic biomarkers identifying disease susceptibility have also been defined in cases where the disruption of epigenetic marks results in detrimental mutations and transcriptional changes leading to disease. This latter type is best illustrated by the "epigenetic progenitor model" (Mirbahai and Chipman 2014; Mirbahai et al. 2011a, b; Portela and Esteller 2010), suggesting that epigenetic changes occur as early as in progenitor cells and facilitate the progression of carcinogenesis (Pogribny 2010; Sharma et al. 2010; Feinberg et al. 2006). This model found support in marine studies combining methylated DNA immunoprecipitation (MeDIP) with de novo high-throughput sequencing to investigate DNA methylation changes in the non-model common flatfish dab (Mirbahai et al. 2013). Accordingly, an unusually high incidence of liver tumors (20% affected in some areas) was found in these organisms, displaying a 1.8-fold decrease in the DNA methylation of adenoma liver tissue cells. Based on these results, it was suggested that chronic exposure to pollutants (including endocrine disruptors and heavy metals) was responsible for the epigenetic changes observed (Bollati and Baccarelli 2010; Huang et al. 2008; Reichard et al. 2007).

The observed cause-effect relationship between environmental stress, epigenetic modifications, and the risk of developing disease later in life supports the relevance of environmental epigenetic studies in aquatic organisms. Although this approach is still hampered by the lack of detailed knowledge regarding epigenetic regulation, some species such as zebrafish and medaka are starting to emerge as model systems (Kim et al. 2016; Mudbhary and Sadler 2011), facilitating the study of transgenerational epigenetics and the impact of environmental stressors on population dynamics. On the other hand, the study of marine mammals can easily benefit from (and even complement) technologies and molecular tools specifically developed in human health research. Accordingly, the use of miRNA in biofluids (e.g., blood and plasma) has already been proposed for biomonitoring and early disease diagnosis of dolphins in aquaria (Segawa et al. 2016). Also, since high levels of circulating nucleosomes have been associated with several types of cancer and other conditions in humans (Chen et al. 2014; McAnena et al. 2017), liquid biopsies targeting histone modifications as potential early biomarkers of different diseases could also be a promising approach in the case of marine mammals (Bauden et al. 2015; Gezer et al. 2015; Abrams et al. 2013). Although the application of these methodologies in ecological studies could be challenging, their implementation in

captive and wild animals would critically contribute to individual and population assessment, supporting management and conservation efforts.

2.1.2 Epigenetic Biomarkers of Stress Exposure

The study of epigenetic biomarkers constitutes a very powerful approach to identify early exposure to pollutants and other environmental stressors, based on the plasticity and sensibility of epigenetic modifications (Jaenisch and Bird 2003). Both genomic and mitochondrial DNAs represent good sources of epigenetic biomarkers, as suggested by studies revealing germ line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location (Byun et al. 2013; Yauk et al. 2008). In marine environments, anthropogenic pollutants are often found in tissues of marine organisms, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDE), and several other chemicals (Tanabe et al. 1983; Stuart-Smith and Jepson 2017; Lascelles et al. 2014). Heavy metals constitute another widely quantified stressor in marine environments (Boening 1999), whose effects include altered DNA methylation states (Baccarelli and Bollati 2009). These elements represent a common threat during dredging associated with the development of coastal areas. During this procedure, the sediment is introduced (along with the pollutants deposited in the soil, particularly trace heavy metals (Calmano et al. 1996)) into the surrounding water column. Under certain conditions these pollutants become bioavailable and subsequently incorporated into the food chain (Latimer et al. 1999; Eggleton and Thomas 2004; Burton et al. 2010), critically impacting species using shallow coastal bay and estuary areas as nurseries, including fish, shrimp, and predatory species such as sharks.

The work toward finding epigenetic biomarkers of exposure for marine organisms has been pioneered by several studies addressing the effects of exposure to various stressors including parasites (Farias et al. 2017), pollutants (Wang et al. 2009), and harmful algal blooms (Suárez-Ulloa et al. 2013; González-Romero et al. 2012a, b). Most of these studies focused on DNA methylation changes with exposure to the various stressors, and all used molluscs as the model organism. Given the intra- and transgenerational persistence of some epigenetic modifications, their study could potentially provide an insight into the variety of environmental exposures that an individual has experienced during its life (Mirbahai and Chipman 2014). With current technology capabilities (i.e., next-generation sequencing, microarrays, bisulfite treatment sequencing), biomarkers of exposure can be identified in species without a reference genome. Simply identifying changes in the epigenome can improve our ability to identify early exposures to detrimental stressors. As more reference genomes become available, biomarker identification will be further facilitated in other marine species as well as our ability to determine the health effects of these stressors upon the organisms and their populations.

2.2 *Epigenetic Study of Population Parameters in Marine Organisms*

Biological conservation requires efficient methods to identify endangered populations in a timely fashion. However, this task is often hindered by the difficulty to record different species attributes. For instance, the study of populations belonging to highly mobile species is more complicated due to their vagility, as it is the case for many marine species. In those scenarios, molecular techniques have proven to be extremely valuable, being in many cases the only approach possible to gather certain types of information, such as sex or populations dynamics. Yet, the information provided by genomic analyses is still limited in many instances. The present section discusses potential applications of epigenetic strategies for gathering additional types of information for population analyses, notably age and sex.

2.2.1 Epigenetic Estimation of Age

Determining the age of individuals is critical to understand their demography and population dynamics (see Fig. 3). Unfortunately, the influence of this trait on trophic interactions (e.g., Lahaye et al. 2006) and on environmental responses (Mashburn and Atkinson 2004; Poloczanska et al. 2016) is generally unknown for many species. This is mainly due to the inherent difficulty in estimating age in these organisms. Consequently, several areas of study (e.g., ecology, physiology, toxicology, etc.; see Fig. 3) would greatly benefit from the incorporation of age into their datasets, improving the resolution of these analyses (Yang et al. 2015; Horvath 2013; Jarman et al. 2015). Large marine mammals such as dolphins and whales are among these difficult species to study. Although different methods have been developed to sample and estimate biological parameters, the age estimation method most widely used in cetaceans is counting growth layer groups (GLGs) on teeth (Perrin and Myrick 1980). This method requires the removal of a tooth from the animal which, in addition of being extremely invasive, is not feasible in the case of population studies involving several animals or in the case of very large species (e.g., large whales).

Molecular methods of age estimation have been therefore developed, notably the analysis of telomere lengths (Fagagna et al. 2003; Hedrick and Lacy 2015). Unfortunately, the high levels of intraindividual variation observed make this approach unreliable for age estimation (Hedrick and Lacy 2015; Olsen et al. 2012). Alternatively, it has been demonstrated that the study of epigenetic modifications such as DNA methylation can provide a reliable method for age estimation (Horvath 2013), based on the correlation between methylation of CpG sites and age (see Fig. 3a, b). This technique has even been calibrated for use with humpback whales, using noninvasive skin tissue samples (Polanowski et al. 2014). More precisely, the humpback epigenetic age assay (HEAA) targets three genes whose DNA

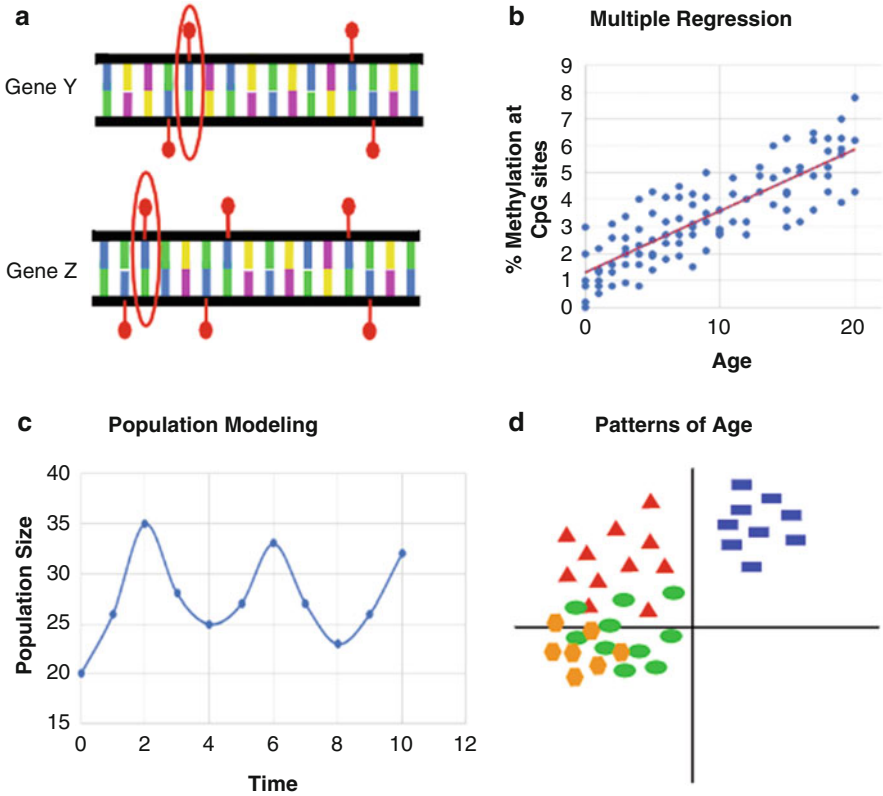

Fig. 3 Epigenetic estimation of age and implications for population ecology. Epigenetics can be used to estimate the age of individuals based on DNA methylation patterns at age-associated loci. (**a**) In mammals, the amount of DNA methylation at specific CpG sites (indicated with red circles) displays a strong correlation with age. (**b**) Using multiple regression based on CpG sites whose DNA methylation changes with age, a model can be created to identify the age of unknown individuals. (**c**) Age identification supports and informs conservation efforts in endangered populations (e.g., helping model and predict population growth through identifying how many individuals are of reproductive age). (**d**) This principal component analysis shows a scenario in which age makes sense of the different groups (e.g., juveniles, blue rectangles; subadults, red triangles; adults, green circles; older nonreproductive individuals, yellow hexagons); this could be a feasible analysis for several fields such as toxicology, DNA methylation patterns and exposures/different environments, or other types of data that may be different across age groups

methylation is highly correlated with aging. The applicability of this tool is not only evident in this species, but it also provides a framework for developing species-specific assays able to efficiently determine age in keystone marine mammals (Hannum et al. 2013). Nonetheless, this goal requires the availability of a reference genome for the targeted species. In case that is not available, the use of high-throughput sequencing might help in finding age markers through massive genome scanning.

2.2.2 Identification of Cryptic Subpopulations Using Epigenetic Markers

One of the most important challenges in conservation biology is to identify when speciation is occurring and to properly manage the incipient subpopulations. Accordingly, when two subpopulations become reproductively isolated, it is expected that genetic mutations will begin to accumulate between the two populations, due to the low gene flow (Bateson 1909; Dobzhansky 1936; Muller 1942). However, little or no genetic differentiation will be evident during the early stages of this process due to not enough time having elapsed since isolation (i.e., mutation rate is low and differentiation is slow). This hinders the correct identification and management of subpopulations of endangered species. Nonetheless, whether population isolation is due to physical barriers or geographical preferences, it is expected that each population will be subject to different environmental conditions, triggering different epigenetic responses. That prediction, which has been supported by studies developed on human monozygotic twins subject to different environments (Fraga et al. 2005), underscores the potential of epigenetic analyses to identify early speciation events and their relevance for management and conservation purposes. This strategy is even more important in populations in which cohorts or social groups may exist which may influence isolation of reproduction, such as dolphins (Viricel and Rosel 2014).

Epigenetics may even contribute to speciation (Blevins et al. 2017). As previously mentioned, rapid acclimatory responses influenced by environmentally responsive epigenetic modifications might provide a basis for rapid adaptation and evolution. For instance, DNA methylation at CpG islands increases the rate of mutation at these sites by as much as tenfold, encompassing implications not only for disease but also for speciation (Sved and Bird 1990; Guerrero-Bosagna et al. 2005). In Blevins et al. (2017), an epiallele for the HISN6 was found that silenced the gene in *Arabidopsis thaliana*. Individuals of the specific ecotype that had this epiallele were found to be incompatible in making viable offspring with individuals of a different ecotype that had a genetic mutation in the gene, HISN6A, that was nonfunctioning. This study evidences that both genetic and epigenetic variation among subpopulations is contributing to their incompatibility. This particular scenario of speciation falls under the mechanism of speciation proposed by Lynch and Force (2000), where gene duplications often lead to the inactivation of one of the duplicates (as a resolution to the duplication). Thus, incompatibilities can occur between individuals from populations with opposing resolutions to gene duplications. Based on this observation, it is now important to look not only at the genotype but also at the epigenotype of populations, in order to identify causes of reduced gene flow. This could be extended into breeding programs to help severely endangered populations such as corals or in the extreme situation where only a handful of individuals of a population remains and captive breeding becomes the only option such as in the case of the attempts to resque the Vaquita (Taylor et al. 2016).

2.2.3 Other Populational and Ecological Applications

There are potentially many different ways in which epigenetics can contribute to the current ecological understanding of marine populations. Thus, when studying a population, it is critical to monitor changes in the genome and also in the epigenome to learn how species respond to their environment. For instance, the study of DNA methylation provides a relatively easy and inexpensive strategy (i.e., storage and handling of samples is less stringent and expensive for DNA vs. RNA) for ascertaining how gene expression is being modified under different environmental regimes. Accordingly, Morán and Pérez-Figueroa (2011) found that epigenetic changes (DNA methylation) participate in the early maturation of male Atlantic salmon (*Salmo salar*) in response to low population densities. Additionally, DNA methylation studies in the three-spined stickleback found that several genes encoding ion channels were differentially methylated between freshwater and saltwater stickleback. They found that genes harboring genetic and epigenetic changes between the two ecotypes were different suggesting that DNA methylation was a complementary mechanism to the adaptation to freshwater (Artemov et al. 2017).

Epigenetics can also help in predicting population parameters or health status in future generations. For example, in European sea bass (*Dicentrarchus labrax*), DNA methylation changes have been found to affect sex determination in response to changes in temperature (Navarro-Martín et al. 2011). More precisely, increased DNA methylation of the promoter region of the aromatase (cyp19a1) gene at higher temperatures decreases its expression, thereby accumulating increased levels of androgen and promoting the formation of testis and a male-biased sex ratio. This is similar to sex differentiation mechanisms found in some reptiles (Matsumoto et al. 2013), and it has been suggested that temperature-dependent sex determination could be inherited transgenerationally (Warner et al. 2013). Knowing this kind of information could help with predicting what new generation population parameters will be like and allow for a better estimation of the breeding population. In addition to predicting sex as a parameter of populations, it can also be informative to be able to just identify the sex of individuals for several fields of study. In species that are hard to differentiate between males and females, epigenetic markers of sex could come in handy. Several studies have documented significant differences in DNA methylation between the sexes (Boks et al. 2009; El-Maarri et al. 2007).

In marine fish, other ecologically relevant traits have been found to be mediated by the epigenome (Bizuayehu and Babiak 2014; Metzger and Schulte 2016). For instance, sea lamprey (*Petromyzon marinus*) go through a metamorphosis from being a filter feeder to a tissue-consuming organism, and it was found that DNA methylation changes were associated with this process in muscle tissue (Covelo-Soto et al. 2015). Similarly, it was found in rainbow trout (*Oncorhynchus mykiss*) alevins that major changes in metabolic gene expression and miRNAs were correlated with the transition from endogenous (yolk sac) to exogenous feeding (Mennigen et al. 2013). miRNAs are thought to contribute greatly to growth in Nile tilapia (*Oreochromis niloticus*). This was demonstrated when skeletal muscle miRNAs were found to be significantly different

between fast- and slow-growing strains (Huang et al. 2012). miRNAs were also found to target IGF-1 in Nile tilapia indicating that they may be of importance to the hypothalamic-pituitary pathway (Yan et al. 2013). Temperature-induced phenotypic plasticity of growth in Senegalese sole (*Solea Senegalensis*) was found to have expression changes in miRNAs as well (Campos et al. 2013). Application of this knowledge could help in the selection of faster-growing individuals for aquaculture. In addition, miRNAs have been found to contribute greatly in immune response of teleost fish similarly to how they have been identified to be important in mammals (Andreassen and Høyheim 2017). Studying this aspect of immunology may provide insights to keeping aquaculture fish healthy or even choosing individuals that have strong immune responses.

2.3 Epigenetic Approaches to Restoration and Management

The primary goal of species reintroductions is to stop population decline and artificially increase the rate of population growth (Seddon et al. 2007). Population decline could be caused by habitat deterioration or loss of population members due to overexploitation. In the latter case, better population management may be enough (Myers et al. 1995) and should be used when the genetic diversity of the population is a concern (Gaffney 2006). On the contrary, the case of habitat deterioration is more complex since the success of reintroduction will depend in the chances of restoring the habitat (Miller and Hobbs 2007; Seaman 2007) or the capacity of the species to acclimate and adapt to the new habitat condition. Because of this and despite its wide implementation, many reintroduction projects have failed to fulfill the aim of establishing self-sustainable populations in marine organisms (Mercado-Molina et al. 2015; Okubo and Omori 2001). Commonly, this failure is attributed to the environmental conditions of the specific site that do not promote species establishment and persistence (Seddon et al. 2007). Unfortunately, under the current pace of climate change, it is possible that those "favorable" conditions are already gone in most regions, deeming restoration a futile effort if organismal acclimatization capabilities are not considered and somehow enhanced.

The role of epigenetic modifications improving acclimatory capabilities has been observed across diverse environmental scenarios. For instance, a role for epigenetic modifications has been proposed in response to invasions (i.e., invasive species, Ardura et al. 2017). Similarly to species reintroduced in a hostile environment, invasive species need to overcome challenges in order to successfully establish self-sustaining populations. Thus, epigenetic modifications were initially proposed as a way to explain how invaders compensate the reduced genetic diversity, derived from the low number of individuals starting the population (Chown et al. 2015; Pérez et al. 2006). Further experimental evidence supporting epigenetic responses to environmental changes, and promoting acclimation/adaptation responses, is also available in terrestrial (Lämke and Bäurle 2017; Sgrò et al. 2016; Galván et al. 2017) and aquatic organisms (Norouzitallab et al. 2014; Palumbi et al. 2014;

Putnam et al. 2016). Based on these results, a change in paradigm for restoration and reintroduction of species populations is starting to be envisioned (van Oppen et al. 2017; Jones and Monaco 2009). The identification and selection of individuals displaying a better ability to respond to environmental stressors or even the induction of "preconditioned" or "hardened" epigenomes constitute one of the central pillars of this new approach.

2.3.1 Epigenetic Basis of Coral Reef Restoration

The application of this strategy (i.e., reintroductions) to marine organisms is best illustrated by coral restoration programs. Hermatypic (i.e., reef-building, stony) corals constitute the structural basis of reef ecosystems, supporting most of marine and coastal biodiversity. Corals are particularly affected by changes in temperature and chemical composition of the oceans (Cai et al. 2016; Hume et al. 2016), evidencing their susceptibility in a global change scenario. Out-planting constitutes one of the principal coral reef restoration strategies implemented by scientists, managers, and local stakeholders in trying to revert the current rate of coral cover loss (Hoegh-Guldberg et al. 2007). Despite being widely implemented, this approach is hampered by the low survival rates of out-planted fragments in many programs worldwide (Okubo and Omori 2001), decreasing significantly after the third year post-out-planting (Garfield 2016). These programs generally select genotypes showing more rapid growth, disregarding other important traits as endurance and resilience. Non-genetic processes such as changes in the microbiome (Hauser et al. 2011; Hernandez-Agreda et al. 2016) and epigenetic mechanisms can accelerate the rate of phenotypic change beyond the limits of genetic adaptation (Fig. 4), helping corals develop traits that permit effective responses to a rapidly changing climate and result in increased survival post-out-planting (Putnam et al. 2016; Roberts and Gavery 2012).

Despite the potential to manipulate epigenetic marks to increase restoration success, little is known about how these non-genetic mechanisms respond to different stressors and their interaction with standing genetic variation to produce acclimatized phenotypes in marine invertebrates (Suarez-Ulloa et al. 2015; Crespi et al. 2012; Beaulieu and Costantini 2014). More so, in many cases, we lack understanding about the mechanisms and their potential to mediate intragenerational plasticity (IGP) and transgenerational plasticity (TGP), critical processes mediating acclimation and adaptation as discussed earlier in this work. Epigenetic analyses in corals have been almost exclusively focused on DNA methylation analyses and its relationship with gene expression (Marsh et al. 2016; Dixon et al. 2014) during responses to environmental change (Dixon et al. 2014; Dimond and Roberts 2016) and the consequences on phenotypic plasticity (Putnam and Gates 2015). These studies showed rapid acclimatory responses in corals during thermal stress (Palumbi et al. 2014; Barshis et al. 2013), including transcriptomic and epigenetic changes in response to nutrient enrichment (Rosic et al. 2014), as well as modifications in DNA methylation levels in response to ocean warming and acidification (Putnam et al. 2016; Putnam and Gates

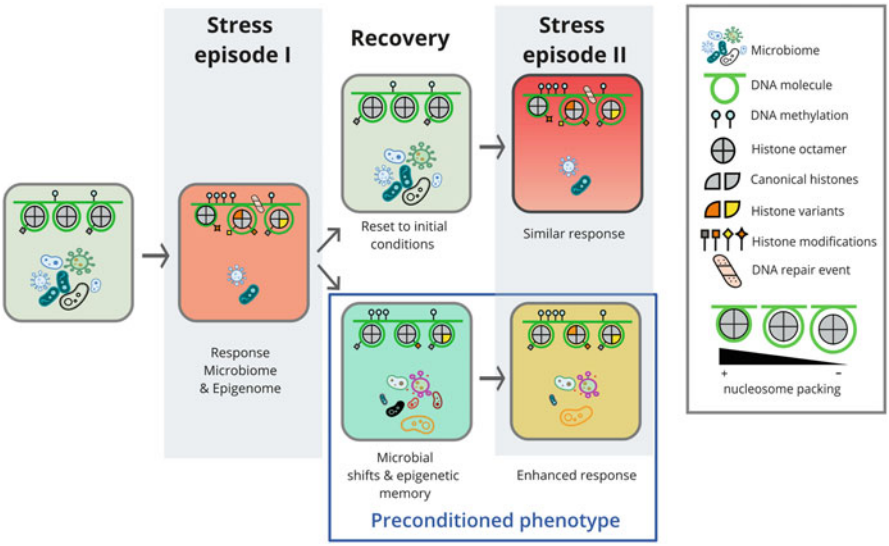

Fig. 4 Epigenetic contribution to environmental preconditioning. Both epigenetic memory and microbiome shifts have the potential to create preconditioned phenotypes displaying enhanced responses to repetitive stress episodes. A stress event will trigger non-genetic responses (epigenetic and microbial shifts) that could be reset to initial conditions or generate persistent changes potentially enhancing organism responses to environmental stress. The present figure depicts these potential scenarios under two pulses of similar stressors

2015). In such a way, they have strengthened the links between DNA methylation and transcriptional plasticity, although the genome-wide distribution of such marks (DNA methylome) and their role in the onset of transgenerational epigenetic memory and adaptive responses are still not clear. Other epigenetic mechanisms, such as noncoding RNAs, histone variants, and their posttranslational modification, have received less attention. This constitutes a research priority based on the very promising results obtained on related species and other marine invertebrates (Moran et al. 2014; Rivera-Casas et al. 2016a, b; Fraune et al. 2016; Reddy et al. 2017) and the implication these mechanisms can have on "preconditioning" of corals.

Several lab-based experiments have increased thermal tolerance of corals through controlled heat-stress exposures (Cunning et al. 2015), mainly promoting thermally tolerant symbionts. However, it is unknown how these manipulations can affect the physiology of the coral holobiont. Towle et al. (2016) showed an increased susceptibility to bleaching in coral preconditioned to increased CO_2. Changes in gene expression (Barshis et al. 2013; Bellantuono et al. 2012) and microbiome composition, derived from the preconditioning, could have a positive effect driving acclimatory processes but at the same time could have unknown negative effects. Further efforts are required to evaluate different strategies for preconditioning both in laboratory and field settings, including the analysis of the interaction between genome, epigenome, and microbiome in the response within and between

generations. Overall, determining whether this "preconditioning" can enhance coral demographic performance in an ecological context constitutes the final step for the development of successful coral reef restoration.

3 Conclusions

Epigenetic analyses encompass many potential applications in the field of marine sciences. Current studies based on DNA methylation analyses are paving the way for the incorporation of epigenetic research into different disciplines within the marine realm, as evidenced by the numerous examples discussed in the present work. Moving forward, there is a need for identifying marine organisms that will represent a new generation of ecologically and environmentally relevant model organisms. These will be fundamental for elucidating how epigenetic mechanisms work, how they change in response to environmental stressors, and how epigenetic signatures are inherited and contribute to phenotype diversity across generations. Currently, little work has been done on assessing epigenomic variation between species and populations for marine organisms, mainly due to the lack of complete genomes for most species as well as because of the high costs associated with single nucleotide resolution studies. In addition, the few works developed were conducted using different methodologies and different types of samples, hampering the comparison of results and making assessment of differences between these species unclear and unreliable (Hofmann 2017). As mentioned earlier, the work comparing three-spined sticklebacks from freshwater and saltwater environments probably constitutes the more comprehensive example of population epigenomic studies in marine organisms. Results from this research suggest that epigenetic adaptation may act as a compensatory regulatory mechanism for the lack of genetic variation, complementing the selection of genetic variants and enhancing phenotypic plasticity in different environments (Artemov et al. 2017).

The current ability to effectively record complex biological traits such as age or sex through epigenetic analyses can potentially revolutionize several research fields, notably ecotoxicology, ecology, and genetics. Within the current context of a highly paced global climate change, it is now evident that epigenetic mechanisms play a key role during organismal acclimatization and adaptation. Further development of marine epigenomics will facilitate a better understanding of how organisms respond to their environment, allowing for stronger restoration efforts to occur as well as fostering the development of a new generation of biomarkers and tools that can be used for conservation.

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References

Abrams ST, Zhang N, Manson J, Liu T, Dart C, Baluwa F, Wang SS, et al. Circulating histones are mediators of trauma-associated lung injury. Am J Respir Crit Care Med. 2013;187(2):160–9.

Adam M, Robert F, Larochelle M, Gaudreau L. H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. Mol Cell Biol. 2001;21 (18):6270–9.

Allis D, Caparros ML, Jenuwein T, Reinberg D. Epigenetics. 2nd ed. Cold Spring Harbor, NY: Cold Spring Laboratory Press; 2015.

Ammar R, Torti D, Tsui K, Gebbia M, Durbic T, Bader GD, Giaever G, Nislow C. Chromatin is an ancient innovation conserved between Archaea and Eukarya. Elife. 2012;1:e00078.

Anastasiadi D, Díaz N, Piferrer F. Small Ocean temperature increases elicit stage-dependent changes in DNA methylation and gene expression in a fish, the European sea bass. Sci Rep. 2017;7(1):12401.

Aniagu SO, Williams TD, Allen Y, Katsiadaki I, Kevin Chipman J. Global genomic methylation levels in the liver and gonads of the three-spine stickleback (Gasterosteus Aculeatus) after exposure to hexabromocyclododecane and 17-beta oestradiol. Environ Int. 2008;34(3):310–7.

Araya I, Nardocci G, Jp M, Mi V, Molina A, Alvarez M. MacroH2A subtypes contribute antagonistically to the transcriptional regulation of the ribosomal cistron during seasonal acclimatization of the carp fish. Epigenetics Chromatin. 2010;3(1):14.

Ardura A, Zaiko A, Morán P, Planes S, Garcia-Vazquez E. Epigenetic signatures of invasive status in populations of marine invertebrates. Sci Rep. 2017;7:42193.

Artemov AV, Mugue NS, Rastorguev SM, Zhenilo S, Mazur AM, Tsygankova SV, Boulygina ES, et al. Genome-wide DNA methylation profiling reveals epigenetic adaptation of stickleback to marine and freshwater conditions. Mol Biol Evol. 2017;34(9):2203–13.

Artyomov MN, Meissner A, Chakraborty AK. A model for genetic and epigenetic regulatory networks identifies rare pathways for transcription factor induced Pluripotency. PLoS Comput Biol. 2010;6(5):e1000785.

Ausio J. Histone variants—the structure behind the function. Brief Funct Genomic Proteomic. 2006;5(3):228–43.

Baccarelli A, Bollati V. Epigenetics and environmental chemicals. Curr Opin Pediatr. 2009;21(2):243–51.

Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res. 2011;21(3):381–95.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR. Genomic basis for coral resilience to climate change. Proc Natl Acad Sci U S A. 2013;110(4):1387–92.

Bateson W. Heredity and variation in modern lights. In: Seward AC, editor. Darwin and modern science. Cambridge: Cambridge University Press; 1909. p. 85–101.

Bauden M, Pamart D, Ansari D, Herzog M, Eccleston M, Micallef J, Andersson B, Andersson R. Circulating nucleosomes as epigenetic biomarkers in pancreatic cancer. Clin Epigenetics. 2015;7:106.

Beaulieu M, Costantini D. Biomarkers of oxidative status: missing tools in conservation physiology. Conserv Physiol. 2014;2(1):cou014.

Bellantuono AJ, Granadoes-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M. Coral thermal tolerance: tuning gene expression to resist thermal stress. PLoS One. 2012;7(11): e50685. https://doi.org/10.1371/journal.pone.0050685.

Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002;16(1):6–21.

Bizuayehu TT, Babiak I. MicroRNA in teleost fish. Genome Biol Evol. 2014;6(8):1911–37.

Bizuayehu TT, Johansen SD, Puvanendran V, Toften H, Babiak I. Temperature during early development has long-term effects on microRNA expression in Atlantic cod. BMC Genomics. 2015;16:305.

Blevins T, Wang J, Pflieger D, Pontvianne F, Pikaard CS. Hybrid incompatibility caused by an epiallele. Proc Natl Acad Sci U S A. 2017;114(14):3702–7.

Boening DW. An Evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. Environ Monit Assess. 1999;55(3):459–70.

Boks MP, Derks EM, Weisenberger DJ, Strengman E, Janson E, Sommer IE, Kahn RS, Ophoff RA. The relationship of DNA methylation with age, gender and genotype in twins and healthy controls. PLoS One. 2009;4(8):e6767.

Bollati V, Baccarelli A. Environmental epigenetics. Heredity. 2010;105(1):105–12.

Burgerhout E, Mommens M, Johnsen H, Aunsmo A, Santi N, Andersen Ø. Genetic background and embryonic temperature affect DNA methylation and expression of Myogenin and muscle development in Atlantic salmon (Salmo salar). PLoS One. 2017;12(6):e0179918.

Burggren W. Epigenetic inheritance and its role in evolutionary biology: re-evaluation and new perspectives. Biology. 2016;5(2). https://doi.org/10.3390/biology5020024.

Burton GA, Allen Burton G, Johnston EL. Assessing contaminated sediments in the context of multiple stressors. Environ Toxicol Chem. 2010;29(12):2625–43.

Byun H-M, Panni T, Motta V, Hou L, Nordio F, Apostoli P, Bertazzi PA, Baccarelli AA. Effects of airborne pollutants on mitochondrial DNA methylation. Part Fibre Toxicol. 2013;10:18.

Cai W-J, Ma Y, Hopkinson BM, Grottoli AG, Warner ME, Ding Q, Xinping H, et al. Microelectrode characterization of coral daytime interior pH and carbonate chemistry. Nat Commun. 2016;7:11144.

Calmano W, Ahlf W, Förstner U. Sediment quality assessment: chemical and biological approaches. In: Calmano W, Förstner U, editors. Sediments and toxic substances: environmental effects and ecotoxity. Berlin: Springer; 1996. p. 1–35.

Campos C, Valente L, Conceição L, Engrola S, Fernandes J. Temperature affects methylation of themyogeninputative promoter, its expression and muscle cellularity in Senegalese sole larvae. Epigenetics. 2013;8(4):389–97.

Capuano F, Mülleder M, Kok R, Blom HJ, Ralser M. Cytosine DNA methylation is found in Drosophila Melanogaster but absent in Saccharomyces Cerevisiae, Schizosaccharomyces Pombe, and other yeast species. Anal Chem. 2014;86(8):3697–702.

Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136 (4):642–55.

Chen R, Kang R, Fan X-G, Tang D. Release and activity of histone in diseases. Cell Death Dis. 2014;5(8):e1370.

Chen B-S, Li C-W. Constructing an integrated genetic and epigenetic cellular network for whole cellular mechanism using high-throughput next-generation sequencing data. BMC Syst Biol. 2016;10:18.

Chown SL, Hodgins KA, Griffin PC, Oakeshott JG, Byrne M, Hoffmann AA. Biological invasions, climate change and genomics. Evol Appl. 2015;8(1):23–46.

Coleman-Derr D, Zilberman D. Deposition of histone variant H2A.Z within gene bodies regulates responsive genes. PLoS Genet. 2012;8(10):e1002988.

Cortessis VK, Thomas DC, Joan Levine A, Breton CV, Mack TM, Siegmund KD, Haile RW, Laird PW. Environmental epigenetics: prospects for studying epigenetic mediation of exposure-response relationships. Hum Genet. 2012;131(10):1565–89.

Cossetti C, Lugini L, Astrologo L, Saggio I, Fais S, Spadafora C. Soma-to-germline transmission of RNA in mice xenografted with human tumour cells: possible transport by exosomes. PLoS One. 2014;9(7):e101629.

Costantini D. Does hormesis foster organism resistance to extreme events? Front Ecol Environ. 2014;12(4):209–10.

Covelo-Soto L, Saura M, Morán P. Does DNA methylation regulate metamorphosis? The case of the sea lamprey (Petromyzon Marinus) as an example. Comp Biochem Physiol B Biochem Mol Biol. 2015;185:42–6.

Crespi EJ, Williams TD, Jessop TS, Delehanty B. Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? Funct Ecol. 2012;27 (1):93–106.

Cunning R, Silverstein RN, Baker AC. Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. Proc R Soc B. 2015;282(1809):20141725. https://doi.org/10.1098/rspb.2014.1725.

Deans C, Maggert KA. What do you mean, 'epigenetic'? Genetics. 2015;199(4):887–96.

Deaton AM, Bird A. CpG Islands and the regulation of transcription. Genes Dev. 2011;25 (10):1010–22.

Destoumieux-Garzón D, Rosa RD, Schmitt P, Barreto C, Vidal-Dupiol J, Mitta G, Gueguen Y, Bachère E. Antimicrobial peptides in marine invertebrate health and disease. Philos Trans R Soc Lond B Biol Sci. 2016;371(1695). https://doi.org/10.1098/rstb.2015.0300.

Dimond JL, Roberts SB. Germline DNA methylation in reef corals: patterns and potential roles in response to environmental change. Mol Ecol. 2016;25(8):1895–904.

Dixon GB, Bay LK, Matz MV. Bimodal signatures of germline methylation are linked with gene expression plasticity in the coral Acropora Millepora. BMC Genomics. 2014;15:1109.

Dobzhansky T. Studies on hybrid sterility. II. Localization of sterility factors in drosophila pseudoobscura hybrids. Genetics. 1936;21:113–35.

Eggleton J, Thomas KV. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ Int. 2004;30(7):973–80.

Eirín-López JM, Ausió J. Origin and evolution of chromosomal sperm proteins. Bioessays. 2009;31 (10):1062–70.

El-Maarri O, Becker T, Junen J, Manzoor SS, Diaz-Lacava A, Schwaab R, Wienker T, Oldenburg J. Gender specific differences in levels of DNA methylation at selected loci from human Total blood: a tendency toward higher methylation levels in males. Hum Genet. 2007;122(5):505–14.

Etchegaray JP, Mostoslavsky R. Interplay between metabolism and epigenetics: a nuclear adaptation to environmental changes. Mol Cell. 2016;62(5):695–711.

Fagagna F, Reaper PM, Clay-Farrace L, et al. A DNA damage checkpoint response in telomere-initiated senescence. Nature. 2003;426:194–8.

Farias ND, de Oliveira NFP, da Silva PM. Perkinsus infection is associated with alterations in the level of global DNA methylation of gills and gastrointestinal tract of the oyster Crassostrea gasar. J Invertebr Pathol. 2017;149:76–81.

Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet. 2012;13(2):97–109.

Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. Nat Rev Genet. 2006;7(1):21–33.

Fellous A, Favrel P, Riviere G. Temperature influences histone methylation and mRNA expression of the Jmj-C histone-demethylase orthologues during the early development of the oyster Crassostrea gigas. Mar Genomics. 2015;19:23–30.

Feng S, Cokus SJ, Zhang X, Chen P-Y, Bostick M, Goll MG, Hetzel J, et al. Conservation and divergence of methylation patterning in plants and animals. Proc Natl Acad Sci U S A. 2010;107 (19):8689–94.

Foo SA, Byrne M. Acclimatization and adaptive capacity of marine species in a changing ocean. Adv Mar Biol. 2016;74:69–116.

Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suñer D, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A. 2005;102 (30):10604–9.

Fraune S, Forêt S, Reitzel AM. Using Nematostella Vectensis to study the interactions between genome, epigenome, and bacteria in a changing environment. Front Mar Sci. 2016;3. https://doi.org/10.3389/fmars.2016.00148.

Gaffney PM. The role of genetics in shellfish restoration. Aquat Living Resour. 2006;19(3):277–82.

Gajigan AP, Conaco C. A microRNA regulates the response of corals to thermal stress. Mol Ecol. 2017;26(13):3472–83.

Galván I, Inácio Â, Romero-Haro AA, Alonso-Alvarez C. Adaptive downregulation of pheomelanin-related Slc7a11 gene expression by environmentally induced oxidative stress. Mol Ecol. 2017;26(3):849–58.

Garfield EN. Case studies in coral restoration: assessing life history and longterm survival patterns in restoration outplants of Acropora cervicornis (Staghorn Coral) and Acropora palmata (Elkhorn Coral) in the Florida Keys and Belize. ProQuest Dissertations Publishing. Boston University. 2016. Retrieved from https://search.proquest.com/docview/1845308002/fulltextPDF/62D313E87714E0FPQ/1?accountid=10901.

Gavery MR, Roberts SB. Predominant intragenic methylation is associated with gene expression characteristics in a bivalve Mollusc. PeerJ. 2013;1:e215.

Gavery MR, Roberts SB. Epigenetic considerations in aquaculture. PeerJ. 2017;5:e4147.

Gezer U, Yörüker EE, Keskin M, Kulle CB, Dharuman Y, Holdenrieder S. Histone methylation marks on circulating nucleosomes as novel blood-based biomarker in colorectal cancer. Int J Mol Sci. 2015;16(12):29654–62.

Gierga G, Voss B, Hess WR. Non-coding RNAs in marine Synechococcus and their regulation under environmentally relevant stress conditions. ISME J. 2012;6(8):1544–57.

Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 2005;74 (1):481–514.

González-Romero R, Rivera-Casas C, Fernández-Tajes J, Ausió J, Méndez J, Eirín-López JM. Chromatin specialization in bivalve molluscs: a leap forward for the evaluation of okadaic acid genotoxicity in the marine environment. Comp Biochem Physiol Toxicol Pharmacol. 2012a;155(2):175–81.

González-Romero R, Rivera-Casas C, Frehlick LJ, Méndez J, Ausió J, Eirín-López JM. Histone H2A (H2A.X and H2A.Z) variants in molluscs: molecular characterization and potential implications for chromatin dynamics. PLoS One. 2012b;7(1):e30006. Public Library of Science

González-Romero R, Suarez-Ulloa V, Rodriguez-Casariego J, Garcia-Souto D, Diaz G, Smith A, Pasantes JJ, Rand G, Eirin-Lopez JM. Effects of Florida Red Tides on histone variant expression and DNA methylation in the eastern oyster Crassostrea Virginica. Aquat Toxicol. 2017;186:196–204.

Greco M, Chiappetta A, Bruno L, Bitonti MB. Effects of light deficiency on genome methylation in Posidonia Oceanica. Mar Ecol Prog Ser. 2013;473:103–14.

Gu M, Naiyachit Y, Wood TJ, Millar CB. H2A.Z marks antisense promoters and has positive effects on antisense transcript levels in budding yeast. BMC Genomics. 2015;16(1):99.

Guerrero-Bosagna C, Sabat P, Valladares L. Environmental signaling and evolutionary change: can exposure of pregnant mammals to environmental estrogens lead to epigenetically induced evolutionary changes in embryos? Evol Dev. 2005;7(4):341–50.

Hannum G, Guinney J, Zhao L, Li Z, Hughes G, Sadda S, Klotzle B, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell. 2013;49 (2):359–67.

Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL. The impacts of climate change in coastal marine systems. Ecol Lett. 2006;9(2):228–41.

Hauser M-T, Aufsatz W, Jonak C, Luschnig C. Transgenerational epigenetic inheritance in plants. Biochim Biophys Acta. 2011;1809(8):459–68.

Hedrick PW, Lacy RC. Measuring relatedness between inbred individuals. J Hered. 2015;106 (1):20–5.

Henikoff S, Ahmad K. Assembly of variant histones into chromatin. Annu Rev Cell Dev Biol. 2005;21(1):133–53.

Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med. 2003;349(21):2042–54.

Hernandez-Agreda A, Leggat W, Bongaerts P, Ainsworth TD. The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. MBio. 2016;7(4):e00560–16. https://doi.org/10.1128/mBio.00560-16.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, et al. Coral reefs under rapid climate change and ocean acidification. Science. 2007;318 (5857):1737–42.

Hofmann G. Ecological epigenetics in marine metazoans. Front Mar Sci. 2017. https://doi.org/10.3389/fmars.2017.00004.

Holoch D, Moazed D. RNA-mediated epigenetic regulation of gene expression. Nat Rev Genet. 2015;16(2):71–84.

Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115.

Huang D, Zhang Y, Qi Y, Chen C, Ji W. Global DNA hypomethylation, rather than reactive oxygen species (ROS), a potential facilitator of cadmium-stimulated K562 cell proliferation. Toxicol Lett. 2008;179(1):43–7.

Huh I, Zeng J, Park T, Soojin VY. DNA methylation and transcriptional noise. Epigenetics Chromatin. 2013;6(1):9.

Hume BCC, Voolstra CR, Arif C, D'Angelo C, Burt JA, Eyal G, Loya Y, Wiedenmann J. Ancestral genetic diversity associated with the rapid spread of stress-tolerant coral symbionts in response to Holocene climate change. Proc Natl Acad Sci U S A. 2016;113(16):4416–21.

Huo D, Sun L, Li X, Xiaoshang R, Liu S, Zhang L, Xing L, Yang H. Differential expression of miRNAs in the respiratory tree of the sea cucumber Apostichopus Japonicus under hypoxia stress. G3. 2017;7(11):3681–92.

Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003;33(Suppl):245–54.

Jarman SN, Polanowski AM, Faux CE, Robbins J, De Paoli-Iseppi R, Bravington M, Deagle BE. Molecular biomarkers for chronological age in animal ecology. Mol Ecol. 2015;24(19):4826–47.

Jiao Y, Zheng Z, Xiaodong D, Wang Q, Huang R, Deng Y, Shi S, Zhao X. Identification and characterization of microRNAs in pearl oyster Pinctada martensii by Solexa deep sequencing. Marine Biotechnol. 2014;16(1):54–62.

Joh RI, Palmieri CM, Hill IT, Motamadi M. Regulation of histone methylation by noncoding RNAs. Biochim Biophys Acta. 2014;1839(12):1385–94.

Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012;13(7):484–92.

Jones TA, Monaco TA. A role for assisted evolution in designing native plant materials for domesticated landscapes. Front Ecol Environ. 2009;7(10):541–7.

Juanchich A, Le Cam A, Montfort J, Guiguen Y, Bobe J. Identification of differentially expressed miRNAs and their potential targets during fish ovarian development. Biol Reprod. 2013;88(5):128.

Kasinsky E, Harold HEK, Eirin-Lopez JM, Ausio J. Protamines: structural complexity, evolution and chromatin patterning. Protein Pept Lett. 2011;18(8):755–71.

Kelly SA, Panhuis TM, Stoehr AM. Phenotypic plasticity: molecular mechanisms and adaptive significance. Compr Physiol. 2012;2(2):1417–39.

Kim B-M, Kim J, Choi I-Y, Raisuddin S, Doris WTA, Leung KMY, Rudolf SSW, Rhee J-S, Lee J-S. Omics of the marine medaka (Oryzias melastigma) and its relevance to marine environmental research. Mar Environ Res. 2016;113:141–52.

Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci. 2006;31(2):89–97.

Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693–705.

Kuhlmann M, Finke A, Mascher M, Mette MF. DNA methylation maintenance consolidates RNA-directed DNA methylation and transcriptional gene silencing over generations in Arabidopsis thaliana. Plant J. 2014;80(2):269–81.

Kumar SV, Wigge PA. H2A.Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell. 2010;140(1):136–47.

Lahaye V, Bustamante P, Dabin W, Van Canneyt O, Dhermain F, Cesarini C, Pierce GJ, Caurant F. New insights from age determination on toxic element accumulation in striped and bottlenose dolphins from Atlantic and Mediterranean waters. Mar Pollut Bull. 2006;52(10):1219–30.

Lämke J, Bäurle I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol. 2017;18(1):124.

Lascelles B, Di Sciara NG, Agardy T, Cuttelod A, Eckert S, Glowka L, Hoyt E, Llewellyn F, Louzao M, Ridoux V, Tetley MJ. Migratory marine species: their status, threats and conservation management needs. Aquat Conserv. 2014. https://doi.org/10.1002/aqc.2512.

Latimer J, Davis W, Keith D. Mobilization of PAHs and PCBs from in-place contaminated marine sediments during simulated resuspension events. Estuar Coast Shelf Sci. 1999;49:577–95.

Lau K, Lai KP, Bao JYJ, Na Z, Tse A, Tong A, Li JW, et al. Identification and expression profiling of microRNAs in the brain, liver and gonads of marine Medaka (Oryzias Melastigma) and in response to hypoxia. PLoS One. 2014;9(10):e110698.

Lee T-F, Zhai J, Meyers BC. Conservation and divergence in eukaryotic DNA methylation. Proc Natl Acad Sci. 2010;107(20):9027–8.

Li E, Zhang Y. DNA methylation in mammals. Cold Spring Harb Perspect Biol. 2014;6(5): a019133.

Li A, Eirín-López JM, Ausió J. H2AX: tailoring histone H2A for chromatin-dependent genomic integrity. Biochem Cell Biol. 2005;83(4):505–15.

Li J-W, Lin X, Tse A, Cheung A, Chan TF, Kong RYC, Lai KP, Rudolf Shiu Sun W. Discovery and functional characterization of novel miRNAs in the marine medaka Oryzias melastigma. Aquat Toxicol. 2016;175:106–16.

Li S, He F, Wen H, Li J, Si Y, Liu M, Huang Y, Meng L. Low salinity affects cellularity, DNA methylation, and mRNA expression of igf1 in the liver of half smooth tongue sole (Cynoglossus semilaevis). Fish Physiol Biochem. 2017;43(6):1587–602.

Liew YJ, Aranda M, Carr A, Baumgarten S, Zoccola D, Tambutté S, Allemand D, Micklem G, Voolstra CR. Identification of microRNAs in the coral Stylophora pistillata. PLoS One. 2014;9(3):e91101.

Liew YJ, Ryu T, Aranda M, Ravasi T. Correction: miRNA Repertoires of Demosponges Stylissa carteri and Xestospongia Testudinaria. PLoS One. 2016;11(4):e0153731.

Lin S, Cheng S, Bo S, Zhong X, Lin X, Li W, Li L, et al. The Symbiodinium kawagutii genome illuminates dinoflagellate gene expression and coral symbiosis. Science. 2015;350(6261):691–4.

Lowdon RF, Jang HS, Wang T. Evolution of epigenetic regulation in vertebrate genomes. Trends Genet. 2016;32(5):269–83.

Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature. 1997;389(6648):251–60.

Luger K, Dechassa ML, Tremethick DJ. New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? Nat Rev Mol Cell Biol. 2012;13(7):436–47.

Lynch M, Force A. The probability of duplicate gene preservation by subfunctionalization. Genetics. 2000;154(1):459–73.

Magistri M, Faghihi MA, Laurent GS 3rd, Wahlestedt C. Regulation of chromatin structure by long noncoding RNAs: focus on natural antisense transcripts. Trends Genet. 2012;28(8):389–96.

Malik HS, Henikoff S. Phylogenomics of the nucleosome. Nat Struct Biol. 2003;10(11):882–91.

Marsh AG, Pasqualone AA. DNA methylation and temperature stress in an Antarctic polychaete, Spiophanes tcherniai. Front Physiol. 2014;5:173.

Marsh AG, Hoadley KD, Warner ME. Distribution of CpG motifs in upstream gene domains in a reef coral and sea Anemone: implications for epigenetics in cnidarians. PLoS One. 2016;11(3): e0150840.

Mashburn KL, Atkinson S. Evaluation of adrenal function in serum and feces of Steller sea lions (Eumetopias Jubatus): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. Gen Comp Endocrinol. 2004;136(3):371–81.

Matsumoto Y, Buemio A, Chu R, Vafaee M, Crews D. Epigenetic control of gonadal aromatase (cyp19a1) in temperature-dependent sex determination of red-eared slider turtles. PLoS One. 2013;8(6):e63599. https://doi.org/10.1371/journal.pone.0063599.

McAnena P, Brown J, Kerin M. Circulating nucleosomes and nucleosome modifications as biomarkers in cancer. Cancer. 2017;9(1):5.

Mercado-Molina AE, Ruiz-Diaz CP, Sabat AM. Demographics and dynamics of two restored populations of the threatened reef-building coral Acropora cervicornis. J Nat Conserv. 2015;24:17–23.

Metzger DCH, Schulte PM. Epigenomics in marine fishes. Mar Genomics. 2016;30:43–54.

Miller JR, Hobbs RJ. Habitat restoration? Do we know what we? Re doing? Restor Ecol. 2007;15 (3):382–90.

Mirbahai L, Chipman JK. Epigenetic memory of environmental organisms: a reflection of lifetime stressor exposures. Mutat Res Genet Toxicol Environ Mutagen. 2014;764–765:10–7.

Mirbahai L, Williams TD, Zhan H, Gong Z, Kevin Chipman J. Comprehensive profiling of zebrafish hepatic proximal promoter CpG Island methylation and its modification during chemical carcinogenesis. BMC Genomics. 2011a;12:3.

Mirbahai L, Yin G, Bignell JP, Li N, Williams TD, Chipman JK. DNA methylation in liver tumorigenesis in fish from the environment. Epigenetics. 2011b;6(11):1319–33.

Mirbahai L, Southam AD, Sommer U, Williams TD, Bignell JP, Lyons BP, Viant MR, Chipman JK. Disruption of DNA methylation via S-adenosylhomocysteine is a key process in high incidence liver carcinogenesis in fish. J Proteome Res. 2013;12(6):2895–904.

Morán P, Pérez-Figueroa A. Methylation changes associated with early maturation stages in the Atlantic salmon. BMC Genet. 2011;12:86.

Moran Y, Fredman D, Praher D, Li XZ, Wee LM, Rentzsch F, Zamore PD, Technau U, Seitz H. Cnidarian microRNAs frequently regulate targets by cleavage. Genome Res. 2014;24 (4):651–63.

Morgan HD, Santos F, Green K, Dean W, Reik W. Epigenetic reprogramming in mammals. Hum Mol Genet. 2005;14(Suppl 1):R47–58.

Mudbhary R, Sadler KC. Epigenetics, development, and cancer: zebrafish make their mark. Birth Defects Res C Embryo Today. 2011;93(2):194–203.

Muller HJ. Isolating mechanisms, evolution and temperature. Biol Symp. 1942;6:71–125.

Myers RA, Barrowman NJ, Hutchings JA, Rosenberg AA. Population dynamics of exploited fish stocks at low population levels. Science. 1995;269(5227):1106–8.

Navarro-Martín L, Viñas J, Ribas L, Díaz N, Gutiérrez A, Di Croce L, Piferrer F. DNA methylation of the gonadal aromatase (cyp19a) promoter is involved in temperature-dependent sex ratio shifts in the European Sea bass. PLoS Genet. 2011;7(12):e1002447.

Norouzitallab P, Baruah K, Vandegehuchte M, Van Stappen G, Catania F, Vanden Bussche J, Vanhaecke L, Sorgeloos P, Bossier P. Environmental heat stress induces epigenetic transgenerational inheritance of robustness in parthenogenetic Artemia model. FASEB J. 2014;28(8):3552–63.

Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99(3):247–57.

Okubo N, Omori M. The review of coral transplantation around the world. J Jpn Coral Reef Soc. 2001;2001(3):31–40.

Olsen MT, Bérubé M, Robbins J, Palsbøll PJ. Empirical evaluation of humpback whale telomere length estimates; quality control and factors causing variability in the Singleplex and multiplex qPCR methods. BMC Genet. 2012;13:77.

Ooi SKT, Qiu C, Bernstein E, Li K, Da J, Yang Z, Erdjument-Bromage H, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature. 2007;448 (7154):714–7.

van Oppen MJH, Gates RD, Blackall LL, Cantin N, Chakravarti LJ, Chan WY, Cormick C, et al. Shifting paradigms in restoration of the world's coral reefs. Glob Chang Biol. 2017;23 (9):3437–48.

Palazzo AF, Lee ES. Non-coding RNA: what is functional and what is junk? Front Genet. 2015;6. https://doi.org/10.3389/fgene.2015.00002.

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA. Mechanisms of reef coral resistance to future climate change. Science. 2014;344(6186):895–8.

Parodi F, Carosio R, Ragusa M, Di Pietro C, Maugeri M, Barbagallo D, Sallustio F, et al. Epigenetic dysregulation in neuroblastoma: a tale of miRNAs and DNA methylation. Biochim Biophys Acta. 2016;1859(12):1502–14.

Patat SA, Carnegie RB, Kingsbury C, Gross PS, Chapman R, Schey KL. Antimicrobial activity of histones from hemocytes of the Pacific white shrimp. Eur J Biochem. 2004;271(23–24):4825–33.

Peat JR, Ortega-Recalde O, Kardailsky O, Hore TA. The elephant shark methylome reveals conservation of epigenetic regulation across jawed vertebrates. F1000Res. 2017;6:526.

Pérez JE, Nirchio M, Alfonsi C, Muñoz C. The biology of invasions: the genetic adaptation paradox. Biol Invasions. 2006;8(5):1115–21.

Perrin WF, Myrick AC. Age determination of toothed whales and sirenians. Vol. 3, Issue 3. Cambridge: International Whaling Commission: Special Issue. Print; 1980.

Picone B, Rhode C, Roodt-Wilding R. Identification and characterization of miRNAs transcriptome in the South African abalone, Haliotis midae. Mar Genomics. 2017;31:9–12.

Pierron F, Baillon L, Sow M, Gotreau S, Gonzalez P. Effect of low-dose cadmium exposure on DNA methylation in the endangered European eel. Environ Sci Technol. 2014;48(1):797–803.

Pogribny IP. Epigenetic events in tumorigenesis: putting the pieces together. Exp Oncol. 2010;32(3):132–6.

Poirier AC, Schmitt P, Rosa RD, Vanhove AS, Kieffer-Jaquinod S, Rubio TP, Charrière GM, Destoumieux-Garzón D. Antimicrobial histones and DNA traps in invertebrate immunity. J Biol Chem. 2014;289(36):24821–31.

Polanowski AM, Robbins J, Chandler D, Jarman SN. Epigenetic estimation of age in humpback whales. Mol Ecol Resour. 2014;14(5):976–87.

Poloczanska ES, Burrows MT, Brown CJ, Molinos JG, Halpern BS, Hoegh-Guldberg O, Kappel CV, et al. Responses of marine organisms to climate change across oceans. Front Mar Sci. 2016;3. https://doi.org/10.3389/fmars.2016.00062.

Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol. 2010;28(10):1057–68.

Pu C, Zhan A. Epigenetic divergence of key genes associated with water temperature and salinity in a highly invasive model ascidian. Biol Invasions. 2017;19(7):2015–28.

Putnam HM, Gates RD. Preconditioning in the reef-building coral Pocillopora Damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. J Exp Biol. 2015;218(Pt 15):2365–72.

Putnam HM, Davidson JM, Gates RD. Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. Evol Appl. 2016;9(9):1165–78.

Rando OJ, Verstrepen KJ. Timescales of genetic and epigenetic inheritance. Cell. 2007;128(4):655–68.

Reddy PC, Ubhe S, Sirwani N, Lohokare R, Galande S. Rapid divergence of histones in Hydrozoa (Cnidaria) and evolution of a novel histone involved in DNA damage response in hydra. Zoology. 2017;123:53–63.

Reichard JF, Schnekenburger M, Puga A. Long term low-dose arsenic exposure induces loss of DNA methylation. Biochem Biophys Res Commun. 2007;352(1):188–92.

Rivera-Casas C, González-Romero R, Cheema MS, Ausió J, Eirín-López JM. The characterization of macroH2A beyond vertebrates supports an ancestral origin and conserved role for histone variants in chromatin. Epigenetics. 2016a;11(6):415–25.

Rivera-Casas C, González-Romero R, Vizoso-Vazquez Á, Cheema MS, Esperanza Cerdán M, Méndez J, Ausió J, Eirin-Lopez JM. Characterization of mussel H2A.Z.2: a new H2A.Z variant preferentially expressed in germinal tissues from Mytilus. Biochem Cell Biol. 2016b;94 (5):480–90.

Rivera-Casas C, González-Romero R, Garduño RA, Cheema MS, Ausio J, Eirin-Lopez JM. Molecular and biochemical methods useful for the epigenetic characterization of chromatin-associated proteins in bivalve molluscs. Front Physiol. 2017;8:490.

Riviere G, Guan-Chung W, Fellous A, Goux D, Sourdaine P, Favrel P. DNA methylation is crucial for the early development in the oyster C. Gigas. Marine Biotechnol. 2013;15(6):739–53.

Riviere G, He Y, Tecchio S, Crowell E, Gras M, Sourdaine P, Guo X, Favrel P. Dynamics of DNA methylomes underlie oyster development. PLoS Genet. 2017;13(6):e1006807.

Roberts SB, Gavery MR. Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates? Front Physiol. 2012;2. https://doi.org/10.3389/fphys.2011.00116.

Rosic N, Kaniewska P, Chan C-KK, Ling EYS, Edwards D, Dove S, Hoegh-Guldberg O. Early transcriptional changes in the reef-building coral Acropora Aspera in response to thermal and nutrient stress. BMC Genomics. 2014;15:1052.

Sano H, Kim H-J. Transgenerational epigenetic inheritance in plants. In: Grafi G, Ohad N, editors. Epigenetic memory and control in plants. Signaling and communication in plants, vol. 18. Berlin, Heidelberg: Springer; 2013. p. 233–53.

Sathyan N, Philip R, Chaithanya ER, Anil Kumar PR. Identification and molecular characterization of Molluskin, a histone-H2A-derived antimicrobial peptide from molluscs. ISRN Mol Biol. 2012;2012:1–6.

Seaman W. Artificial habitats and the restoration of degraded marine ecosystems and fisheries. Hydrobiologia. 2007;580(1):143–55.

Seddon PJ, Armstrong DP, Maloney RF. Developing the science of reintroduction biology. Conserv Biol. 2007;21(2):303–12.

Segawa T, Kobayashi Y, Inamoto S, Suzuki M, Endoh T, Itou T. Identification and expression profiles of microRNA in dolphin. Zoolog Sci. 2016;33(1):92–7.

Sgrò CM, Terblanche JS, Hoffmann AA. What can plasticity contribute to insect responses to climate change? Annu Rev Entomol. 2016;61:433–51.

Shao C, Li Q, Chen S, Zhang P, Lian J, Qiaomu H, Sun B, et al. Epigenetic modification and inheritance in sexual reversal of fish. Genome Res. 2014;24(4):604–15.

Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010;31(1):27–36.

Shukla S, Kavak E, Gregory M, Imashimizu M, Shutinoski B, Kashlev M, Oberdoerffer P, Sandberg R, Oberdoerffer S. CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. Nature. 2011;479(7371):74–9.

Siklenka K, Erkek S, Godmann M, Lambrot R, McGraw S, Lafleur C, Cohen T, Xia J, Hallett M, Trasler J, Peters AH, Kimmins S. Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. Science. 2015;350(6261):aab2006. https://doi.org/10.1126/science.aab2006.

Simonet NG, Reyes M, Nardocci G, Molina A, Alvarez M. Epigenetic regulation of the ribosomal Cistron seasonally modulates enrichment of H2A.Z and H2A.Zub in response to different environmental inputs in carp (Cyprinus Carpio). Epigenetics Chromatin. 2013;6(1):22.

Skjærven KH, Hamre K, Penglase S, Finn RN, Olsvik PA. Thermal stress alters expression of genes involved in one carbon and DNA methylation pathways in Atlantic cod embryos. Comp Biochem Physiol A Mol Integr Physiol. 2014;173C:17–27.

Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nat Rev Genet. 2013;14(3):204–20.

Smith VJ, Desbois AP, Dyrynda EA. Conventional and unconventional antimicrobials from fish, marine invertebrates and micro-algae. Mar Drugs. 2010;8(4):1213–62.

Song K, Li L, Zhang G. The association between DNA methylation and exon expression in the Pacific oyster Crassostrea gigas. PLoS One. 2017;12(9):e0185224.

Stuart-Smith J, Jepson P. Persistent threats need persistent counteraction: responding to PCB pollution in marine mammals. Mar Policy. 2017;84:69–75.

Suárez-Ulloa V, Fernández-Tajes J, Aguiar-Pulido V, Rivera-Casas C, González-Romero R, Ausio J, Méndez J, Dorado J, Eirín-López JM. The CHROMEVALOA database: a resource for the evaluation of Okadaic acid contamination in the marine environment based on the chromatin-associated transcriptome of the mussel Mytilus galloprovincialis. Mar Drugs. 2013;11(3):830–41.

Suarez-Ulloa V, González-Romero R, Eirin-Lopez JM. Environmental epigenetics: a promising venue for developing next-generation pollution biomonitoring tools in marine invertebrates. Mar Pollut Bull. 2015;98(1–2):5–13.

Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet. 2008;9(6):465–76.
Suzuki MM, Kerr ARW, De Sousa D, Bird A. CpG methylation is targeted to transcription units in an invertebrate genome. Genome Res. 2007;17(5):625–31.
Sved J, Bird A. The expected equilibrium of the CpG dinucleotide in vertebrate genomes under a mutation model. Proc Natl Acad Sci U S A. 1990;87(12):4692–6.
Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324(5929):930–5.
Talbert PB, Henikoff S. Environmental responses mediated by histone variants. Trends Cell Biol. 2014;24(11):642–50.
Tanabe S, Mori T, Tatsukawa R, Miyazaki N. Global pollution of marine mammals by PCBs, DDTs, and HCHs (BHCs). Chemosphere. 1983;12:1269–75.
Taylor BL, Rojas-Bracho L, Moore J, Jaramillo-Legorreta A, Ver Hoef JM, Cardenas-Hinojosa G, Nieto-Garcia E, et al. Extinction is imminent for Mexico's endemic porpoise unless fishery bycatch is eliminated. Conserv Lett. 2016;10(5):588–95.
Towle EK, Baker AC, Langdon C. Preconditioning to high CO2 exacerbates the response of the Caribbean branching coral Porites Porites to high temperature stress. Mar Ecol Prog Ser. 2016;546:75–84.
Trautner JH, Reiser S, Blancke T, Unger K, Wysujack K. Metamorphosis and transition between developmental stages in European eel (Anguilla Anguilla, L.) involve epigenetic changes in DNA methylation patterns. Comp Biochem Physiol Part D Genomics Proteomics. 2017;22:139–45.
Tricker PJ. Transgenerational inheritance or resetting of stress-induced epigenetic modifications: two sides of the same coin. Front Plant Sci. 2015;6:699.
Tse AC-K, Li J-W, Wang SY, Chan T-F, Lai KP, Rudolf Shiu-Sun W. Hypoxia alters testicular functions of marine medaka through microRNAs regulation. Aquat Toxicol. 2016;180:266–73.
Turner BM. Histone acetylation and an epigenetic code. Bioessays. 2000;22(9):836–45.
Tweedie S, Charlton J, Clark V, Bird A. Methylation of genomes and genes at the invertebrate-vertebrate boundary. Mol Cell Biol. 1997;17(3):1469–75.
van Holde KE. Chromatin. Springer series in molecular biology. New York, NY: Springer; 1989.
Vandegehuchte MB, Kyndt T, Vanholme B, Haegeman A, Gheysen G, Janssen CR. Occurrence of DNA methylation in Daphnia magna and influence of multigeneration Cd exposure. Environ Int. 2009;35(4):700–6.
Varriale A, Bernardi G. DNA methylation and body temperature in fishes. Gene. 2006;385:111–21.
Verhoeven KJF, vonHoldt BM, Sork VL. Epigenetics in ecology and evolution: what we know and what we need to know. Mol Ecol. 2016;25(8):1631–8.
Vidigal JA, Ventura A. The biological functions of miRNAs: lessons from in vivo studies. Trends Cell Biol. 2015;25(3):137–47.
Vignet C, Joassard L, Lyphout L, Guionnet T, Goubeau M, Le Menach K, Brion F, et al. Exposures of zebrafish through diet to three environmentally relevant mixtures of PAHs produce behavioral disruptions in unexposed F1 and F2 descendant. Environ Sci Pollut Res Int. 2015;22(21):16371–83.
Viricel A, Rosel PE. Hierarchical population structure and habitat differences in a highly mobile marine species: the Atlantic spotted dolphin. Mol Ecol. 2014;23(20):5018–35.
Waddington CH. The epigenotype. Endeavour. 1942;1:18–20.
Wan Y, Saleem RA, Ratushny AV, Roda O, Smith JJ, Lin C-H, Chiang J-H, Aitchison JD. Role of the histone variant H2A.Z/Htz1p in TBP recruitment, chromatin dynamics, and regulated expression of oleate-responsive genes. Mol Cell Biol. 2009;29(9):2346–58.
Wang Y, Wang C, Zhang J, Chen Y, Zuo Z. DNA hypomethylation induced by tributyltin, triphenyltin, and a mixture of these in Sebastiscus marmoratus liver. Aquat Toxicol. 2009;95(2):93–8.
Warner DA, Uller T, Shine R. Transgenerational sex determination: the embryonic environment experienced by a male affects offspring sex ratio. Sci Rep. 2013;3:2709.

Wu L, Zhou H, Zhang Q, Zhang J, Ni F, Liu C, Qi Y. DNA methylation mediated by a microRNA pathway. Mol Cell. 2010;38(3):465–75.

Xu F, Wang X, Feng Y, Huang W, Wang W, Li L, Fang X, Que H, Zhang G. Identification of conserved and novel microRNAs in the Pacific oyster Crassostrea gigas by deep sequencing. PLoS One. 2014;9(8):e104371.

Yang J, Huang T, Petralia F, Long Q, Zhang B, Argmann C, Zhao Y, et al. Synchronized age-related gene expression changes across multiple tissues in human and the link to complex diseases. Sci Rep. 2015;5:15145.

Yauk C, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, Van Schooten FJ, Berndt ML, et al. Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. Proc Natl Acad Sci. 2008;105 (2):605–10.

Zemach A, McDaniel IE, Silva P, Zilberman D. Genome-wide evolutionary analysis of eukaryotic DNA methylation. Science. 2010;328(5980):916–9.

Zhang R, Zhang L, Wenqiang Y. Genome-wide expression of non-coding RNA and global chromatin modification: figure 1. Acta Biochim Biophys Sin. 2011;44(1):40–7.

Zhang X, Li H, Burnett JC, Rossi JJ. The role of antisense long noncoding RNA in small RNA-triggered gene activation. RNA. 2014;20(12):1916–28.

Zhang J, Li S, Li L, Li M, Guo C, Yao J, Mi S. Exosome and exosomal microRNA: trafficking, sorting, and function. Genomics Proteomics Bioinformatics. 2015;13(1):17–24.

Zhao Y, Sun H, Wang H. Long noncoding RNAs in DNA methylation: new players stepping into the old game. Cell Biosci. 2016a;6:45. https://doi.org/10.1186/s13578-016-0109-3.

Zhao X, Yu H, Kong L, Liu S, Li Q. High throughput sequencing of small RNAs transcriptomes in two Crassostrea oysters identifies microRNAs involved in osmotic stress response. Sci Rep. 2016b;6:22687.

Zilberman D, Coleman-Derr D, Ballinger T, Henikoff S. Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. Nature. 2008;456(7218):125–9.

Part VI
Protection, Conservation, and Management of Marine Organisms

Marine Invasion Genomics: Revealing Ecological and Evolutionary Consequences of Biological Invasions

S. D. Bourne, J. Hudson, L. E. Holman, and M. Rius

Abstract Genomic approaches are increasingly being used to study biological invasions. Here, we first analyse how high-throughput sequencing has aided our understanding of the mechanisms associated with biological invasions. These include the transport of propagules to pre-invaded areas, an exploration of the consequences of hybridisation during range expansions, and the pre- and post-invasion adaptation of colonising populations. We then explore how contemporary genomic methods have been used to probe and monitor the spread of non-indigenous species. More specifically, we focus on the detection of species richness from environmental samples, measures of quantitative traits that may promote invasiveness, analysis of rapid adaptation, and the study of phenotypic plasticity. Finally, we look to the future, exploring how genomic approaches will assist future biodiversity conservationists in their efforts to mitigate the spread and effects of biological invasions. Ultimately, although the use of genomic tools to study non-indigenous species has so far been rather limited, studies to date indicate that genomic tools offer unparalleled research opportunities to continually improve our understanding of marine biological invasions.

Keywords Adaptation · eDNA · Environmental DNA · Hybridisation · Nonindigenous species · Population genomics · Propagule pressure · Transcriptomics

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1 Introduction

Anthropogenic activities are increasingly affecting global biodiversity patterns (Halpern et al. 2008; Williams et al. 2015; Waters et al. 2016), with artificial transport of species away from their native ranges contributing to this change (Simberloff 2013; Boivin et al. 2016). This anthropogenic translocation of species is enhancing the presence of new colonisations by non-indigenous species (NIS) worldwide (Carlton 1999; Mead et al. 2011; Ricciardi et al. 2017; Seebens et al. 2017). In the last few decades, global trade has exponentially increased (Hulme 2009) and with this, marine shipping (Corbett and Winebrake 2008; IMO 2012), which has grown fourfold in the past 25 years (Tournadre 2014). Shipping acts as a vector for marine NIS that are often transported in ballast water tanks (which can carry up to 10,000 species at any one time; Carlton 1999), on ship hulls (Minchin and Gollasch 2003; Drake and Lodge 2007), or inside sea chests (Frey et al. 2014). Further significant vectors include aquaculture (Naylor et al. 2001; Molnar et al. 2008), the opening of artificial channels such as the Suez Canal (Golani and Ben-Tuvia 1989; Golani 1993), and the aquarium species trade (Padilla and Williams 2004). These vectors have been so effective that only 16% of marine ecoregions are recorded as unaffected by NIS (Molnar et al. 2008). NIS are responsible for major changes in the composition and structure of marine ecosystems (Ehrenfeld 2010; Ricciardi and MacIsaac 2010), as well as causing severe impacts on regional and global economies (Pimentel et al. 2005; Williams et al. 2010). This creates an urgent need to understand the phenotypic and genetic attributes that enable their global spread and success in novel ranges.

The publication of *The Genetics of Colonizing Species* (Baker and Stebbins 1965) is considered the beginning of the field of invasion genetics (Barrett 2015). Since then research in the field has been promoted by a dramatic decrease in the cost of DNA sequencing over recent years (NHGRI 2016), driving a rapid growth in the number of studies utilising genetic tools to study marine biological invasions (Rius et al. 2015a). As genetic approaches have recently progressed into more comprehensive genome-wide techniques (Rius et al. 2015b; Viard et al. 2016), researchers are increasingly using a diverse range of genomic tools to study marine biological invasions (Jombart 2008; Zheng et al. 2012; Catchen et al. 2013; Reitzel et al. 2013; Pfeifer et al. 2014). Macro-scale population processes such as connectivity and spread are now being assessed using genomic tools (Wagner et al. 2013; Vera et al. 2016; Narum et al. 2017), as well as studies on adaptation (Stapley et al. 2010, 2015), including marine NIS (Richardson and Sherman 2015; Tepolt 2015; Tepolt and Palumbi 2015; Bernardi et al. 2016; Wellband and Heath 2017). Additionally, new genetic tools allow the genomic analyses of previously-understudied taxa. Genomic studies have been traditionally restricted to the study of model organisms, which represent only a small fraction of total global biodiversity (Sullivan 2015). Recent technological advances have opened up genome-wide analyses to non-model organisms (Ekblom and Galindo 2011; Reitzel et al. 2013; da Fonseca et al. 2016) which are now routinely studied without prior knowledge of reference

genome data (Elshire et al. 2011; Catchen et al. 2013). This increasing accessibility of genomics allows invasion biologists to address a wider set of research questions on any type of taxa.

Here we review current progress in genomic studies of NIS, highlighting studies that use genomic approaches to better understand the mechanisms ruling marine biological invasions. We first focus on how genetic and genomic techniques assist researchers in exploring key mechanisms driving biological invasions, including pre- and post-invasion adaptation and hybridisation of NIS. We then show how methods that invasion biologists use to study NIS have been enhanced by the application of genomic techniques. These include methods to improve early detection of NIS, as well as detailed population-level analyses of NIS. Finally, we show that although the uptake of genomic tools to investigate marine biological invasions has been limited, their recent use to study both aquatic and terrestrial ecosystems suggests that they have great potential for future studies focusing on marine biological invasions.

2 Mechanisms Associated with Biological Invasions

The introduction of species away from their native ranges has been extensively discussed in the literature over the past decade (Rossman 2001; Sakai et al. 2001; Hulme 2009; Lowry et al. 2013), with much work attempting to characterise the invasion process into distinct stages (Williamson 1993; Colautti and MacIsaac 2004; Catford et al. 2009; Blackburn et al. 2011; Tsoar et al. 2011). It is now generally accepted that biological invasions undergo four different stages: transport, introduction, establishment, and spread (Fig. 1). The process begins with survival during transport of NIS; followed by introduction of species and their propagules into a novel habitat, with some individuals establishing to form a small population; and finally thriving to form a self-sustaining population that may spread and cause impacts (Richardson et al. 2000). NIS populations can undergo a lag phase of variable duration (Crooks et al. 1999), which may influence the genetic composition of introduced populations (Gaither et al. 2012). In the following sections we will briefly present the importance of the mechanisms associated with biological invasions considering the different invasion stages.

2.1 Propagule Pressure

The number of individuals introduced to a novel environment (propagule size) and the total number of introduction events (propagule number) are important factors determining the invasion success of an incipient population (Roman and Darling 2007; Simberloff 2009; Rius and Darling 2014). Propagule pressure may involve a variety of life-history stages (Fig. 2) and often include larval stages that spread

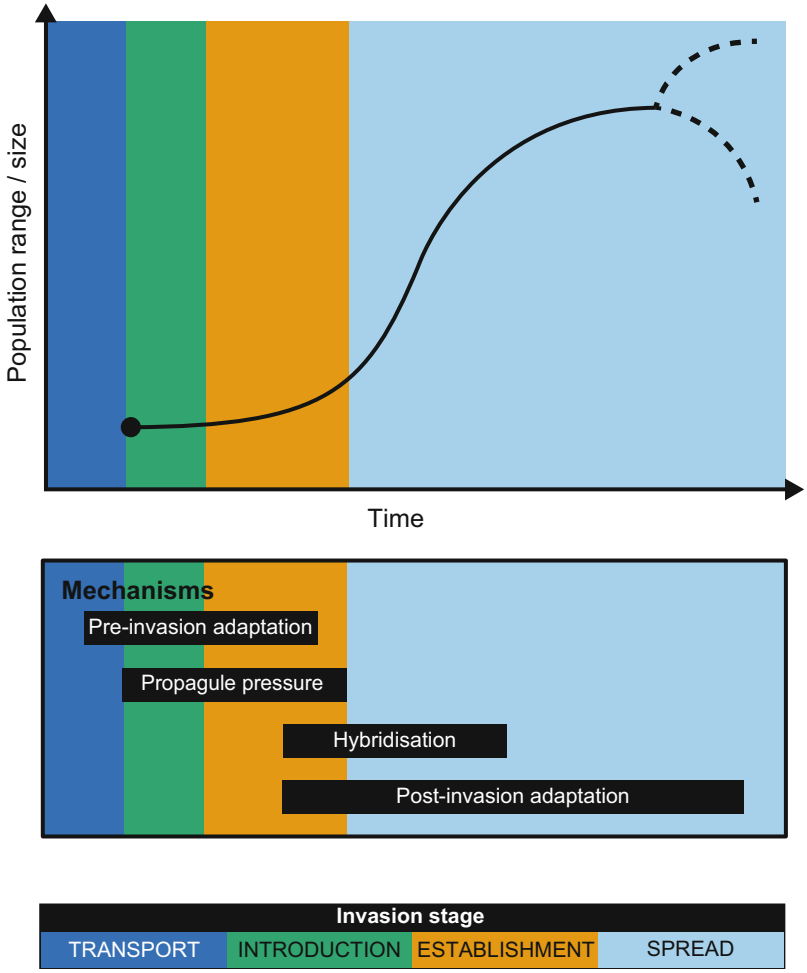

Fig. 1 The invasion stages (different colours) and main mechanisms (black boxes) shaping biological invasions. Dotted lines indicate different trajectories that introduced populations may follow once they become invasive

naturally when released to the introduced range (Johnston et al. 2009). Whilst studies of biological invasions have historically focused on species and recipient ecosystem traits (Blossey and Notzold 1995; Rejmánek and Richardson 1996; van Kleunen et al. 2015), the past two decades have seen an increase in studies assessing the role of propagule pressure on invasion success (Lockwood et al. 2005; Simberloff 2009). When a small number of individuals are artificially transported to a new location, a founder effect may reduce both the number of rare alleles and overall heterozygosity in a given introduced population (Widmer et al. 1998; Allendorf and Lundquist 2003; Roderick and Navajas 2003; Weber et al. 2004; Colautti et al. 2005). In addition, the establishment of a small introduced population may promote

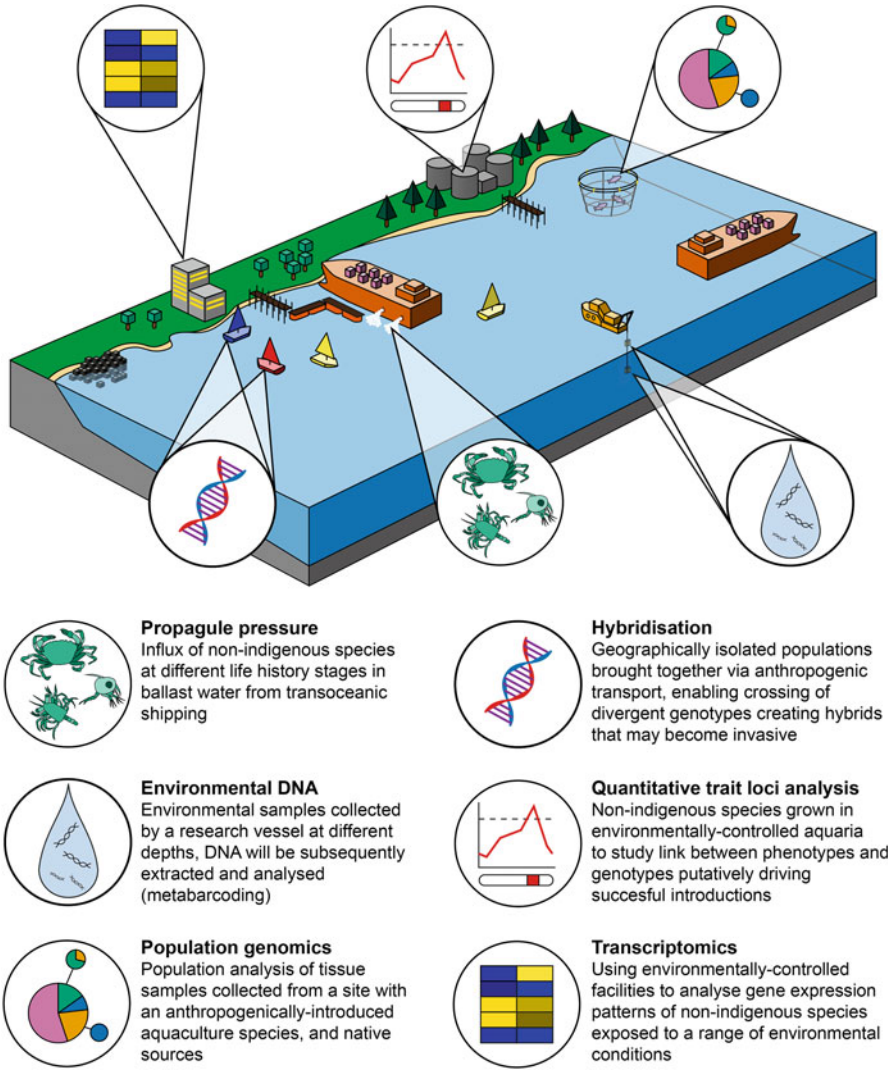

Fig. 2 Generalised schematic showing how marine NIS introductions can be studied in a coastal setting using genomic tools

mechanisms that concentrate alleles at the invasion front, such as allele surfing (Excoffier and Ray 2008; Hallatschek and Nelson 2008), further reducing genetic diversity. If the genetic bottleneck is transient, it would reduce the number of rare alleles present, whilst a longer bottleneck would reduce both levels of heterozygosity within the population and the number of rare alleles. Moreover, this bottlenecked population may be affected by inbreeding (Furlan et al. 2012), further reducing heterozygosity and leading to the accumulation of unfavourable recessive alleles.

This will ultimately affect population fitness in a phenomenon called inbreeding depression (Keller and Waller 2002). Therefore, low propagule pressure can lead to a genetic bottleneck that may limit the ability of an introduced population to establish and spread. Unsurprisingly, increased propagule pressure has been shown to mitigate these bottleneck-related effects, with an increasing number of studies showing similar levels of genetic diversity between native and introduced populations (Kelly et al. 2006; Rius et al. 2012, 2015b).

Roman and Darling (2007) comprehensively discussed how propagule pressure can affect genetic diversity of NIS and reiterated the importance of elucidating the relative contribution of the different components of propagule pressure. For example, it is known that increasing propagule size can benefit introduced populations twofold. Firstly, an increased propagule size will raise the genetic diversity of the introduced population (Suarez and Tsutsui 2008; Simberloff 2009; Wilson et al. 2009), as seen for example in introduced golden mussels (Ghabooli et al. 2013). Secondly, an increased propagule size with large effective population size may be more resistant to hostile conditions in the introduced range (Holle and Simberloff 2005). Regarding propagule number, an increase may improve the resistance of introduced populations to environmental stochasticity (sudden changes in the environment such as natural disasters or freak weather events) (Simberloff 2009). Additional introduction events, from shipping for example, could occur from numerous genetically distinct source populations, increasing genetic diversity in the incipient introduced population (Voisin et al. 2005; Gillis et al. 2009).

The relative role of propagule pressure on invasion success has been studied using experiments under different levels of propagule pressure and controlled conditions. Clark and Johnston (2009) found that on presettled plates, high propagule pressure was needed for NIS recruits to survive past 3 months, but was needed in tandem with disturbance (clearing presettled organisms from a third of each plate) to be successful. Such disturbance was essential to create space for recruits, indicating that high propagule pressure alone is not enough to ensure enhanced recruitment. Another example comes from Hedge et al. (2012), who studied the role of propagule pressure in the recruitment of the Pacific oyster, *Magallana gigas* (formerly *Crassostrea gigas*; Salvi et al. 2014), concluding that small frequent introductions were the most effective method for successful invasion. However, Sinclair and Arnott (2016) demonstrated that propagule size rather than number determined invasion success in the introduced mysid *Hemimysis anomala*.

Propagule size and number can also be strong indicators of an organism's genetic diversity, as Romiguier et al. (2014) showed in their extensive study linking genetic diversity and species ecological strategies. Propagule size was the strongest parameter of all studied ecological strategies to predict the genetic diversity in multiple species and families. Thus, high genetic diversity, ascertained through population genetic techniques, could indicate the presence of high propagule flow. Genomic techniques can also probe propagule pressure, as Narum et al. (2017) showed when they used reduced-representation techniques to genotype two salmonid species between native and introduced ranges. High propagule pressure and associated

mechanisms (e.g. genetic admixture, multiple introductions) maintained high genetic diversity within the introduced range of both species.

Genomic techniques offer the ability to scrutinise genomes to a much finer resolution than genetic approaches (more on this below), allowing a more representative picture of genetic diversity and propagule size to be constructed. Overall, propagule pressure has a major influence in shaping the genetic makeup of NIS, regulating their invasion success. Genetic and genomic techniques are well placed to investigate the genetic constitution of native and introduced ranges, indicating the strength of gene flow within and between them.

2.2 Hybridisation

The spread of NIS provides unprecedented opportunity for previously-isolated genotypes to contact and hybridise (Fig. 2), which can lead to elevated invasiveness (Ellstrand and Schierenbeck 2000). Hybrids may exhibit phenotypic superiority over their parents due to heterozygote advantage – a phenomenon known as heterosis (Lippman and Zamir 2007). In sexually reproducing species, heterosis is seen as transient, affecting only the F1 offspring due to the effects of genetic segregation (Lee 2002). Heterosis can remove inbreeding depression by purging accumulated deleterious recessive alleles (Keller and Waller 2002), enabling a population to "catapult" in size rapidly to overcome disadvantages associated with founder effects (Drake 2006). Crossing between divergent genotypes also provides an opportunity for increasing genetic variation and a larger pool of genotypes on which selection can act (Hegarty 2012). Hybridisation can also promote adaptive variation in NIS (Rius and Darling 2014; Stelkens et al. 2014), as it can occur between native and introduced species (Hänfling et al. 2005; Meilink et al. 2015; Gardner et al. 2016; Oyarzún et al. 2016), leading to displacement of native populations (Huxel 1999). Recent population genomic approaches have found evidence of selection both for and against hybridisation (Saarman and Pogson 2015; Kovach et al. 2016; Jeffery et al. 2017).

Hybridisation can also lead to the genesis of novel genotypes/phenotypes, which may provide a selective advantage due to transgressive segregation (Fig. 3). The generation of phenotypes that are extreme compared to the parental phenotypes and that affect the F2 generation onwards (de Vicente and Tanksley 1993) has been demonstrated in a wide range of organisms. Rieseberg et al. (1999) reviewed 171 hybridisation studies, finding that 155 of them reported at least one transgressive trait. In some cases, transgressive traits provide potential for hybrids to inhabit niches unused by either parent. This is evidenced by the case of *Spartina* spp. in San Francisco Bay, California, where the introduced *Spartina alterniflora* hybridises with the native *Spartina foliosa* (Sloop et al. 2009). Late generation *Spartina* hybrids are larger than either parents and produce more seeds as a result of transgressive segregation. Due to such peculiar gene complexes developed via transgressive segregation, it became detrimental for the hybrid to outcross, leading to selection

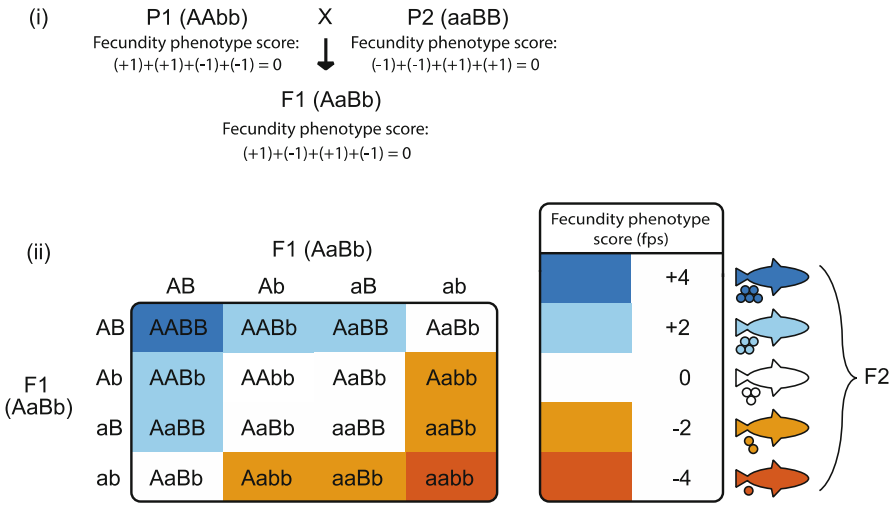

Fig. 3 Transgressive segregation during biological invasions. (i) Two parents, each with a different fixed allele at two unlinked loci. Alleles increase or decrease fecundity phenotype score (fps) by one unit, depending on uppercase (+1) or lowercase (−1). Both parental species are homozygous at both loci, resulting in net score of 0. F1 offspring are all heterozygous at both loci (AaBb), also resulting in a score of 0 (A + a + B + b = (+1) + (−1) + (+1) + (−1) = 0). (ii) F2 hybrids' fps range between +4 and −4, indicating varying phenotype fitness. Adapted from Bell and Travis (2005)

in favour of inbreeding (Sloop et al. 2009). Indeed, hybrids can evolve self-fertility, which neither parent nor F1 hybrids are capable of, rapidly assisting colonisation of novel ecosystems (Sloop et al. 2009).

Progeny from the hybridisation of closely-related lineages or species are often infertile, sterile, or inviable – termed hybrid breakdown (Orr and Turelli 2001; Arcella et al. 2014; Stelkens et al. 2015). This hybrid incompatibility is commonly attributed to deleterious epistatic interactions between alleles at different loci of parental genomes (Coyne and Orr 2004), or the breakdown of coadapted gene complexes during recombination, as seen in the copepod *Tigriopus californicus* (Edmands et al. 2009). Hybridisation may also be selected against in introduced populations (Arcella et al. 2014; Saarman and Pogson 2015; Kovach et al. 2016). If recipient and parental environments are similar, hybridisation of ecologically divergent subpopulations (e.g. a preadapted parent with a non-preadapted parent) will disrupt preadapted gene complexes and reduce offspring fitness (Rius and Darling 2014). Additionally, even if hybrids are genetically compatible, reproduction may not occur, or be reduced, due to prezygotic reproductive isolation. For example, hybrid male fur seals (hybridised between Antarctic *Arctocephalus gazella*, subantarctic *A. tropicalis*, and New Zealand *A. forsteri* fur seals) have been shown to exhibit lower reproductive success than pure-species males, putatively due to phenotypic traits affecting mate choice (Lancaster et al. 2007).

Hybridisation may also lead to extinction of native populations (Rhymer and Simberloff 1996; Arcella et al. 2014). An example of this is unfolding with crayfish

(*Orconectes rusticus* and *O. propinquus*) introductions into North American lakes (Arcella et al. 2014). F1 generation hybrids of the native *O. propinquus* and the introduced *O. rusticus* display hybrid vigour (Perry et al. 2001). This dynamic is extirpating native *O. propinquus* populations, as they are outcompeted by F1 hybrids, which then decrease in fitness over subsequent generations and enable the introduced *O. rusticus* to migrate in and outcompete them. As *O. propinquus* is outcompeted by F1 hybrids and *O. rusticus*, it is being removed from lakes in the region.

Genomic approaches offer a strong suite of tools to probe hybridisation (Twyford and Ennos 2012; Payseur and Rieseberg 2016), with population genomics well-suited for marine NIS (Hohenlohe et al. 2011; Hand et al. 2015; Saarman and Pogson 2015; Kovach et al. 2016). Reduced-representation approaches (i.e. sequencing of just a portion of the genome) such as RAD-Seq are particularly useful as the high number of markers returned means they are sensitive to weak levels of hybridisation, allowing them to confidently detect when selection is occurring against hybridisation (Saarman and Pogson 2015; Kovach et al. 2016). Selection against hybridisation may be occurring between native and introduced ascidian species in regions of sympatry. Whilst hybridisation occurs in the laboratory between the native Sea Vase tunicate (*Ciona intestinalis*) and the introduced *Ciona robusta* (Sato and Bishop 2012), genomic analyses indicate that naturally occurring hybridisation is rare (Bouchemousse et al. 2016a; Nydam et al. 2017a).

At the whole genome level, hybridisation can modify the architecture of genomes in complex ways. An F1 individual is essentially heterozygous at all loci, but over subsequent generations selection for coadapted gene complexes and the removal of unfit allelic combinations occurs, resulting in drastic (and rapid) changes in patterns of genomic variation (Ungerer et al. 1998). Important changes in gene expression can also be detected in hybrid transcriptomes using high-throughput sequencing. For example, comparisons of expression profiles of parent species and hybrid individuals show highly-dissimilar gene expression profiles (Wolf et al. 2010). Variation in gene expression can occur with changing ecological conditions (May et al. 2013) and be adaptive (Fisher and Oleksiak 2007), driving adaptation to rapid environmental changes, which may be facilitated by hybridisation (Chown et al. 2015). It is therefore clear that the study of genomes and transcriptomes is key to investigating the genetic basis and consequences of NIS hybridisation.

2.3 Pre-colonisation Adaptation

Species traits that have evolved in the native range can sometimes facilitate colonisation success in the introduced range. These are often referred to as preadapted traits (Curnutt 2000) and occur if NIS are introduced to an area that is ecologically or environmentally similar to their native range. Preadaptation may also help NIS more easily withstand environmental challenges in the introduced range (Schlaepfer et al. 2010; Bock et al. 2015; Elst et al. 2016). An example of preadaptation aiding

biological invasions has been detected in the invasion of the European common reed, *Phragmites australis,* in North America. Guo et al. (2014) compared morphological and ecophysiological traits, showing that the introduced genotype was preadapted and outcompeted native congeners due to elevated photosynthetic capacity. A second aquatic example comes from Zhang et al. (2010), who found that a genotype of the water hyacinth *Eichhornia crassipes* that dominates native high-altitude populations monopolised cold parts of the introduced range. Lastly, dominant species of the Red Sea's sandy shores have successfully colonised similar habitats (shallow sandy and muddy shores) in the Mediterranean, suggesting that preadaptation facilitated their establishment into the new range (Golani and Ben-Tuvia 1989; Golani 1993).

Although DNA-based approaches are severely underutilised in studying preadaptation in marine NIS, some studies have inferred preadaptation from genotype distribution (Zhang et al. 2010; Guo et al. 2014). Another approach is to directly use genomic tools to identify the presence of preadapted genes (Ometto et al. 2013; Gleason and Burton 2015; Gleason and Burton 2016a), such as the use of RNA-seq to detect the preadaptation of a whole suite of genes (Wang et al. 2009). These genomic approaches have been effectively used to detect preadaptation in native marine species. For example, Gleason and Burton (2016a) used reduced-representation genomics to probe the marine snail, *Chlorostoma funebralis,* finding strong divergence linked to temperature adaptation. RNA-seq revealed that lower-latitude populations were preadapted to cope with thermal stress by employing unique gene expression profiles compared to higher latitude populations (Gleason and Burton 2015). Subsequently, Gleason and Burton (2016b) reinforced these findings by investigating the actual temperatures encountered in the studied populations. They found that lower latitude populations were three times more likely to experience temperatures causing a heat-shock response. These studies show the utility of genomic approaches to identify preadaptation and how this approach can greatly increase our understanding of forces shaping adaptation pre-arrival.

2.4 Post-colonisation Adaptation

Post-colonisation adaptation after establishment can also happen when NIS enter a novel ecosystem (Guo et al. 2014; Lin et al. 2017). Studies show that post-colonisation adaptation may occur extremely rapidly (Huey et al. 2000; Dlugosch and Parker 2008; Whitney and Gabler 2008; Moran and Alexander 2014; Stapley et al. 2015) and is often referred to as contemporary evolution (Stockwell et al. 2002). Post-colonisation adaptation is essential for understanding genomic changes that may occur throughout the invasion process.

Rapid adaptation of NIS can be driven by several mechanisms (Gilchrist and Lee 2007), including transposable elements (TE) and epigenetics. Discovered in 1950 (McClintock 1950), TEs are a large group of highly variable loci that are able to move from one location to another on the genome (Pray 2008). They are known to

have a significant role inducing rapid adaptation in response to changes in the environment (Casacuberta and González 2013) and subsequently drive the adaptation of NIS (Schrader et al. 2014; Stapley et al. 2015). TEs are heavily influenced by epigenetics, another mechanism that can drive rapid adaptation in NIS (Ardura et al. 2017). Epigenetics is an umbrella term that refers to a group of heritable effects, such as DNA methylation or chromatin remodelling, that are unrelated to variation in DNA sequence variation (Huang et al. 2017). There is increasing evidence suggesting that epigenetic mechanisms contribute to phenotypic variation in ecologically relevant traits (Bossdorf et al. 2008). This is particularly pertinent to NIS where genetic variation may be very low and population expansion may occur on timescales not conducive to DNA sequence evolution (Dlugosch and Parker 2008). NIS may therefore encounter highly variable conditions to which they are not preadapted and therefore be forced to rely on their epigenetic variation (Pu and Zhan 2017). Current work indicates that the strength of epigenetic signals in NIS overcomes the signal from environmental conditions, supporting the hypothesis that early-invasion NIS, as part of their post-colonisation adaptation, increase phenotypic plasticity as a result of reduced methylation (Ardura et al. 2017). There is still much to study concerning the influence that TEs and epigenetics have on promoting post-colonisation adaptation in NIS. Genomic approaches are well placed to examine them in natural populations (Casacuberta and González 2013; Stapley et al. 2015; Trucchi et al. 2016; Hofmann 2017) as they open up new research avenues such as sequencing both the transcriptome and methylome simultaneously (Hofmann 2017).

Post-colonisation adaptation is known to enable single-source introduction NIS to rapidly respond to local environmental conditions (Dlugosch and Parker 2008; Prentis et al. 2008; Bock et al. 2015) and overcome the negative effects resultant from genetic bottlenecks (Colautti and Barrett 2013). This is often referred to as evolutionary rescue (Gonzalez et al. 2013; Carlson et al. 2014; Lau and terHorst 2015) and is based on the idea that rapid adaptation may aid NIS in mitigating the deleterious effects of low genetic diversity. Pre- and post-invasion adaptation are not mutually exclusive (Sakai et al. 2001; Bernardi et al. 2016), as species may be preadapted before arrival, enabling a successful introduction, and then may undergo rapid post-invasion adaptation to further optimise fitness in the introduced area. This has been reported in the common reed wetland grass (*Phragmites australis*), wherein a more efficient photosynthetic apparatus of a preadapted strain colonised North America and then underwent further post-colonisation morphological and ecophysiological adaptation to maximise fitness and thrive in the introduced area (Guo et al. 2014). This has also been reported in other terrestrial plant introductions (Henery et al. 2010). Introduced species can also induce post-colonisation adaptation in native inhabitants (Carroll 2007; Oduor 2013). The introduction of toxic cane toads (*Bufo marinus*) into Australia induced an adaptation in native snake species (*Pseudechis porphyriacus* and *Dendrelaphis punctulatus*) to restrict their predation of these toxic toads. The snakes adapted morphological traits to lessen risk of consumption and ill effects (decreased gape size, and increased body lengths), whilst snakes that did not prey upon the toads (*Hemiaspis signata* and *Tropidonophis mairii*) exhibited no consistent change after introduction (Phillips and Shine 2004).

NIS introductions also create competition, which can induce morphological changes, as occurred when the native lizard (*Anolis carolinensis*) adapted larger toepads in order to cling to higher perches after the introduction of another lizard – *Anolis sagrei* – (Stuart et al. 2014).

Genetic and genomic techniques provide a strong complement to morphological and ecophysiological analyses to test questions on NIS adaptation. In a study of the introduced Lessepsian migrant bluespotted cornetfish (*Fistularia commersonii*), genomic regions associated with disease resistance and osmoregulation were identified as putatively being under selective pressure in the introduced range (Bernardi et al. 2016). Considering the relatively short time since the introduction of this NIS, it was suggested that both pre- and post-colonisation adaptation may have occurred.

3 Methods Used to Study Marine Invasion Genomics

3.1 Environmental DNA

Organisms naturally shed DNA into the environment in which they live. This environmental DNA (eDNA) can be filtered or precipitated out and analysed to infer the presence or absence of a given organism. Recent work indicates that eDNA is made up of a range of different-sized particles, from very large multicellular fragments (>180 μm) down to extracellular DNA (Turner et al. 2014; Sassoubre et al. 2016). Estimates of eDNA persistence in seawater have been limited to fish (Thomsen et al. 2012; Maruyama et al. 2014; Sassoubre et al. 2016; Andruszkiewicz et al. 2017) and crustacean (Forsström and Vasemägi 2016) species, showing degradation on the order of days. Current eDNA research is progressing from monitoring presence/absence towards quantifying species abundance (Lacoursière-Roussel et al. 2016) – applications that otherwise require large sampling effort. For example, Thomsen et al. (2016) showed that eDNA assessments were comparable to trawling sampling when probing deep-sea fish faunas. Thus, eDNA shows great potential for detecting and monitoring aquatic NIS, in both presence and quantity. eDNA has thus far been used to probe introduced gobies (Adrian-Kalchhauser and Burkhardt-Holm 2016), aquatic plants (Scriver et al. 2015), bullfrogs (Ficetola et al. 2008; Dejean et al. 2012), pythons (Piaggio et al. 2014), carp (Jerde et al. 2013; Mahon et al. 2013; Klymus et al. 2015), crayfish (Larson et al. 2017), bivalves (Ardura et al. 2015a), snails (Goldberg et al. 2013), and turtles (Davy et al. 2015). eDNA has also been used to monitor vectors transporting NIS (Collins et al. 2013; Mahon et al. 2014; Nathan et al. 2015).

Work using eDNA has begun to study the initial stages of colonisation, such as transport and establishment (Fig. 1), where traditional techniques lack the required sensitivity for early detection of both successful and unsuccessful species introductions (Takahara et al. 2013; Forsström and Vasemägi 2016; Tucker et al. 2016; Simpson et al. 2017). Early detection is pivotal for a rapid response to NIS (Pyšek and Richardson 2010) and is more economically efficient than removing a more-

progressed NIS (Williams et al. 2010). For example, the invasion of the black striped mussel (*Mytilopsis sallei*) in 1999 to Darwin harbour, Australia, threatened marine infrastructures, the surrounding environment, and a pearl fishery worth A$40 million (Bax et al. 2002). A rapid response required chemical treatment of marinas to eradicate the introduced mussel and caused transient mortality in a significant proportion of native fauna. However, the cost associated with eradication (A$2.2 million) was deemed minimal compared to the potential environmental and economic damage the mussel could have caused (Willan et al. 2000; Bax et al. 2002). eDNA is also a potent tool for ascertaining which NIS are in transit and understanding which shipping routes or ports are prolific transporters, being well-suited to testing ballast water (Egan et al. 2015; Tucker et al. 2016; Darling and Frederick 2017). Current techniques enable detection of eDNA from species at very low densities (e.g. using qPCR; Foote et al. 2012; Bergman et al. 2016), providing a tool to learn more about initial stages of colonisation that previously evaded investigation.

Although eDNA shows great potential in investigating NIS, limitations exist. DNA decays at different rates under varying environmental conditions, biasing the detection of NIS (Barnes and Turner 2016). eDNA is also susceptible to false positives (Ficetola et al. 2016) and negatives (Schultz and Lance 2015), as it can travel long distances (Thomsen et al. 2012; Deiner and Altermatt 2014). Current progress in eDNA research is increasing its accuracy and reliability (Amberg et al. 2015; Ficetola et al. 2016; Lahoz-Monfort et al. 2016; Guillera-Arroita et al. 2017), especially when eDNA surveys are combined with traditional survey approaches (Yamamoto et al. 2016; Sigsgaard et al. 2017).

Innovations in metabarcoding (i.e. using universal primers to amplify and sequence a conserved region in an environmental sample for species identification) are increasingly being applied to marine biological invasions (Ardura et al. 2015b; Zaiko et al. 2015). The metabarcoding of eDNA, when used judiciously (Goldberg et al. 2016), not only allows for the rapid and accurate detection of NIS during introduction and establishment, but also the collection of information on currently nontarget species and community composition.

3.2 Quantitative Trait Loci Mapping

A pertinent method for determining the genetic basis of invasiveness and rapid adaptation is the study of quantitative trait loci (QTL) (Stapley et al. 2015). QTLs are genomic regions that correlate with the expression of quantitative traits. Whilst there is not a suite of traits representative of all NIS, quantitative traits such as those that enhance fecundity, growth, and spawning time can play an important role in determining colonisation of novel environments (van Kleunen et al. 2015; McKnight et al. 2017). The main purpose of QTL analysis is to link genotype loci to a phenotype (determining the genetic basis for an observed quantitative trait) and is especially adept at investigating how genomic regions are responsible for adaptive

loci (Pardo-Diaz et al. 2015). QTL analyses have been used to identify the basis of adaptation in introduced terrestrial species (Weinig et al. 2007; Hamilton et al. 2015; Whitney et al. 2015), native marine species (Johnston et al. 2014), and even native's response to NIS (Yu and Andrés 2014). However, much like other genomic techniques, these methods are severely underutilised in the study of marine NIS. QTL mapping could be used to study marine NIS by crossing native and introduced individuals of a marine species that differ in a trait of interest (e.g. size, fecundity, or spawning time). By scoring the phenotype and genotype of the derived individuals, molecular markers linked to QTL will show significant associations with phenotypes, allowing the identification of regions of the genome putatively associated with key traits. QTL mapping may also play an important role in the further study of TEs and their influence on rapid adaptation (Stapley et al. 2015).

3.3 Population Genomics

Population genetics represents a robust approach to assess the demographics and population structure of NIS (Riquet et al. 2013; Rius et al. 2015b; Bouchemousse et al. 2016b; Wrange et al. 2016; Cordero et al. 2017) – also see boxed case study. The low costs of using mitochondrial DNA and polymorphic markers such as microsatellites, together with the relative computational simplicity of their analyses, means that they are still the dominant approach used in marine invasion genetics (Rius et al. 2015b).

Recent advances in DNA sequencing technology have allowed the transition from population genetics to population genomics (Stinchcombe and Hoekstra 2008; Hemmer-Hansen et al. 2014). High-throughput sequencing approaches on NIS can now return tens to hundreds of thousands of polymorphic markers such as single nucleotide polymorphisms (SNPs) across the genome (Puzey and Vallejo-Marin 2014; Vandepitte et al. 2014; Hand et al. 2015). The ability to sequence large numbers of loci simultaneously enables the detection and categorisation of positive and neutral selection (Luikart et al. 2003). Two general methods exist to identify outlier loci (Hoban et al. 2016): differentiation outlier analyses, which identify loci that are abnormally differentiated between populations, and genotype-environment outliers, in which loci are correlated to local environmental factors (White et al. 2013). The analysis of genotype-environment interactions allows researchers to study how environmental conditions influence adaptation of introduced populations. For example, White et al. (2013), looking at the water vole (*Myodes glareolus*), used geographical distance from the point of introduction as the environmental variable and found that strong signals of selection were affected by rapid range expansion.

The use of a large number of loci also allows greater discrimination of differentiation between populations (Rašić et al. 2014). This is especially important to differentiate native and introduced populations, and to reliably identify source populations of a highly homogenous introduced range. This is clearly shown in a genomic study of the global distribution of the introduced ascidian *Microcosmus*

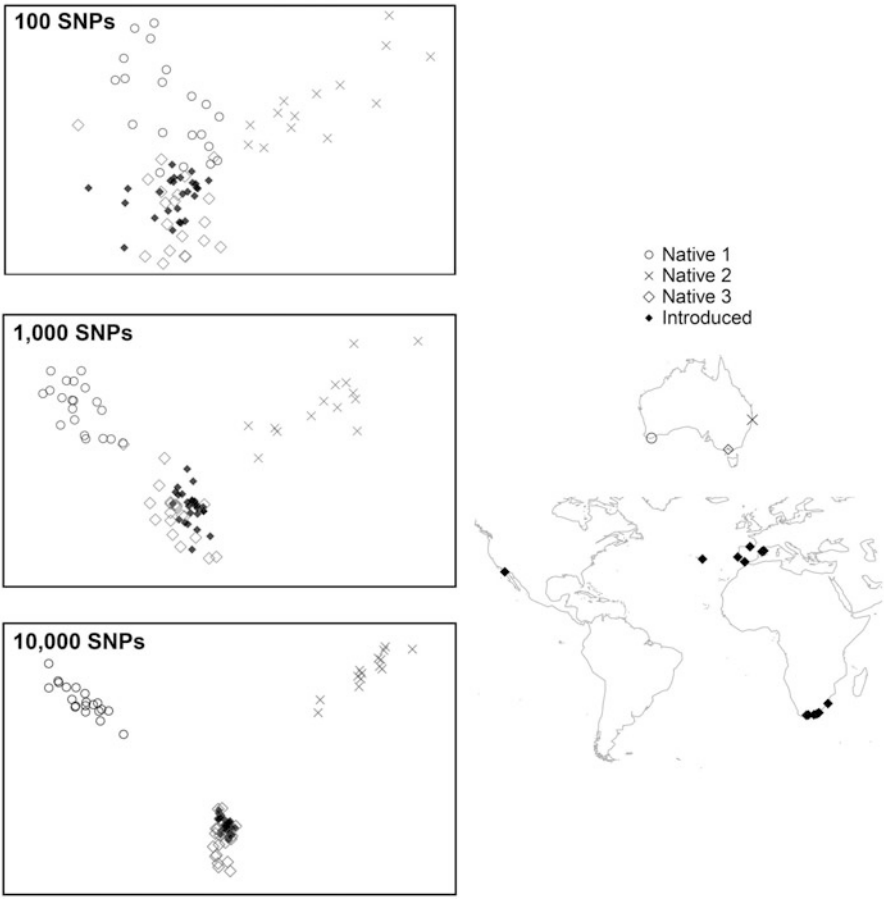

Fig. 4 Discriminant analysis of principal component plots showing how an increase in the number of single nucleotide polymorphism markers for the ascidian *Microcosmus squamiger* (Bourne et al., unpublished) alters the population structure of globally sampled sites. The location of the sampled sites is indicated on the right-side maps, with the native (Australia) and introduced (South Africa, Europe, and Baja California) ranges denoted with different symbols. Note that sites within the native range are genetically divergent, whilst the ones found in the introduced range are panmictic

squamiger (Fig. 4) that shows the beneficial effects of a larger number of markers (Bourne et al. unpublished). A discriminant analysis of principal components shows increasing segregation between native populations as the number of markers increases (Fig. 4). Also clearly elucidated from a higher number of markers is the putative source of introduced populations.

Population genomic approaches are being used with increasing regularity in the study of NIS, having been applied to crabs (Jeffery et al. 2017), salmonids (Narum et al. 2017), toads (Trumbo et al. 2016), mosquitofish (Vera et al. 2016),

trout (Hand et al. 2015; Kovach et al. 2016), and mussels (Saarman and Pogson 2015), finding evidence of reduction or no change in diversity between native and introduced ranges (Vera et al. 2016; Narum et al. 2017). Although these approaches are being incorporated into invasion biology, they are still largely underutilised when studying marine biological invasions (Sherman et al. 2016).

Boxed Case Study

Native to the Indo-Pacific, the lionfishes *Pterois volitans* (Linnaeus 1758) and *P. miles* (Bennett 1828) are highly invasive species. Officially first recorded in the western North Atlantic in 2000 (Whitfield et al. 2002), although observed since at least 1985 in Florida (Florida Fish and Wildlife Conservation Commission 2017), these species have since expanded towards lower latitudes, including South America (Ferreira et al. 2015). Subsequently, they have extensively expanded within the Mediterranean (Golani and Sonin 1992; Azzurro et al. 2017). Thought to be vagrants of the aquarium trade (Hare and Whitfield 2003), lionfish significantly affect invaded ecosystems, including drastically reducing the recruitment (Albins and Hixon 2008) and abundance (Green et al. 2012; Ballew et al. 2016) of native fish species.

Much population genetic work has been undertaken to understand their expansion in the western Atlantic, with Hamner et al. (2007) discovering evidence of a strong founder effect upon colonisation. They also found that the majority were *P. volitans*, with few *P. miles* individuals present. The western Atlantic origin of introduction was found to be the Florida coast by Betancur et al. (2011), who also found that *P. miles* is restricted to northernmost locations, whilst *P. volitans* is much more ubiquitous, with strong population structure evident between northern and Caribbean populations.

(continued)

Valdez-Moreno et al. (2012) reinforced this by finding only *P. volitans* individuals in the Mexican Caribbean and furthermore, after identifying stomach contents, confirmed that they were engaging in cannibalistic behaviour – a first recorded instance in this species. Similarly, only *P. volitans* individuals were found in Puerto Rico by Toledo-Hernández et al. (2014), who also found a secondary founder effect present from the original nonindigenous population. Tracking their spread into South America, Ferreira et al. (2015) used DNA analyses to match individuals to haplotypes found in North America and Mexico, indicating that the nonindigenous population is spreading and that the Brazilian population is not resultant from an independent invasion from the Indo-Pacific, but natural larval dispersal from the invaded Caribbean region. However, a recent DNA analysis has proposed that the invaded region may be the recipient of multiple introductions, and not the single introduction as previously thought (Butterfield et al. 2015), showing that DNA-based approaches can be confounded by the choice of sampled populations. Lastly, comprehensive high-resolution genomic SNP work undertaken by Pérez-Portela et al. (2018) show complete panmixia in the region. Interestingly, this contrasts previous genetic results that found differentiation (Butterfield et al. 2015; Johnson et al. 2016). Pérez-Portela et al. (2018) suggest two reasons for this difference. Firstly, a fundamental difference in findings between genetic and genomic work (or mitochondrial and nuclear DNA), as has been previously-reported (Toews and Brelsford 2012). Or secondly, that high gene flow has eroded the previous genetic signals and created regional homogenisation. Further to the contribution of high gene flow, they also proposed that local adaptation is further contributing to the panmixia.

Genetic work has also identified that hybridisation within the native range may be a contributing factor to the invasion success of nonindigenous populations in the West Atlantic (Wilcox et al. 2018). The invasive lineage may be resultant from hybridisation between the Indian and Pacific lineages, raising the interesting prospect that heterosis may be enhancing the success of the invasive *P. volitans* in the West Atlantic.

The genetics of the nonindigenous Mediterranean population has also been recently probed, with the introduced *P. miles* individuals related to Red Sea populations, indicating a Lessepsian invasion (Bariche et al. 2017). A low genetic diversity also implied the occurrence of another founder effect, though the success to which the Mediterranean has been colonised indicates that low genetic diversity has not proven a barrier to their invasion.

Future genomic studies can assist investigators in mitigating the lionfish expansion. Currently, they must be observed to be recorded in a novel area, but the use of eDNA monitoring could enable conservationists to be more reactive to the lionfish's spread. This in turn allows an accurate picture to be constructed of their nonindigenous range and expanding front, aiding managers in deciding where to allocate their eradication efforts for optimum

(continued)

effectiveness. Future higher-resolution population genomic approaches (see Future Directions), along with more comprehensive sampling, will enable scientists to resolve whether the invaded region has been subjected to multiple introductions or a single expanding front. However, it is possible that the intense homogenisation observed by the high-resolution approach of Pérez-Portela et al. (2018) could mask previous signals of invasion history. If, however, invasion pathways can be elucidated, this would enable investigators to understand where nonindigenous populations are sourced from, again allowing managers to allocate resources to disrupting the pathway supplying the nonindigenous populations. After eradication efforts, further monitoring using eDNA can effectively scrutinise the previous range, confirming the success of failure of such efforts in curbing nonindigenous lionfish populations.

Ultimately, genomic approaches will prove a major contributor in the conservation efforts against lionfish, first enabling scientists and managers to understand their spread, then assisting in their mitigation, and finally monitoring the success of management efforts.

Genomic data may also be used to infer invasion pathways and colonisation histories (Estoup and Guillemaud 2010; Guillemaud et al. 2010; Cristescu 2015). Several methods are available to reconstruct routes of invasion, such as population genetic and genomic inferences using phylogenetic trees (Estoup and Guillemaud 2010; Cristescu 2015). A particularly fruitful approach has been to compare specific invasion scenarios using the approximate Bayesian computation (ABC) method (Beaumont et al. 2002). Multiple software are now available to reconstruct colonisation histories using genetic data and ABC (for review see Estoup and Guillemaud 2010), which have been used extensively in terrestrial studies (e.g. Lombaert et al. 2010; Brown et al. 2011; Boissin et al. 2012; Konečný et al. 2013) and increasingly in aquatic species [ascidians (Rius et al. 2012; Teske et al. 2015; Nydam et al. 2017b), mosquitofish (Purcell et al. 2012), cyprinids (Simon et al. 2011), mussels (Marescaux et al. 2016), red shiners (Glotzbecker et al. 2016), and pike (Pedreschi et al. 2014)]. These studies have found evidence of population bottlenecks (Purcell et al. 2012), secondary introductions (Pedreschi et al. 2014), genetic admixture (Rius et al. 2012; Glotzbecker et al. 2016), and both independent (Marescaux et al. 2016) and non-independent invasions (Rius et al. 2012). These studies have mostly used genetic data, and the few that have used genomic data have quickly overwhelmed computing resources (Marx 2013; Ocaña and de Oliveira 2015). In order to tackle this issue, invasion-inference techniques that use less computing effort are now being developed to handle larger genomic datasets (Pudlo et al. 2016) such as the random forest statistical technique (Breiman 2001). We expect that when coupling these techniques with broadened access to higher computation power (Marx 2013), genomic data will be more commonly used to infer colonisation histories of marine NIS.

3.4 Gene Expression

Transcriptomic approaches have been greatly enhanced by the genomic revolution (Marguerat et al. 2008), providing a useful tool for marine invasion biologists (Fig. 2) (Rius et al. 2015a). The ability to look at gene expression allows researchers to study adaptation of NIS at a more detailed level. Differential gene expression studies across environments can help researchers understand the role of phenotypic plasticity (Aubin-Horth and Renn 2009) in marine NIS (Wellband and Heath 2017). Phenotypic plasticity, or the ability of a genotype to produce different phenotypes in response to environmental conditions (Pigliucci 2001), frequently occurs in NIS (Davidson et al. 2011; Lande 2015; Sassenhagen et al. 2015; Guardiola et al. 2016). Wellband and Heath (2017) compared two introduced goby species, each of varying colonisation success (measured as the extent of geographic spread), finding that success could be related to phenotypic plasticity. The more successful species of the two was more phenotypically responsive to temperature, in both gene transcription magnitude and function. The biological processes altered by the successful goby species were consistent with reported phenotypic gene expression responses to temperature, whereas the less successful goby species exhibited maladaptive phenotypic plasticity. A similar scenario was shown in the mussels *Mytilus galloprovincialis* and *M. trossulus* (Lockwood et al. 2010). *M. galloprovincialis,* which outcompetes *M. trossulus* in warmer habitats (including in the native range of *M. trossulus*), showed an elevated response to acute heat stress, with the most differentially expressed gene between the two being a heat-shock protein. Finally, transcriptomic approaches have been combined with genome assembly to explain the resistance of some introduced marine species to xenobiotic chemicals. An example of this is the highly invasive catfish, *Pterygoplichthys anisitsi*, which has been found to have an expanded defensome (i.e. genes that code for defence mechanisms to chemical environmental stressors), which may assist it in invading polluted areas (Parente et al. 2017).

4 Future Directions

As the cost of high-throughput sequencing is continually decreasing, the inhibitive stage in genomic studies has shifted from data acquisition to storage and analysis, with the vast amount of data generated becoming the new limiting factor on the experiment pipeline (Marx 2013). Indeed, it takes less than a year for the amount of sequence data stored by the European Bioinformatics Institute to double (European Bioinformatics Institute 2012). Researchers must grapple with these new demands, and on a broad scale, the development of associated technology will continue to mitigate the challenges of genomic data storage and analysis, including the introduction of overarching approaches that effectively integrate the four pillars of

genomic studies: data acquisition, storage, distribution, and analysis (Stephens et al. 2015). Progress in this direction will promote a productive future for marine invasion genomic studies.

One approach that is predicted to be especially productive in the future for invasion biologists is eDNA (Barnes and Turner 2016). It has already been applied to the detection of individual NIS, as well as assessing entire community composition (Kelly et al. 2014) or addressing species abundance (Takahara et al. 2012; Lacoursière-Roussel et al. 2016). Other exciting developments include the employment of remote vehicles or stations fitted with eDNA technology (Scholin 2010; McQuillan and Robidart 2017), allowing unprecedented spatial and temporal resolution. Although currently an expensive option, progressing autonomous vehicle development will enable invasion biologists to reliably and regularly sample areas prone to invasion, giving a time-linked genomic profile of the areas and signalling the arrival of NIS. Another exciting future prospect is the use of eDNA to assess population genetic inference of NIS. This concept has recently been proved, with the population genetics of whaleshark populations derived from eDNA-sampled mitochondrial fragments (Sigsgaard et al. 2016). The leading edge of eDNA NIS research involves applying this concept to NIS. It has recently been shown that eDNA can accurately reconstruct the proportion of different genotypes in a water body using qPCR (Uchii et al. 2016). This dramatically increases our ability to understand the distribution of well-studied NIS. Additionally, combining this approach with metabarcoding of variable regions allows researchers to discover previously unknown genotypes (Sigsgaard et al. 2016). Furthermore, it may be possible to infer population parameters using haplotype frequency and diversity data in species where mutation rates are known, which when combined with the added ease of eDNA sampling will prove a robust tool for future invasion biologists.

Another potentially fruitful future concerns the investigation of environmental adaptation in NIS using genomic scans. Genome-wide scans give investigators unparalleled power, as genomic outlier loci can be identified from any genomic region using fixation statistics such as F_{ST}. These outlier loci can then be associated with the environmental conditions in which they are prevalent, such as altitude (Dong et al. 2014), climate (Yoder et al. 2014), salinity, and temperature (Guo et al. 2015). However, Hoban et al. (2016) raise three considerations for which future invasion biologists should be aware of. Firstly, the genomic data must be placed in context with the demographics and population history of the studied NIS. Secondly, the use of reference data will always provide a benefit to genomic projects, and as such efforts should be made to develop reference genomes with high-quality gene annotations. Lastly, concerning experimental design, a priori environmental information should be used to inform sample sites, to broaden our knowledge of the environmental response of the organism. The need for a comprehensive sampling strategy when probing NIS genomics is reiterated by Viard et al. (2016). There is also a pressing need to integrate the current distinct approaches into a single framework, which would prove especially useful when assessing the impact of environment on adaptation in marine NIS (Bragg et al. 2015). When following the

recommendations of Hoban et al. (2016) and Bragg et al. (2015), invasion biologists can reap the rewards of a robust population genomic approach, including the future benefit that will come from full genomes being sequenced for each studied individual. This increasing genomic resolution will enable invasion biologists to disentangle complex invasion histories. Furthermore, a future shaped by a rapidly changing climate will likely see different effects on NIS (Stachowicz et al. 2002; Dukes 2010; Masters and Norgrove 2010; Muhlfeld et al. 2014). Interactions between climate change and NIS can be complex (Kolar and Lodge 2000) and are likely to be context and species dependent, involving multiple factors mediating such interaction (Rius et al. 2014). Greater understanding of the genetic changes of NIS during adaptation to warming is critical for predicting dynamics of future invasions (Somero 2010) and identifying management strategies (Rahel et al. 2008).

Furthermore, the availability of third-generation sequencers is expected to drastically boost evolutionary and invasion research. This new sequencing technology will further promote de novo genome sequencing and assembly, leading to genomic resources being developed for more non-model organisms and increasing the genomic repertoire available to invasion biologists. An increase in read length also augments current population genomic approaches as longer reads can be compared between individuals, further increasing extracted information and genome resolution, therefore add a whole suite of tools to the biologists' toolbox (Sedlazeck et al. 2018). Another benefit of the new sequencing technology is the ability to more efficiently probe post-translational modifications (Garalde et al. 2018). The ability to study post-translational modifications opens up many opportunities when examining the occurrence of the rapid localised adaptation frequently accompanying NIS.

5 Conclusions

Biological invasions are one of the greatest threats facing the preservation of global biodiversity, causing vast ecological and economic impacts (Pimentel et al. 2005; Kumschick et al. 2015). In turn, they also provide excellent models for understanding ecological and evolutionary mechanisms (Sax et al. 2005) such as rapid adaptation over contemporary time scales (Huey et al. 2005). The knowledge gained from studying invasion biology in these instances can be pertinent to broadly applicable processes, such as natural range expansions (Colautti and Barrett 2013).

The suite of genomic tools now available to invasion biologists has considerably enhanced the understanding of processes underpinning biological invasions (Stapley et al. 2015; Viard et al. 2016). For example, the role that hybridisation plays in promoting NIS can now be disentangled using genomic approaches, and the study of genomic regions linked to quantitative traits (Dlugosch et al. 2015) is revealing genetic attributes linked to invasiveness (Weinig et al. 2007). NIS can be detected earlier with eDNA (Dougherty et al. 2016), allowing detection of NIS before they cause substantial environmental and economic damage (Williams et al. 2010). The effects of adaptation on marine NIS can be explored (Bernardi et al. 2016) along with

associated mechanisms such as TEs and epigenetics (Casacuberta and González 2013; Stapley et al. 2015; Hofmann 2017). The role of gene expression in marine NIS can be evaluated with transcriptomics (Lockwood et al. 2010; Wellband and Heath 2017), and the ability of marine NIS to thrive in polluted conditions probed (Parente et al. 2017). More fundamentally, genomic resources can be developed for NIS that are unrelated to well-studied model organisms (da Fonseca et al. 2016). Furthermore, the ability to reconstruct recent invasion pathways strengthens our ability to undertake invasion risk assessments. Other genomic applications are also possible, such as the genome-editing technology that is currently under development (Ricciardi et al. 2017) and could alter the genomes of marine NIS. This has the potential to reduce their impact and halt their spread (Esvelt et al. 2014; Webber et al. 2015; Harvey-Samuel et al. 2017). Ultimately, marine NIS can now be studied much more comprehensively than ever before, a result of recent developments in genomic tools.

The current increase in numbers of invasion biology studies using genomic approaches (Barrett et al. 2016) and the reduction of associated sequencing costs (Rius et al. 2015a), show that studies focusing on marine invasion genomics will increasingly help scientists and policymakers better manage this major driver of global biodiversity change.

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References

Adrian-Kalchhauser I, Burkhardt-Holm P. An eDNA assay to monitor a globally invasive fish species from flowing freshwater. PLoS One. 2016;11:e0147558.

Albins MA, Hixon MA. Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. Mar Ecol Prog Ser. 2008;367:233–8.

Allendorf FW, Lundquist LL. Introduction: population biology, evolution, and control of invasive species. Conserv Biol. 2003;17:24–30.

Amberg JJ, Grace McCalla S, Monroe E, Lance R, Baerwaldt K, Gaikowski MP. Improving efficiency and reliability of environmental DNA analysis for silver carp. J Great Lakes Res. 2015;41:367–73.

Andruszkiewicz EA, Sassoubre LM, Boehm AB. Persistence of marine fish environmental DNA and the influence of sunlight. PLoS One. 2017;12:e0185043.

Arcella TE, Perry WL, Lodge DM, Feder JL. The role of hybridization in a species invasion and extirpation of resident Fauna: hybrid vigor and breakdown in the rusty crayfish, *Orconectes rusticus*. J Crustac Biol. 2014;34:157–64.

Ardura A, Zaiko A, Martinez JL, Samulioviene A, Semenova A, Garcia-Vazquez E. eDNA and specific primers for early detection of invasive species – a case study on the bivalve *Rangia cuneata*, currently spreading in Europe. Mar Environ Res. 2015a;112(Part B):48–55.

Ardura A, Zaiko A, Martinez JL, Samuiloviene A, Borrell Y, Garcia-Vazquez E. Environmental DNA evidence of transfer of North Sea molluscs across tropical waters through ballast water. J Moll Stud. 2015b;81:495–501.

Ardura A, Zaiko A, Moran P, Planes S, Garcia-Vazquez E. Epigenetic signatures of invasive status in populations of marine invertebrates. Sci Rep. 2017;7:42193.

Aubin-Horth N, Renn SCP. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. Mol Ecol. 2009;18:3763–80.

Azzurro E, Stancanelli B, Di Martino V, Bariche M. Range expansion of the common lionfish *Pterois miles* (Bennett, 1828) in the Mediterranean Sea: an unwanted new guest for Italian waters. Bioinvasions Rec. 2017;6:95–8.

Baker H, Stebbins G. The genetics of colonizing species. New York: Academic Press; 1965.

Ballew NG, Bacheler NM, Kellison GT, Schueller AM. Invasive lionfish reduce native fish abundance on a regional scale. Sci Rep. 2016;6:32169.

Bariche M, Kleitou P, Kalogirou S, Bernardi G. Genetics reveal the identity and origin of the lionfish invasion in the Mediterranean Sea. Sci Rep. 2017;7:6782.

Barnes MA, Turner CR. The ecology of environmental DNA and implications for conservation genetics. Conserv Genet. 2016;17:1–17.

Barrett SCH. Foundations of invasion genetics: the Baker and Stebbins legacy. Mol Ecol. 2015;24:1927–41.

Barrett SCH, Colautti RI, Dlugosch KM, Rieseberg LH. Invasion genetics: the Baker and Stebbins Legacy. Hoboken, NJ: Wiley; 2016.

Bax NJ, Hayes K, Marshall A, Parry D, Thresher R. Man-made marinas as sheltered islands for alien marine organisms: establishment and eradication of an alien invasive marine species. In: Veitch CR, Clout MN, editors. Turning the tide: the eradication of invasive species. Gland, Switzerland: IUCN SSC Invasive Species Specialist Group; 2002. p. 26–39.

Beaumont MA, Zhang W, Balding DJ. Approximate Bayesian computation in population genetics. Genetics. 2002;162:2025–35.

Bell MA, Travis MP. Hybridization, transgressive segregation, genetic covariation, and adaptive radiation. Trends Ecol Evol. 2005;20:358–61.

Bergman PS, Schumer G, Blankenship S, Campbell E. Detection of adult green sturgeon using environmental DNA analysis. PLoS One. 2016;11:e0153500.

Bernardi G, Azzurro E, Golani D, Miller MR. Genomic signatures of rapid adaptive evolution in the bluespotted cornetfish, a Mediterranean Lessepsian invader. Mol Ecol. 2016;25:3384–96.

Betancur RR, Hines A, Acero PA, Ortí G, Wilbur AE, Freshwater DW. Reconstructing the lionfish invasion: insights into greater Caribbean biogeography. J Biogeogr. 2011;38:1281–93.

Blackburn TM, Pysek P, Bacher S, Carlton JT, Duncan RP, Jarosik V, Wilson JR, Richardson DM. A proposed unified framework for biological invasions. Trends Ecol Evol. 2011;26:333–9.

Blossey B, Notzold R. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol. 1995;83:887–9.

Bock DG, Caseys C, Cousens RD, Hahn MA, Heredia SM, Hübner S, Turner KG, Whitney KD, Rieseberg LH. What we still don't know about invasion genetics. Mol Ecol. 2015;24:2277–97.

Boissin E, Hurley B, Wingfield MJ, Vasaitis R, Stenlid J, Davis C, de Groot P, Ahumada R, Carnegie A, Goldarazena A, Klasmer P, Wermelinger B, Slippers B. Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* woodwasp. Mol Ecol. 2012;21:5728–44.

Boivin NL, Zeder MA, Fuller DQ, Crowther A, Larson G, Erlandson JM, Denham T, Petraglia MD. Ecological consequences of human niche construction: examining long-term anthropogenic shaping of global species distributions. Proc Natl Acad Sci. 2016;113:6388–96.

Bossdorf O, Richards CL, Pigliucci M. Epigenetics for ecologists. Ecol Lett. 2008;11:106–15.

Bouchemousse S, Liautard-Haag C, Bierne N, Viard F. Distinguishing contemporary hybridization from past introgression with postgenomic ancestry-informative SNPs in strongly differentiated *Ciona* species. Mol Ecol. 2016a;25:5527–42.

Bouchemousse S, Bishop JDD, Viard F. Contrasting global genetic patterns in two biologically similar, widespread and invasive *Ciona* species (Tunicata, Ascidiacea). Sci Rep. 2016b;6:24875.

Bragg JG, Supple MA, Andrew RL, Borevitz JO. Genomic variation across landscapes: insights and applications. New Phytol. 2015;207:953–67.

Breiman L. Random forests. Mach Learn. 2001;45:5–32.

Brown PMJ, Thomas CE, Lombaert E, Jeffries DL, Estoup A, Lawson Handley L-J. The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. BioControl. 2011;56:623.

Butterfield JSS, Díaz-Ferguson E, Silliman BR, Saunders JW, Buddo D, Mignucci-Giannoni AA, Searle L, Allen AC, Hunter ME. Wide-ranging phylogeographic structure of invasive red lionfish in the western Atlantic and greater Caribbean. Mar Biol. 2015;162:773–81.

Carlson SM, Cunningham CJ, Westley PAH. Evolutionary rescue in a changing world. Trends Ecol Evol. 2014;29:521–30.

Carlton JT. The scale and ecological consequences of biological invasions in the world's oceans. In: Sandlund O, Schei P, Viken Å, editors. Invasive species and biodiversity management. Dordrecht: Kluwer; 1999. p. 195–212.

Carroll SP. Natives adapting to invasive species: ecology, genes, and the sustainability of conservation. Ecol Res. 2007;22:892–901.

Casacuberta E, González J. The impact of transposable elements in environmental adaptation. Mol Ecol. 2013;22:1503–17.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. Stacks: an analysis tool set for population genomics. Mol Ecol. 2013;22:3124–40.

Catford JA, Jansson R, Nilsson C. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers Distrib. 2009;15:22–40.

Chown SL, Hodgins KA, Griffin PC, Oakeshott JG, Byrne M, Hoffmann AA. Biological invasions, climate change and genomics. Evol Appl. 2015;8:23–46.

Clark GF, Johnston EL. Propagule pressure and disturbance interact to overcome biotic resistance of marine invertebrate communities. Oikos. 2009;118:1679–86.

Colautti RI, Barrett SCH. Rapid adaptation to climate facilitates range expansion of an introduced plant. Science. 2013;342:364–6.

Colautti RI, MacIsaac HJ. A neutral terminology to define "invasive" species. Divers Distrib. 2004;10:135–41.

Colautti RI, Manca M, Viljanen M, Ketelaars HAM, Bürgi H, Macisaac HJ, Heath DD. Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and gene flow using microsatellites. Mol Ecol. 2005;14:1869–79.

Collins RA, Armstrong KF, Holyoake AJ, Keeling S. Something in the water: biosecurity monitoring of ornamental fish imports using environmental DNA. Biol Invasions. 2013;15:1209–15.

Corbett JJ, Winebrake J. The impacts of globalisation on international maritime transport activity. Global forum on transport and environment in a globalising world. Guadalajara, Mexico: OECD; 2008.

Cordero D, Delgado M, Liu B, Ruesink J, Saavedra C. Population genetics of the Manila clam (*Ruditapes philippinarum*) introduced in North America and Europe. Sci Rep. 2017;7:39745.

Coyne JA, Orr HA. Speciation. Sunderland, MA: Sinauer Associates; 2004.

Cristescu ME. Genetic reconstructions of invasion history. Mol Ecol. 2015;24:2212–25.

Crooks JA, Soulé ME, Sandlund O. Lag times in population explosions of invasive species: causes and implications. In: Sandlund O, Schei P, Viken A, editors. Invasive species and biodiversity management. Population and Community Biology Series. 1st ed. vol 24. Netherlands: Kluwer Academic Publishers; 1999. pp. 103–25.

Curnutt JL. Host-area specific climatic-matching. Biol Conserv. 2000;94:341–51.

da Fonseca RR, Albrechtsen A, Themudo GE, Ramos-Madrigal J, Sibbesen JA, Maretty L, Zepeda-Mendoza ML, Campos PF, Heller R, Pereira RJ. Next-generation biology: sequencing and data analysis approaches for non-model organisms. Mar Genomics. 2016;30:3–13.

Darling JA, Frederick RM. Nucleic acids-based tools for ballast water surveillance, monitoring, and research. J Sea Res. 2017;33:43–52.

Davidson AM, Jennions M, Nicotra AB. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol Lett. 2011;14:419–31.

Davy CM, Kidd AG, Wilson CC. Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. PLoS One. 2015;10:e0130965.

Deiner K, Altermatt F. Transport distance of invertebrate environmental DNA in a Natural River. PLoS One. 2014;9:e88786A.

Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. J Appl Ecol. 2012;49:953–9.

de Vicente MC, Tanksley SD. QTL analysis of Transgressive segregation in an interspecific tomato cross. Genetics. 1993;134:585–96.

Dlugosch KM, Parker IM. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol. 2008;17:431–49.

Dlugosch KM, Anderson SR, Braasch J, Cang FA, Gillette HD. The devil is in the details: genetic variation in introduced populations and its contributions to invasion. Mol Ecol. 2015;24:2095–111.

Dong K, Yao N, Pu Y, He X, Zhao Q, Luan Y, Guan W, Rao S, Ma Y. Genomic scan reveals loci under altitude adaptation in Tibetan and Dahe pigs. PLoS One. 2014;9:e110520.

Dougherty MM, Larson ER, Renshaw MA, Gantz CA, Egan SP, Erickson DM, Lodge DM. Environmental DNA (eDNA) detects the invasive rusty crayfish *Orconectes rusticus* at low abundances. J Appl Ecol. 2016;53:722–32.

Drake JM. Heterosis, the catapult effect and establishment success of a colonizing bird. Biol Lett. 2006;2:304–7.

Drake JM, Lodge DM. Hull fouling is a risk factor for intercontinental species exchange in aquatic ecosystems. Aquat Invasions. 2007;2:121–31.

Dukes JS. Responses of invasive species to a changing climate and atmosphere. In: Richardson DM, editor. Fifty years of invasion ecology: the legacy of Charles Elton. 1st ed. Oxford: Wiley-Blackwell; 2010.

Edmands S, Northrup SL, Hwang AS. Maladapted gene complexes within populations of the intertidal copepod *Tigriops californicus*. Evolution. 2009;63:2184–92.

Egan SP, Grey E, Olds B, Feder JL, Ruggiero ST, Tanner CE, Lodge DM. Rapid molecular detection of invasive species in ballast and harbor water by integrating environmental DNA and light transmission spectroscopy. Environ Sci Technol. 2015;49:4113–21.

Ehrenfeld JG. Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst. 2010;41:59–80.

Ekblom R, Galindo J. Applications of next-generation sequencing in molecular ecology of non-model organisms. Heredity (Edinb). 2011;107:1–15.

Ellstrand NC, Schierenbeck KA. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc Natl Acad Sci. 2000;97:7043–50.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One. 2011;6:e19379.

Elst EM, Acharya KP, Dar PA, Reshi ZA, Tufto J, Nijs I, Graae BJ. Pre-adaptation or genetic shift after introduction in the invasive species *Impatiens glandulifera*? Acta Oecol. 2016;70:60–6.

Estoup A, Guillemaud T. Reconstructing routes of invasion using genetic data: why, how and so what? Mol Ecol. 2010;19:4113–30.

Esvelt KM, Smidler AL, Catteruccia F, Church GM. Concerning RNA-guided gene drives for the alteration of wild populations. Elife. 2014;3:e03401.

European Bioinformatics Institute. EMBL-EBI annual scientific report EMBL–EBI, 2013. 2012.

Excoffier L, Ray N. Surfing during population expansions promotes genetic revolutions and structuration. Trends Ecol Evol. 2008;23:347–51.

Ferreira CEL, Luiz OJ, Floeter SR, Lucena MB, Barbosa MC, Rocha CR, Rocha LA. First record of invasive lionfish (*Pterois volitans*) for the Brazilian coast. PLoS One. 2015;10:e0123002.

Ficetola GF, Miaud C, Pompanon F, Taberlet P. Species detection using environmental DNA from water samples. Biol Lett. 2008;4:423–5.

Ficetola GF, Taberlet P, Coissac E. How to limit false positives in environmental DNA and metabarcoding? Mol Ecol Resour. 2016;16:604–7.

Fisher MA, Oleksiak MF. Convergence and divergence in gene expression among natural populations exposed to pollution. BMC Genomics. 2007;8:108.

Florida Fish and Wildlife Conservation Commission (2017) Lionfish – *Pterois volitans*. http://myfwc.com/wildlifehabitats/nonnatives/marine-species/lionfish/.

Foote AD, Thomsen PF, Sveegaard S, Wahlberg M, Kielgast J, Kyhn LA, Salling AB, Galatius A, Orlando L, Gilbert MT. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. PLoS One. 2012;7:e41781.

Forsström T, Vasemägi A. Can environmental DNA (eDNA) be used for detection and monitoring of introduced crab species in the Baltic Sea? Mar Pollut Bull. 2016;109:350–5.

Frey M, Simard N, Robichaud D, Martin J, Therriault T. Fouling around: vessel sea-chests as a vector for the introduction and spread of aquatic invasive species. Manag Biol Invasions. 2014;5:21–30.

Furlan EM, Stoklosa J, Griffiths J, Gust N, Ellis R, Huggins RM, Weeks AR. Small population size and extremely low levels of genetic diversity in island populations of the platypus, *Ornithorhynchus anatinus*. Ecol Evol. 2012;2:844–57.

Gaither MR, Toonen RJ, Bowen BW. Coming out of the starting blocks: extended lag time rearranges genetic diversity in introduced marine fishes of Hawai'i. Proc R Soc B Biol Sci. 2012;279:3948–57.

Garalde DR, Snell EA, Jachimowicz D, Sipos B, Lloyd JH, Bruce M, Pantic N, Admassu T, James P, Warland A, Jordan M, Ciccone J, Serra S, Keenan J, Martin S, McNeill L, Wallace EJ, Jayasinghe L, Wright C, Blasco J, Young S, Brocklebank D, Juul S, Clarke J, Heron AJ, Turner DJ. Highly parallel direct RNA sequencing on an array of nanopores. Nat Meth. 2018;15:201–6.

Gardner JPA, Zbawicka M, Westfall KM, Wenne R. Invasive blue mussels threaten regional scale genetic diversity in mainland and remote offshore locations: the need for baseline data and enhanced protection in the Southern Ocean. Glob Chang Biol. 2016;22:3182–95.

Ghabooli S, Zhan A, Sardiña P, Paolucci E, Sylvester F, Perepelizin PV, Briski E, Cristescu ME, MacIsaac HJ. Genetic diversity in introduced golden mussel populations corresponds to vector activity. PLoS One. 2013;8:e59328.

Gilchrist GW, Lee CE. All stressed out and nowhere to go: does evolvability limit adaptation in invasive species? Genetica. 2007;129:127–32.

Gillis NK, Walters LJ, Fernandes FC, Hoffman EA. Higher genetic diversity in introduced than in native populations of the mussel *Mytella charruana*: evidence of population admixture at introduction sites. Divers Distrib. 2009;15:784–95.

Gleason LU, Burton RS. RNA-seq reveals regional differences in transcriptome response to heat stress in the marine snail *Chlorostoma funebralis*. Mol Ecol. 2015;24:610–27.

Gleason LU, Burton RS. Genomic evidence for ecological divergence against a background of population homogeneity in the marine snail *Chlorostoma funebralis*. Mol Ecol. 2016a;25:3557–73.

Gleason LU, Burton RS. Regional patterns of thermal stress and constitutive gene expression in the marine snail *Chlorostoma funebralis* in northern and southern California. Mar Ecol Prog Ser. 2016b;556:143–59.

Glotzbecker GJ, Alda F, Broughton RE, Neely DA, Mayden RL, Blum MJ. Geographic independence and phylogenetic diversity of red shiner introductions. Conserv Genet. 2016;17:795–809.

Golani D. The sandy shore of the Red Sea-launching pad for Lessepsian (Suez Canal) migrant fish colonizers of the eastern Mediterranean. J Biogeogr. 1993;20:579–85.

Golani D, Ben-Tuvia A. Characterisation of Lessepsian (Suez Canal) fish migrants. In: Spanier E, Steinberger Y, Lurie M, editors. Environmental quality and ecosystem stability: 1V-B. Jerusalem, Israel: ISEEQS; 1989. p. 235–43.

Golani D, Sonin O. New records of the Red Sea fishes, *Pterois miles* (Scorpaenidae) and *Pteragogus pelycus* (Labridae) from the eastern Mediterranean Sea. Jpn J Ichthyol. 1992;39:167–9.

Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). Freshwater Sci. 2013;32:792–800.

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-McCance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS, Strickler KM, Waits LP, Fremier AK, Takahara T, Herder JE, Taberlet P. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol. 2016;7:1299–307.

Gonzalez A, Ronce O, Ferriere R, Hochberg ME. Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. Philos Trans R Soc B Biol Sci. 2013;368:20120404.

Green SJ, Akins JL, Maljković A, Côté IM. Invasive lionfish drive Atlantic coral reef fish declines. PLoS One. 2012;7:e32596.

Guardiola M, Frotscher J, Uriz MJ. High genetic diversity, phenotypic plasticity, and invasive potential of a recently introduced calcareous sponge, fast spreading across the Atlanto-Mediterranean basin. Mar Biol. 2016;163:123.

Guillemaud T, Beaumont MA, Ciosi M, Cornuet JM, Estoup A. Inferring introduction routes of invasive species using approximate Bayesian computation on microsatellite data. Heredity (Edinb). 2010;104:88–99.

Guillera-Arroita G, Lahoz-Monfort JJ, van Rooyen AR, Weeks AR, Tingley R. Dealing with false-positive and false-negative errors about species occurrence at multiple levels. Methods Ecol Evol. 2017;8:1081–91.

Guo W-Y, Lambertini C, Nguyen LX, Li X-Z, Brix H. Preadaptation and post-introduction evolution facilitate the invasion of *Phragmites australis* in North America. Ecol Evol. 2014;4:4567–77.

Guo B, DeFaveri J, Sotelo G, Nair A, Merilä J. Population genomic evidence for adaptive differentiation in Baltic Sea three-spined sticklebacks. BMC Biol. 2015;13:19.

Hallatschek O, Nelson DR. Gene surfing in expanding populations. Theor Popul Biol. 2008;73:158–70.

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R. A global map of human impact on marine ecosystems. Science. 2008;319:948–52.

Hamilton JA, Okada M, Korves T, Schmitt J. The role of climate adaptation in colonization success in *Arabidopsis thaliana*. Mol Ecol. 2015;24:2253–63.

Hamner RM, Freshwater DW, Whitfield PE. Mitochondrial cytochrome b analysis reveals two invasive lionfish species with strong founder effects in the western Atlantic. J Fish Biol. 2007;71:214–22.

Hand BK, Hether TD, Kovach RP, Muhlfeld CC, Amish SJ, Boyer MC, O'Rourke SM, Miller MR, Lowe WH, Hohenlohe PA, Luikart G. Genomics and introgression: discovery and mapping of thousands of species-diagnostic SNPs using RAD sequencing. Curr Zool. 2015;61:146–54.

Hänfling B, Bolton P, Harley M, Carvalho GR. A molecular approach to detect hybridisation between crucian carp (*Carassius carassius*) and non-indigenous carp species (*Carassius* spp. and *Cyprinus carpio*). Freshw Biol. 2005;50:403–17.

Hare JA, Whitfield PE. An integrated assessment of the introduction of lionfish (*Pterois volitans/miles* complex) to the western Atlantic Ocean. NOAA Technical Memorandum NOS NCCOS 2. 2003. 21 pp.

Harvey-Samuel T, Ant T, Alphey L. Towards the genetic control of invasive species. Biol Invasions. 2017;19:1683–703.

Hedge LH, O'Connor WA, Johnston EL. Manipulating the intrinsic parameters of propagule pressure: implications for bio-invasion. Ecosphere. 2012;3:1–13.

Hegarty MJ. Invasion of the hybrids. Mol Ecol. 2012;21:4669–71.

Hemmer-Hansen J, Therkildsen NO, Pujolar JM. Population genomics of marine fishes: next-generation prospects and challenges. Biol Bull. 2014;227:117–32.

Henery ML, Bowman G, Mráz P, Treier UA, Gex-Fabry E, Schaffner U, Müller-Schärer H. Evidence for a combination of pre-adapted traits and rapid adaptive change in the invasive plant *Centaurea stoebe*. J Ecol. 2010;98:800–13.

Hoban S, Kelley D, Lotterhos K, Antolin M, Bradburd G, Lowry DB, Poss M, Reed L, Storfer A, Whitlock M. Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. Am Nat. 2016;188:379–97.

Hofmann GE. Ecological epigenetics in marine metazoans. Front Mar Sci. 2017;4:4.

Hohenlohe PA, Amishm SJ, Catchen JM, Allendorf FW, Luikart G. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Mol Ecol Resour. 2011;11:117–22.

Holle BV, Simberloff D. Ecological resistance to biological invasion overwhelmed by Propagule pressure. Ecology. 2005;86:3212–8.

Huang X, Li S, Ni P, Gao Y, Jiang B, Zhou Z, Zhan A. Rapid response to changing environments during biological invasions: DNA methylation perspectives. Mol Ecol. 2017;26:6621–33.

Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L. Rapid evolution of a geographic cline in size in an introduced fly. Science. 2000;287:308–9.

Huey RB, Gilchrist A, Hendry AP. Using invasive species to study evolution. In: Sax DF, Stachowicz JJ, Gaines SD, editors. Species invasions: insights into ecology, evolution and biogeography. Sunderland, MA: Sinauer Associates; 2005. p. 139–64.

Hulme PE. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J Appl Ecol. 2009;46:10–8.

Huxel GR. Rapid displacement of native species by invasive species: effects of hybridization. Biol Conserv. 1999;89:143–52.

IMO. International facts and figures – information resources on trade, safety, security, environment. Maritime Knowledge Centre; 2012.

Jeffery NW, DiBacco C, Van Wyngaarden M, Hamilton LC, Stanley RRE, Bernier R, FitzGerald J, Matheson K, McKenzie CH, Nadukkalam Ravindran P, Beiko R, Bradbury IR. RAD sequencing reveals genomewide divergence between independent invasions of the European green crab (*Carcinus maenas*) in the Northwest Atlantic. Ecol Evol. 2017;7:2513–24.

Jerde CL, Chadderton WL, Mahon AR, Renshaw MA, Corush J, Budny ML, Mysorekar S, Lodge DM. Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. Can J Fish Aquat Sci. 2013;70:522–6.

Johnston EL, Piola RF, Clark GF. The role of propagule pressure in invasion success. In: Rilov G, Crooks JA, editors. Biological invasions in marine ecosystems, ecological studies 204. Berlin: Springer; 2009.

Johnston SE, Orell P, Pritchard VL, Kent MP, Lien S, Niemelä E, Erkinaro J, Primmer CR. Genome-wide SNP analysis reveals a genetic basis for sea-age variation in a wild population of Atlantic salmon (*Salmo salar*). Mol Ecol. 2014;23:3452–68.

Johnson J, Bird CE, Johnston MA, Fogg AQ, Hogan JD. Regional genetic structure and genetic founder effects in the invasive lionfish: comparing the Gulf of Mexico, Caribbean and North Atlantic. Mar Biol. 2016;163:216.

Jombart T. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 2008;24(11):1403–5.

Keller L, Waller D. Inbreeding effects in wild populations. Trends Ecol Evol. 2002;17:230–41.

Kelly DW, Muirhead JR, Heath DD, Macisaac HJ. Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters. Mol Ecol. 2006;15:3641–53.

Kelly RP, Port JA, Yamahara KM, Crowder LB. Using environmental DNA to census marine fishes in a large mesocosm. PLoS One. 2014;9:e86175.

Klymus KE, Richter CA, Chapman DC, Paukert C. Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. Biol Conserv. 2015;183:77–84.

Kolar CS, Lodge DM. Freshwater nonindigenous species: interactions with other global changes. In: Mooney HA, Hobbs RJ, editors. Invasive species in a changing world. Washington, DC: Island Press; 2000. p. 3–30.

Konečný A, Estoup A, Duplantier J-M, Bryja J, Bâ K, Galan M, Tatard C, Cosson J-F. Invasion genetics of the introduced black rat (*Rattus rattus*) in Senegal, West Africa. Mol Ecol. 2013;22:286–300.

Kovach RP, Hand BK, Hohenlohe PA, Cosart TF, Boyer MC, Neville HH, Muhlfeld CC, Amish SJ, Carim K, Narum SR, Lowe WH, Allendorf FW, Luikart G. Vive la résistance: genome-wide selection against introduced alleles in invasive hybrid zones. Proc R Soc B Biol Sci. 2016;283:1843.

Kumschick S, Gaertner M, Vilà M, Essl F, Jeschke JM, Pyšek P, Ricciardi A, Bacher S, Blackburn TM, Dick JTA, Evans T, Hulme PE, Kühn I, Mrugała A, Pergl J, Rabitsch W, Richardson DM, Sendek A, Winter M. Ecological impacts of alien species: quantification, scope, caveats, and recommendations. Bioscience. 2015;65:55–63.

Lacoursière-Roussel A, Côté G, Leclerc V, Bernatchez L. Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. J Appl Ecol. 2016;53:1148–57.

Lancaster ML, Bradshaw CJA, Goldsworthy SD, Sunnucks P. Lower reproductive success in hybrid fur seal males indicates fitness costs to hybridization. Mol Ecol. 2007;16:3187–97.

Lahoz-Monfort JJ, Guillera-Arroita G, Tingley R. Statistical approaches to account for false-positive errors in environmental DNA samples. Mol Ecol Resour. 2016;16:673–85.

Lande R. Evolution of phenotypic plasticity in colonizing species. Mol Ecol. 2015;24:2038–45.

Larson ER, Renshaw MA, Gantz CA, Umek J, Chandra S, Lodge DM, Egan SP. Environmental DNA (eDNA) detects the invasive crayfishes *Orconectes rusticus* and *Pacifastacus leniusculus* in large lakes of North America. Hydrobiologia 2017;800:173–85.

Lau JA, terHorst CP. Causes and consequences of failed adaptation to biological invasions: the role of ecological constraints. Mol Ecol. 2015;24:1987–98.

Lee CE. Evolutionary genetics of invasive species. Trends Ecol Evol. 2002:386–91.

Lin Y, Chen Y, Yi C, Fong JJ, Kim W, Rius M, Zhan A. Genetic signatures of natural selection in a model invasive ascidian. Sci Rep. 2017;7:44080.

Lippman ZB, Zamir D. Heterosis: revisiting the magic. Trends Genet. 2007;23:60–6.

Lockwood JL, Cassey P, Blackburn T. The role of propagule pressure in explaining species invasions. Trends Ecol Evol. 2005;20:223–8.

Lockwood BL, Sanders JG, Somero GN. Transcriptomic responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. J Exp Biol. 2010;213(20):3548–58.

Lombaert E, Guillemaud T, Cornuet J-M, Malausa T, Facon B, Estoup A. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLoS One. 2010;5:e9743.

Lowry E, Rollinson EJ, Laybourn AJ, Scott TE, Aiello-Lammens ME, Gray SM, Mickley J, Gurevitch J. Biological invasions: a field synopsis, systematic review, and database of the literature. Ecol Evol. 2013;3:182–96.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet. 2003;4:981–94.

Mahon AR, Jerde CL, Galaska M, Bergner JL, Chadderton WL, Lodge DM, Hunter ME, Nico LG. Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments. PLoS One. 2013;8:e58316.

Mahon AR, Nathan LR, Jerde CL. Meta-genomic surveillance of invasive species in the bait trade. Conserv Genet Resour. 2014;6:563–7.

Marescaux J, von Oheimb KCM, Etoundi E, von Oheimb PV, Albrecht C, Wilke T, Van Doninck K. Unravelling the invasion pathways of the quagga mussel (*Dreissena rostriformis*) into Western Europe. Biol Invasions. 2016;18:245–64.

Marguerat S, Wilhelm Brian T, Bähler J. Next-generation sequencing: applications beyond genomes. Biochem Soc Trans. 2008;36(Pt 5):1091–6.

Maruyama A, Nakamura K, Yamanaka H, Kondoh M, Minamoto T. The release rate of environmental DNA from juvenile and adult fish. PLoS One. 2014;9:e114639.

Marx V. Biology: the big challenges of big data. Nature. 2013;498:255–60.

Masters G, Norgrove L. Climate change and invasive alien species. CABI Working Paper 1. 2010.

May P, Liao W, Wu Y, Shuai B, Richard McCombie W, Zhang MQ, Liu QA. The effects of carbon dioxide and temperature on microRNA expression in *Arabidopsis* development. Nat Commun. 2013;4:2145.

McClintock B. The origin and behavior of mutable loci in maize. Proc Natl Acad Sci. 1950;36:344–55.

McKnight E, García-Berthou E, Srean P, Rius M. Global meta-analysis of native and nonindigenous trophic traits in aquatic ecosystems. Glob Chang Biol. 2017;23:1861–70.

McQuillan JS, Robidart JC. Molecular-biological sensing in aquatic environments: recent developments and emerging capabilities. Curr Opin Biotechnol. 2017;45(Suppl C):43–50.

Mead A, Carlton JT, Griffiths CL, Rius M. Revealing the scale of marine bioinvasions in developing regions, a south African re-assessment. Biol Invasions. 2011;13:1991–2008.

Meilink WRM, Arntzen JW, van Delft JJCW, Wielstra B. Genetic pollution of a threatened native crested newt species through hybridization with an invasive congener in the Netherlands. Biol Conserv. 2015;184:145–53.

Minchin D, Gollasch S. Fouling and Ships' Hulls: how changing circumstances and spawning events may result in the spread of exotic species. Biofouling. 2003;19:111–22.

Molnar JL, Gamboa RL, Revenga C, Spalding MD. Assessing the global threat of invasive species to marine biodiversity. Front Ecol Environ. 2008;6:485–92.

Moran EV, Alexander JM. Evolutionary responses to global change: lessons from invasive species. Ecol Lett. 2014;17:637–49.

Muhlfeld CC, Kovach RP, Jones LA, Al-Chokhachy R, Boyer MC, Leary RF, Lowe WH, Luikart G, Allendorf FW. Invasive hybridization in a threatened species is accelerated by climate change. Nat Clim Change. 2014;4:620–4.

Narum SR, Gallardo P, Correa C, Matala A, Hasselman D, Sutherland BJG, Bernatchez L. Genomic patterns of diversity and divergence of two introduced salmonid species in Patagonia, South America. Evol Appl. 2017;10:402–16.

Nathan LR, Jerde CL, Budny ML, Mahon AR. The use of environmental DNA in invasive species surveillance of the Great Lakes commercial bait trade. Conserv Biol. 2015;29:430–9.

Naylor RL, Williams SL, Strong DR. Aquaculture – a gateway for exotic species. Science. 2001;294:1655–6.

NHGRI. The cost of sequencing a human genome. 2016. https://www.genome.gov/sequencingcosts/.

Nydam ML, Yanckello LM, Bialik SB, Giesbrecht KB, Nation GK, Peak JL. Introgression in two species of broadcast spawning marine invertebrate. Biol J Linn Soc. 2017a;120:879–90.

Nydam ML, Giesbrecht KB, Stephenson EE. Origin and dispersal history of two colonial ascidian clades in the *Botryllus schlosseri* species complex. PLoS One. 2017b;12:e0169944.

Ocaña K, de Oliveira D. Parallel computing in genomic research: advances and applications. Adv Appl Bioinforma Chemistry. 2015;8:23–35.

Oduor AMO. Evolutionary responses of native plant species to invasive plants: a review. New Phytol. 2013;200:986–92.

Ometto L, Cestaro A, Ramasamy S, Grassi A, Revadi S, Siozios S, Moretto M, Fontana P, Varotto C, Pisani D, Dekker T, Wrobel N, Viola R, Pertot I, Cavalieri D, Blaxter M, Anfora G, Rota-Stabelli O. Linking genomics and ecology to investigate the complex evolution of an invasive Drosophila Pest. Genome Biol Evol. 2013;5:745–57.

Orr HA, Turelli M. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. Evolution. 2001;55:1085–94.

Oyarzún PA, Toro JE, Cañete JI, Gardner JPA. Bioinvasion threatens the genetic integrity of native diversity and a natural hybrid zone: smooth-shelled blue mussels (*Mytilus* spp.) in the strait of Magellan. Biol J Linn Soc. 2016;117:574–85.

Padilla DK, Williams SL. Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Front Ecol Environ. 2004;2:131–8.

Pardo-Diaz C, Salazar C, Jiggins CD. Towards the identification of the loci of adaptive evolution. Methods Ecol Evol. 2015;6:445–64.

Parente TE, Moreira DA, Magalhães MGP, de Andrade PCC, Furtado C, Haas BJ, Stegeman JJ, Hahn ME. The liver transcriptome of suckermouth armoured catfish (*Pterygoplichthys anisitsi*, Loricariidae): identification of expansions in defensome gene families. Mar Pollut Bull. 2017;115:352–61.

Payseur BA, Rieseberg LH. A genomic perspective on hybridization and speciation. Mol Ecol. 2016;25:2337–60.

Pedreschi D, Kelly-Quinn M, Caffrey J, O'Grady M, Mariani S. Genetic structure of pike (*Esox lucius)* reveals a complex and previously unrecognized colonization history of Ireland. J Biogeogr. 2014;41:548–60.

Pérez-Portela R, Bumford A, Coffman B, Wedelich S, Davenport M, Fogg A, Swenarton MK, Coleman F, Johnston MA, Crawford DL, Oleksiak MF. Genetic homogeneity of the invasive lionfish across the Northwestern Atlantic and the Gulf of Mexico based on single nucleotide polymorphisms. Sci Rep. 2018;8:5062.

Perry WL, Feder JL, Lodge DM. Implications of hybridization between introduced and resident *Orconectes* crayfishes. Conserv Biol. 2001;15:1656–66.

Pfeifer B, Wittelsburger U, Ramos-Onsins SE, Lercher MJ. PopGenome: an efficient Swiss army knife for population genomic analyses in R. Mol Biol Evol. 2014;31:1929–36.

Phillips BL, Shine R. Adapting to an invasive species: toxic cane toads induce morphological change in Australian snakes. Proc Natl Acad Sci U S A. 2004;101:17150–5.

Piaggio AJ, Engeman RM, Hopken MW, Humphrey JS, Keacher KL, Bruce WE, Avery ML. Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. Mol Ecol Resour. 2014;14:374–80.

Pigliucci M. Phenotypic plasticity: beyond nature and nurture. Baltimore: JHU Press; 2001.

Pimentel D, Zuniga R, Morrison D. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ. 2005;52:273–88.

Pray LA. Transposons: the jumping genes. Nat Educ. 2008;1:204.

Prentis PJ, Wilson JR, Dormontt EE, Richardson DM, Lowe AJ. Adaptive evolution in invasive species. Trends Plant Sci. 2008;13:288–94.

Pu C, Zhan A. Epigenetic divergence of key genes associated with water temperature and salinity in a highly invasive model ascidian. Biol Invasions. 2017;19:2015–28.

Pudlo P, Marin J-M, Estoup A, Cornuet J-M, Gautier M, Robert CP. Reliable ABC model choice via random forests. Bioinformatics. 2016;32:859–66.

Purcell KM, Ling N, Stockwell CA. Evaluation of the introduction history and genetic diversity of a serially introduced fish population in New Zealand. Biol Invasions. 2012;14:2057–65.

Puzey J, Vallejo-Marin M. Genomics of invasion: diversity and selection in introduced populations of monkeyflowers (*Mimulus guttatus*). Mol Ecol. 2014;23:4472–85.

Pyšek P, Richardson DM. Invasive species, environmental change and management, and health. Annu Rev Env Resour. 2010;35:25–55.

Rahel FJ, Bierwagen B, Taniguchi Y. Managing aquatic species of conservation concern in the face of climate change and invasive species Manejo de Especies Acuáticas de Interés para la Conservación ante el Cambio Climático y las Especies Invasoras. Conserv Biol. 2008;22:551–61.

Rašić G, Filipović I, Weeks AR, Hoffmann AA. Genome-wide SNPs lead to strong signals of geographic structure and relatedness patterns in the major arbovirus vector, *Aedes aegypti*. BMC Genomics. 2014;15:275.

Reitzel AM, Herrera S, Layden MJ, Martindale MQ, Shank TM. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Mol Ecol. 2013;22:2953–70.

Rejmánek M, Richardson DM. What attributes make some plant species more invasive? Ecology. 1996;77:1655–61.

Rhymer JM, Simberloff D. Extinction by hybridization and introgression. Annu Rev Ecol Syst. 1996;27:83–109.

Ricciardi A, Macisaac HJ. Impacts of biological invasions on freshwater ecosystems. In: Fifty years of invasion ecology. Wiley-Blackwell; 2010. p. 211–24.

Ricciardi A, Blackburn TM, Carlton JT, Dick JTA, Hulme PE, Iacarella JC, Jeschke JM, Liebhold AM, Lockwood JL, MacIsaac HJ, Pyšek P, Richardson DM, Ruiz GM, Simberloff D, Sutherland WJ, Wardle DA, Aldridge DC. Invasion science: a horizon scan of emerging challenges and opportunities. Trends Ecol Evol. 2017;32:464–74.

Richardson MF, Sherman CDH. De novo assembly and characterization of the invasive northern Pacific Seastar Transcriptome. PLoS One. 2015;10:e0142003.

Richardson DM, Pysek P, Rejmánek M, Barbour MG, Panetta FD, West CJ. Naturalization and invasion of alien plants-concepts and definitions. Divers Distrib. 2000;6:93–107.

Rieseberg LH, Archer MA, Wayne RK. Transgressive segregation, adaptation and speciation. Heredity (Edinb). 1999;83:363–72.

Riquet F, Daguin-Thiebaut C, Ballenghien M, Bierne N, Viard F. Contrasting patterns of genome-wide polymorphism in the native and invasive range of the marine mollusc *Crepidula fornicata*. Mol Ecol. 2013;22:1003–18.

Rius M, Darling JA. How important is intraspecific genetic admixture to the success of colonising populations? Trends Ecol Evol. 2014;29:233–42.

Rius M, Turon X, Ordonez V, Pascual M. Tracking invasion histories in the sea: facing complex scenarios using multilocus data. PLoS One. 2012;7:e35815.

Rius M, Clusella-Trullas S, McQuaid CD, Navarro RA, Griffiths CL, Matthee CA, von der Heyden S, Turon X. Range expansions across ecoregions: interactions of climate change, physiology and genetic diversity. Glob Ecol Biogeogr. 2014;23:76–88.

Rius M, Bourne S, Hornsby H, Chapman D. Applications of next-generation sequencing to the study of biological invasions. Curr Zool. 2015a;61:488–504.

Rius M, Turon X, Bernardi G, Volckaert FAM, Viard F. Marine invasion genetics: from spatio-temporal patterns to evolutionary outcomes. Biol Invasions. 2015b;17:869–85.

Roderick GK, Navajas M. Genes in new environments: genetics and evolution in biological control. Nat Rev Genet. 2003;4:889–99.

Roman J, Darling JA. Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol. 2007;22:454–64.

Romiguier J, Gayral P, Ballenghien M, Bernard A, Cahais V, Chenuil A, Chiari Y, Dernat R, Duret L, Faivre N, Loire E, Lourenco JM, Nabholz B, Roux C, Tsagkogeorga G, Weber AAT, Weinert LA, Belkhir K, Bierne N, Glemin S, Galtier N. Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature. 2014;515:261–3.

Rossman AY. A special issue on global movement of invasive plants and fungi. Bioscience. 2001;51:93–4.

Saarman NP, Pogson GH. Introgression between invasive and native blue mussels (genus *Mytilus*) in the Central California hybrid zone. Mol Ecol. 2015;24:4723–38.

Salvi D, Macali A, Mariottini P. Molecular phylogenetics and systematics of the bivalve family Ostreidae based on rRNA sequence-structure models and multilocus species tree. PLoS One. 2014;9:e108696.

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG. The population biology of invasive species. Annu Rev Ecol Evol Syst. 2001;32:305–32.

Sassenhagen I, Wilken S, Godhe A, Rengefors K. Phenotypic plasticity and differentiation in an invasive freshwater microalga. Harmful Algae. 2015;41:38–45.

Sassoubre LM, Yamahara KM, Gardner LD, Block BA, Boehm AB. Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. Environ Sci Technol. 2016;50:10456–64.

Sato A, Bishop JD. Field identification of "types" A and B of the ascidian *Ciona intestinalis* in a region of sympatry. Mar Biol. 2012;159:1611–9.

Sax DF, Stachowicz JJ, Gaines SD. Introduction. In: Stachowicz JJ, Gaines SD, editors. Species invasions: insights into ecology, evolution, and biogeography. Sunderland, MA: Sinauer Associates Inc.; 2005.

Schlaepfer DR, Glättli M, Fischer M, van Kleunen M. A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. New Phytol. 2010;185:1087–99.

Scholin CA. What are "ecogenomic sensors?" A review and thoughts for the future. Ocean Sci. 2010;6:51–60.

Schrader L, Kim JW, Ence D, Zimin A, Klein A, Wyschetzki K, Weichselgartner T, Kemena C, Stökl J, Schultner E, Wurm Y, Smith CD, Yandell M, Heinze J, Gadau J, Oettler J. Transposable element islands facilitate adaptation to novel environments in an invasive species. Nat Commun. 2014;5:5495.

Schultz MT, Lance RF. Modeling the sensitivity of field surveys for detection of environmental DNA (eDNA). PLoS One. 2015;10:e0141503.

Scriver M, Marinich A, Wilson C, Freeland J. Development of species-specific environmental DNA (eDNA) markers for invasive aquatic plants. Aquat Bot. 2015;122:27–31.

Sedlazeck FJ, Lee H, Darby CA, Schatz MC. Piercing the dark matter: bioinformatics of long-range sequencing and mapping. Nat Rev Gen. 2018. https://doi.org/10.1038/s41576-018-0003-4.

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F. No saturation in the accumulation of alien species worldwide. Nat Commun. 2017;8:14435.

Sherman CDH, Lotterhos KE, Richardson MF, Tepolt CK, Rollins LA, Palumbi SR, Miller AD. What are we missing about marine invasions? Filling in the gaps with evolutionary genomics. Mar Biol. 2016;163:198.

Sigsgaard EE, Nielsen IB, Bach SS, Lorenzen ED, Robinson DP, Knudsen SW, Pedersen MW, Jaidah MA, Orlando L, Willerslev E, Møller PR, Thomsen PF. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. Nat Ecol Evol. 2016;1:0004.

Sigsgaard EE, Nielsen IB, Carl H, Krag MA, Knudsen SW, Xing Y, Holm-Hansen TH, Møller PR, Thomsen PF. Seawater environmental DNA reflects seasonality of a coastal fish community. Mar Biol. 2017;164:128.

Simberloff D. The role of propagule pressure in biological invasions. Annu Rev Ecol Evol Syst. 2009;40:81–102.

Simberloff D. Biological invasions: prospects for slowing a major global change. Elem Sci Anth. 2013;1:000008.

Simon A, Britton R, Gozlan R, van Oosterhout C, Volckaert FAM, Hänfling B. Invasive cyprinid fish in Europe originate from the single introduction of an admixed source population followed by a complex pattern of spread. PLoS One. 2011;6:e18560.

Simpson TJS, Dias PJ, Snow M, Muñoz J, Berry T. Real-time PCR detection of *Didemnum perlucidum* (Monniot, 1983) and *Didemnum vexillum* (Kott, 2002) in an applied routine marine biosecurity context. Mol Ecol Resour. 2017;17:443–53.

Sinclair JS, Arnott SE. Strength in size not numbers: propagule size more important than number in sexually reproducing populations. Biol Invasions. 2016;18:497–505.

Sloop CM, Ayres DR, Strong DR. The rapid evolution of self-fertility in *Spartina* hybrids (*Spartina alterniflora* × *foliosa*) invading San Francisco Bay, CA. Biol Invasions. 2009;11:1131–44.

Somero GN. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers". J Exp Biol. 2010;213:912–20.

Stachowicz JJ, Terwin JR, Whitlatch RB, Osman RW. Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. Proc Natl Acad Sci. 2002;99:15497–500.

Stapley J, Reger J, Feulner PG, Smadja C, Galindo J, Ekblom R, Bennison C, Ball AD, Beckerman AP, Slate J. Adaptation genomics: the next generation. Trends Ecol Evol. 2010;25:705–12.

Stapley J, Santure AW, Dennis SR. Transposable elements as agents of rapid adaptation may explain the genetic paradox of invasive species. Mol Ecol. 2015;24:2241–52.

Stelkens RB, Brockhurst MA, Hurst GDD, Miller EL, Greig D. The effect of hybrid transgression on environmental tolerance in experimental yeast crosses. J Evol Biol. 2014;27:2507–19.

Stelkens RB, Schmid C, Seehausen O. Hybrid breakdown in Cichlid fish. PLoS One. 2015;10: e0127207.

Stephens ZD, Lee SY, Faghri F, Campbell RH, Zhai C, Efron MJ, Iyer R, Schatz MC, Sinha S, Robinson GE. Big data: astronomical or genomical? PLoS Biol. 2015;13:e1002195.

Stinchcombe JR, Hoekstra HE. Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. Heredity (Edinb). 2008;100:158–70.

Stockwell CA, Hendry AP, Kinnison MT. Contemporary evolution meets conservation biology. Trends Ecol Evol. 2002;18:94–101.

Stuart YE, Campbell TS, Hohenlohe PA, Reynolds RG, Revell LJ, Losos JB. Rapid evolution of a native species following invasion by a congener. Science. 2014;346:463–6.

Suarez AV, Tsutsui ND. The evolutionary consequences of biological invasions. Mol Ecol. 2008;17:351–60.

Sullivan W. The institute for the study of non-model organisms and other fantasies. Mol Biol Cell. 2015;26:387–9.

Takahara T, Minamoto T, Yamanaka H, Doi H, Zi K. Estimation of fish biomass using environmental DNA. PLoS One. 2012;7:e35868.

Takahara T, Minamoto T, Doi H. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. PLoS One. 2013;8:e56584.

Tepolt CK. Adaptation in marine invasion: a genetic perspective. Biol Invasions. 2015;17:887–903.

Tepolt CK, Palumbi SR. Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. Mol Ecol. 2015;24:4145–58.

Teske PR, Sandoval-Castillo J, Sasaki M, Beheregaray LB. Invasion success of a habitat-forming marine invertebrate is limited by lower-than-expected dispersal ability. Mar Ecol Prog Ser. 2015;536:221–7.

Thomsen PF, Kielgast J, Iversen LL, Moller PR, Rasmussen M, Willerslev E. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One. 2012;7: e41732.

Thomsen PF, Møller PR, Sigsgaard EE, Knudsen SW, Jørgensen OA, Willerslev E. Environmental DNA from seawater samples correlate with trawl catches of subarctic, Deepwater fishes. PLoS One. 2016;11:e0165252.

Toews DP, Brelsford A. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol. 2012;21:3907–30.

Toledo-Hernández C, Vélez-Zuazo X, Ruiz-Diaz CP, Patricio AR, Mège P, Navarro M, Sabat AM, Betancur RR, Papa R. Population ecology and genetics of the invasive lionfish in Puerto Rico. Aquat Invasions. 2014;9:227–37.

Tournadre J. Anthropogenic pressure on the open ocean: the growth of ship traffic revealed by altimeter data analysis. Geophys Res Lett. 2014;41:7924–32.

Trucchi E, Mazzarella AB, Gilfillan GD, Lorenzo MT, Schönswetter P, Paun O. BsRADseq: screening DNA methylation in natural populations of non-model species. Mol Ecol. 2016;25:1697–713.

Trumbo DR, Epstein B, Hohenlohe PA, Alford RA, Schwarzkopf L, Storfer A. Mixed population genomics support for the central marginal hypothesis across the invasive range of the cane toad (*Rhinella marina*) in Australia. Mol Ecol. 2016;25:4161–76.

Tsoar A, Shohami D, Nathan R. A movement ecology approach to study seed dispersal and plant invasion: an overview and application of seed dispersal by Fruit Bats. In: Richardson DM, editor. Fifty years of invasion ecology: the legacy of Charles Elton. Oxford, UK: Blackwell Publishing Ltd; 2011. p. 103–19.

Tucker AJ, Chadderton WL, Jerde CL, Renshaw MA, Uy K, Gantz C, Mahon AR, Bowen A, Strakosh T, Bossenbroek JM, Sieracki JL, Beletsky D, Bergner J, Lodge DM. A sensitive environmental DNA (eDNA) assay leads to new insights on Ruffe (*Gymnocephalus cernua*) spread in North America. Biol Invasions. 2016;18:3205–22.

Turner CR, Barnes MA, Xu CCY, Jones SE, Jerde CL, Lodge DM. Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol Evol. 2014;5:676–84.

Twyford AD, Ennos RA. Next-generation hybridization and introgression. Heredity. 2012;108:179–89.

Uchii K, Doi H, Minamoto T. A novel environmental DNA approach to quantify the cryptic invasion of non-native genotypes. Mol Ecol Resour. 2016;16:415–22.

Ungerer MC, Baird SJE, Pan J, Rieseberg LH. Rapid hybrid speciation in wild sunflowers. Proc Natl Acad Sci. 1998;95:11757–62.

Valdez-Moreno M, Quintal-Lizama C, Gómez-Lozano R, García-Rivas MC. Monitoring an Alien invasion: DNA barcoding and the identification of lionfish and their prey on coral reefs of the Mexican Caribbean. PLoS One. 2012;7:e36636.

van Kleunen M, Dawson W, Maurel N. Characteristics of successful alien plants. Mol Ecol. 2015;24:1954–68.

Vandepitte K, de Meyer T, Helsen K, van Acker K, Roldan-Ruiz I, Mergeay J, Honnay O. Rapid genetic adaptation precedes the spread of an exotic plant species. Mol Ecol. 2014;23:2157–64.

Vera M, Díez-del-Molino D, García-Marín J-L. Genomic survey provides insights into the evolutionary changes that occurred during European expansion of the invasive mosquitofish (*Gambusia holbrooki*). Mol Ecol. 2016;25:1089–105.

Viard F, David P, Darling JA. Marine invasions enter the genomic era: three lessons from the past, and the way forward. Curr Zool. 2016;62:629–42.

Voisin M, Engel CR, Viard F. Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. Proc Natl Acad Sci. 2005;102 (15):5432–7.

Wagner CE, Keller I, Wittwer S, Selz OM, Mwaiko S, Greuter L, Sivasundar A, Seehausen O. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Mol Ecol. 2013;22:787–98.

Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009;10:57–63.

Waters CN, Zalasiewicz J, Summerhayes C, Barnosky AD, Poirier C, Gałuszka A, Cearreta A, Edgeworth M, Ellis EC, Ellis M, Jeandel C, Leinfelder R, McNeill JR, Richter DD, Steffen W, Syvitski J, Vidas D, Wagreich M, Williams M, Zhisheng A, Grinevald J, Odada E, Oreskes N, Wolfe AP. The Anthropocene is functionally and stratigraphically distinct from the Holocene. Science. 2016;351(6269).

Webber BL, Raghu S, Edwards OR. Opinion: is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? Proc Natl Acad Sci. 2015;112:10565–7.

Weber DS, Stewart BS, Lehman N. Genetic consequences of a severe population bottleneck in the Guadalupe Fur seal (*Arctocephalus townsendi*). J Hered. 2004;95:144–53.

Weinig C, Brock MT, Dechaine JA, Welch SM. Resolving the genetic basis of invasiveness and predicting invasions. Genetica. 2007;129:205–16.

Wellband KW, Heath DD. Plasticity in gene transcription explains the differential performance of two invasive fish species. Evol Appl. 2017;10:563–76.

White TA, Perkins SE, Heckel G, Searle JB. Adaptive evolution during an ongoing range expansion: the invasive bank vole (*Myodes glareolus*) in Ireland. Mol Ecol. 2013;22:2971–85.

Whitfield PE, Gardner T, Vives SP, Gilligan MR, Courtenay WR Jr, Ray C, Hare JA. Biological invasion of the Indo-Pacific lionfish *Pterois volitans* along the Atlantic coast of North America. Mar Ecol Prog Ser. 2002;235:289–97.

Whitney KD, Gabler CA. Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. Divers Distrib. 2008;14:569–80.

Whitney KD, Broman KW, Kane NC, Hovick SM, Randell RA, Rieseberg LH. Quantitative trait locus mapping identifies candidate alleles involved in adaptive introgression and range expansion in a wild sunflower. Mol Ecol. 2015;24:2194–211.

Widmer A, Schmid-Hempel P, Estoup A, Scholl A. Population genetic structure and colonization history of *Bombus terrestris* s.l. (Hymenoptera: Apidae) from the Canary Islands and Madeira. Heredity. 1998;81:563–72.

Wilcox CL, Motomura H, Matsunuma M, Bowen BW. Phylogeography of lionfishes (*Pterois*) indicate taxonomic over splitting and hybrid origin of the invasive *Pterois volitans*. J Hered. 2018;14:162–75.

Willan RC, Russell BC, Murfet NB, Moore KL, McEnnulty FR, Horner SK, Hewitt CL, Dally GM, Campbell ML, Bourke ST. Outbreak of *Mytilopsis sallei* (Récluz, 1849) (Bivalvia: Dreissenidae) in Australia. Molluscan Res. 2000;20:25–30.

Williams F, Eschen R, Harris A, Djeddour D, Pratt C, Shaw R, Varia S, Lamontagne-Godwin J, Thomas S, Murphy S. The economic cost of invasive non-native species on Great Britain. CABI Project Number VM10066. 2010. p. 1–99.

Williams M, Zalasiewicz J, Haff P, Schwägerl C, Barnosky AD, Ellis EC. The Anthropocene biosphere. Anthropocene Rev. 2015;2:196–219.

Williamson M. Invaders, weeds and the risk from genetically manipulated organisms. Experientia. 1993;49:219–24.

Wilson JR, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM. Something in the way you move: dispersal pathways affect invasion success. Trends Ecol Evol. 2009;24:136–44.

Wolf JBW, Bayer T, Haubold B, Schilhabel M, Rosenstiel P, Tautz D. Nucleotide divergence vs. gene expression differentiation: comparative transcriptome sequencing in natural isolates from the carrion crow and its hybrid zone with the hooded crow. Mol Ecol. 2010:162–75.

Wrange A-L, Charrier G, Thonig A, Alm Rosenblad M, Blomberg A, Havenhand JN, Jonsson PR, André C. The story of a hitchhiker: population genetic patterns in the invasive barnacle *Balanus* (*Amphibalanus*) *improvisus* Darwin 1854. PLoS One. 2016;11:e0147082.

Yamamoto S, Minami K, Fukaya K, Takahashi K, Sawada H, Murakami H, Tsuji S, Hashizume H, Kubonaga S, Horiuchi T, Hongo M, Nishida J, Okugawa Y, Fujiwara A, Fukuda M, Hidaka S, Suzuki KW, Miya M, Araki H, Yamanaka H, Maruyama A, Miyashita K, Masuda R, Minamoto T, Kondoh M. Environmental DNA as a "Snapshot" of fish distribution: a case study of Japanese Jack Mackerel in Maizuru Bay, Sea of Japan. PLoS One. 2016;11:e0149786.

Yoder JB, Stanton-Geddes J, Zhou P, Briskine R, Young ND, Tiffin P. Genomic signature of adaptation to climate in *Medicago truncatula*. Genetics. 2014;196:1263–75.

Yu Y, Andrés JA. Genetic architecture of contemporary adaptation to biotic invasions: quantitative trait locus mapping of beak reduction in soapberry bugs. G3 (Bethesda). 2014;4:255–64.

Zaiko A, Martinez JL, Schmidt-Petersen J, Ribicic D, Samuiloviene A, Garcia-Vazquez E. Metabarcoding approach for the ballast water surveillance – an advantageous solution or an awkward challenge? Mar Pollut Bull. 2015;92:25–34.

Zhang Y-Y, Zhang D-Y, Barrett SCH. Genetic uniformity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. Mol Ecol. 2010;19:1774–86.

Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics. 2012;28:3326–8.

Population Genomics Applied to Fishery Management and Conservation

Laura Benestan

Abstract In times of overfishing and climate change, marine resources are extremely vulnerable, and the vast majority of the world's fish stocks have already collapsed. In this fragile context, the need to drive fisheries toward sustainability has become a priority. Population genomics methods, which compare DNA of individuals from different populations occupying distinct environments, are promising tools to address such need. Indeed, these methods provide new knowledge on the demographic and adaptive history of marine resources, which allows fisheries-specific issues to be resolved, so that delineation of stocks coincides with actual population boundaries and genetic diversity is maintained, ensuring the long-term sustainability of resources. In addition, the field of population genomics applied to fisheries management, or commonly referred to as "fisheries genomics," has benefited from emerging molecular approaches that can now address fisheries management issues that could not previously be addressed. This genomics revolution is accompanied by an apparent increase in information and resolution on the main causes of marine population differentiation, which makes it possible to assess the persistence of marine species in the face of climate change and overfishing, two major threats at the heart of fisheries management issues. In this chapter, I synthesize information on empirical examples of the application of population genomics to fisheries and provide suggestions as to how modern population genomics approaches could address some of the most urgent challenges in fisheries management and conservation. I discuss the application of genomics to fishery management and conservation from four main angles: stock structure, climate change, forensics, and fishery-induced evolution.

Keywords Climate change · Conservation · Fisheries-induced evolution · Fishery forensics · Fishery management · Outlier detection · Population genomics · Stock structure

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1 Introduction

Fisheries are of substantial social and economic importance, employing around 56.6 million people worldwide and totaling more than 40 billion US$ per annum, according to the FAO (Food and Agriculture Organization) report of 2015. Nowadays, a large proportion of the world's marine resources are fully exploited, overexploited, depleted, or in need of recovery (McCauley et al. 2015). Overfishing may lead to a reduction of genetic diversity and eventually to the disappearance of marine populations in the world's oceans (Pinsky and Palumbi 2014). Moreover, exploited species are often essential components of the diet of marine megafauna species, such that their decline can cause marked cascading effects on marine food webs, which may then impact the integrity of marine ecosystems (Scheffer et al. 2005). Consequently, sustainable management and genetic resource conservation are required to ensure that fisheries will persist over time, preventing further collapse of stocks – many fisheries remain overexploited or near collapse (Pauly and Zeller 2016) – and, thus, avoiding significant ecological, economic, and human consequences.

Recent developments in genomics gave rise to a versatile and powerful set of tools for monitoring marine populations, such as providing information pertaining to their demographic and adaptive history (Kelley et al. 2016). Yet, only a few genetics and genomics research outcomes, such as for Pacific salmon *Oncorhynchus* spp. and Atlantic salmon (*Salmo salar*), have been successfully implemented into existing management plans as pointed out many times over the past years (Waples et al. 2008; Reiss et al. 2009; Bernatchez et al. 2017). Then, despite the profuse studies on the topic, the considerable need for genetically based scientific information for marine resource management and the demonstrated ability of genomic data to address questions of direct management relevance, concrete integration of genetic information into fisheries management has been questioned (Shafer et al. 2015; but see Garner et al. 2016). Arguably, there is a risk that this gap between the fields of population genomics and fishery management could become wider if researchers focus more on applying the newest technological advances without considering that actual needs of fishery management (Bernatchez et al. 2017). While there is no question that genomics approaches need to be developed cautiously regarding the expansion of the field and the novelty of the tools used, it is now time for this field to fill the gap between the results and their application in a context of marine resource management and conservation. More particularly, the integration of genomic knowledge into fisheries management policymaking and development would be facilitated by population genomics studies that are conducted collaboratively with managers and meet management needs. Here, I synthesize some of the most convincing empirical examples of population genomics applied to fisheries, as they have the potential to illustrate how genomics information can be effectively integrated into fisheries management policies. Then I provide suggestions of modern genomics approaches that are likely to address some of the most urgent fisheries management challenges.

2 Fishery Genomics, from a Genetic to a Genomic Perspective: The Need to Open Up

Fishery genomics is a growing area that aims to solve fisheries-specific questions using relevant genomics tools (Valenzuela-Quiñonez 2016). As proposed by Bernatchez et al. (2017), in population genetics, the term genomics is typically used as a shorthand to describe studies applying large and genome-wide datasets, with a classic, yet arbitrary, threshold of >1000s versus 10s–100s of markers to distinguish between genomics and genetics studies, respectively. Initially, the fishery genetics explosion began in the 1980s and accelerated with the boom of massive parallel sequencing technologies (MPS), which enables researchers to genotype thousands of single nucleotide polymorphism (SNP) markers in non-model marine species (Kelley et al. 2016) and brings new opportunities for conservation applications. Moving from a genetic to a genomic perspective, fishery genomics has also widened its range of applications (Willette et al. 2014; Ovenden et al. 2015). In this chapter, we focus on the application of population genomics to fishery management and conservation under four main angles: (1) stock structure, (2) climate change, (3) forensics, and (4) fishery-induced evolution. Despite the diversity of themes, stock structure is arguably the most common topic covered in this field (Abdelrahman et al. 2017). Indeed, populations are the fundamental units of conservation and management (Palsbøll et al. 2007), and the delineation of stock boundaries is a major goal of fishery management (Waples and Naish 2009; Waples 1998). In the future, the field of fishery genomics is expected to open to a broader range of fishery management issues as several authors have already suggested (Waples and Naish 2009; Ovenden et al. 2015; Bernatchez et al. 2017) as well as considered more taxa out of the Actinopterygii (ray-finned fishes) phylum (Fig. 1).

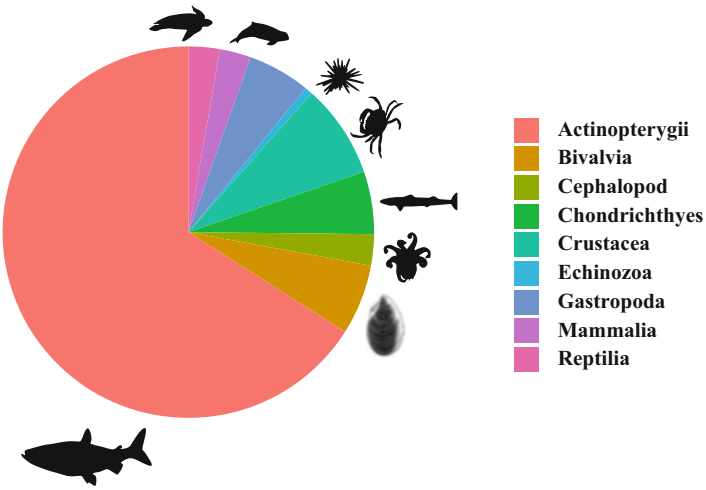

Fig. 1 Representation of taxonomic group considering the 127 fishery genomics studies published between 1997 and 2016 and listed in Web of Science database. Only research articles were retained

Indeed, some taxa at lower trophic levels, such as the echinoderm (Echinozoa), are poorly documented whereas fishing down the food web tends to increase with important effects on the abundance of these lower trophic-level species (Pauly et al. 1998; Essington et al. 2006). Documenting genetic structure of all exploited marine species, including the low-trophic level species, will be crucial for assessing how this trophic level shift will impact the entire ecosystem.

3 Fishery Stock Structure: How to Delineate Population Boundaries

Box 1 Proposed Definitions of Common Terms Used in Population Genomics Applied to Fishery Management

Stock: Demographically cohesive group of individuals of one species exploited in a specific area.

Population: Group of individuals that have similar demographic or genetic characteristics and thus will respond uniquely and independently to fishing.

Demographic connectivity: Exchange of individuals among populations.

Genetic connectivity: Exchange of genes among populations.

Local adaptation: Refers to the concept that individuals of local populations tend to have a higher mean fitness in their native environment than in other environments. This phenomenon results from the interaction between multiple evolutionary forces (e.g., genetic drift, migration, mutation, and selection).

Sustainable fishing: Fishing activity at a level which ensures the fishery can persist over time, with minimal environment impact and an effective fisheries management.

Genetic erosion: Process in which a species faces a gradual or drastic reduction of its genetic diversity. This loss of genetic diversity weakens the species and can contribute to accelerate extinction.

Genetic diversity: Variety of alleles and genotypes present in a population, which is reflected in morphological, physiological, and behavioral differences between individuals and populations.

Understanding how populations are interconnected across geographic, temporal, and political landscapes is challenging since most of the marine species can migrate long distances, crossing international boundaries, therefore, making their exploitation of global concern (Ovenden et al. 2015). During the twentieth century, fishery scientists gradually began to consider that marine species may be potentially divided into multiple self-sustaining stocks. Delineating the spatial distribution of these entities became a major goal for stock assessment, quota allocation, or monitoring in order to predict sustainable catch limits (Ward 2000). Sustainability requires a

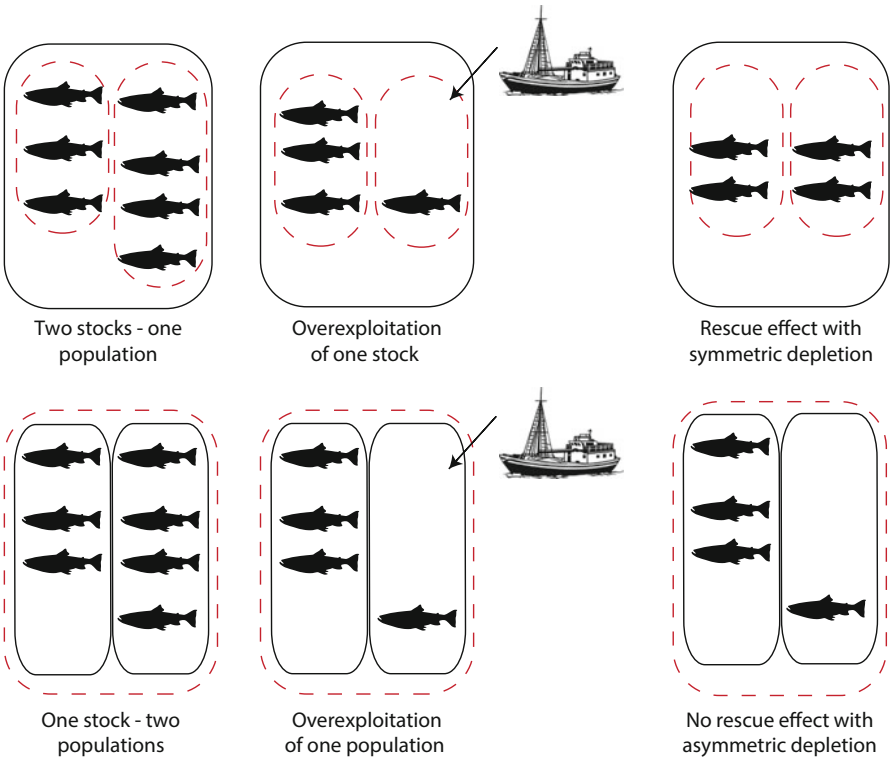

Fig. 2 Schematic representation of two major issues resulting from a mismatch between populations (black outlines) and management units (red outlines)

match between biological process and management actions, whereas assessment models and fishery management designs were predominantly conceived based on "administrative units" that do not necessarily reflect the delineation of meaningful biological units (Reiss et al. 2009; Fig. 2).

While a discrepancy between independent breeding stocks and management units may lead to overexploitation and disappearance of many local stocks (Reiss et al. 2009; Valenzuela-Quiñonez 2016), the integration of genetic data has been stagnated. Yet, in a situation when two demographically isolated populations are managed as one stock, the management decisions in one unit may strongly influence the viability of the other. For instance, overexploitation of one stock may lead to the depletion of both stocks, which underlines the need for the development of management plans that consider these demographic realities and their concordance with the geographic stock and population distribution (Waples and Naish 2009; Reiss et al. 2009; Valenzuela-Quiñonez 2016). Inversely, where a demographically isolated stock tends to decline – population dynamics depends more on local births and deaths than on immigration (Funk et al. 2012) – the recovery of this stock would fail due to too low number of migrants (i.e., no "rescue effect"; Bowler and Benton

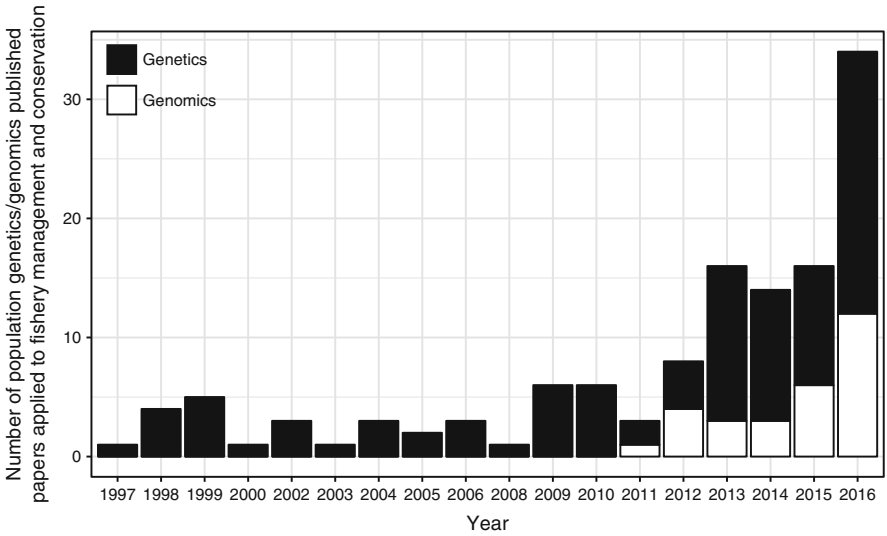

Fig. 3 Frequency distribution of studies published between 1997 and 2016 that used population genetics (in black) genomics (in white) in a context of fishery management and conservation. An initial raw list of 127 publications was generated from a Web of Science literature search. Only research articles were retained

2005). The recognition that molecular tools may help to provide insights into the demographic independence of stocks led to an exponential increase of fishery genetics/genomics studies over the past decades (Fig. 3), but mismatches between genetic and management units still persist (Reiss et al. 2009; Ovenden et al. 2015; Table 1).

3.1 Uncovering Finer-Scale Population Structure

Delineating appropriate conservation units, which lies at the heart of short-term management programs, is a difficult task in marine systems characterized by weakly genetically differentiated marine populations (Waples and Gaggiotti 2006). During the "genetics era," small F_{ST} values have rarely been translated into an accurate estimate of N_em, partly because of (1) the non-respect of several biological assumptions under the Wright model (Whitlock and Mccauley 1999) and (2) the fact that such values fall within the asymptotic region of the nonlinear relationship between F_{ST} and N_em, where resolution (i.e., large variance surrounding the estimated value) has classically been hampered by a small number of genetic markers (Waples and Gaggiotti 2006). This also explains why traditional genetic markers have often failed to detect genetic divergence (e.g., confidence intervals comprising $F_{ST} = 0$) even among very distant populations, whereas thousands of markers succeed. Indeed,

Table 1 Illustrative genomic studies defining population structure on exploited marine species: author, species, scientific name, geographic area studied, number of genetic units observed versus number of current management units, commercial value of species, and the match between the population genetic structure observed and the current management units being used

Author	Species	Latin name	Geographic area	Genetic units versus management units	Commercial value	Genomic versus management
Benestan et al. (2015)	American lobster	*Homarus americanus*	Northeast Atlantic (Canada, USA)	11 genetic units versus 16 management units	High (industrial fishery)	Match and mismatch
Carreras et al. (2017)	East Atlantic peacock wrasse	*Symphodus tinca*	Adriatic and Ionian seas	3 genetic units *across* 3 countries	Little (small-scale fishery)	Match and mismatch
Jackson et al. (2014)	Nassau grouper	*Epinephelus striatus*	Caribbean Sea	3 genetic units *across* 9 countries	Moderate – Redlist IUCN	Mismatch
Lal et al. (2016)	Black-lip pearl oyster	*Pinctada margaritifera*	Fiji Islands	1 genetic unit versus 1 management unit	High (production and growth)	Match
Lal et al. (2017)	Black-lip pearl oyster	*Pinctada margaritifera*	Indian and Pacific Ocean	5 genetic units *across* 11 countries	High (production and growth)	Match and mismatch
Larson et al. (2014)	Chinook salmon	*Oncorhynchus tshawytscha*	Alaska	5 genetic units	Moderate – recreational fishery	Match
Le Moan et al. (2016)	European anchovy	*Engraulis encrasicolus*	Atlantic and Mediterranean regions	2 genetic units versus 1 management unit	High (industrial fishery)	Mismatch
Miller et al. (2016)	Blacklip abalone	*Haliotis rubra*	Southeastern Australia	1 genetic unit versus 3 management units	Moderate – Redlist IUCN	Mismatch
Moore et al. (2014)	Atlantic salmon	*Salmo salar*	West Atlantic	12 genetic units	Moderate – recreational fishery	Match
Pecoraro et al. (2016)	Yellowfin tuna	*Thunnus albacares*	Atlantic, Pacific, and Indian Ocean	3 genetic units versus no clear management units (offshore fishery)	High (industrial fishery)	Mismatch
Poćwierz-Kotus et al. (2015)	Atlantic cod	*Gadus morhua*	Eastern Baltic (Poland, Germany)	2 genetic units versus 2 management units	High (industrial fishery)	Match

(continued)

Table 1 (continued)

Author	Species	Latin name	Geographic area	Genetic units versus management units	Commercial value	Genomic versus management
Rodríguez-Ezpeleta et al. (2016)	Atlantic mackerel	*Scomber scombrus*	Atlantic and Mediterranean regions	2 genetic units *across* 5 countries	High (industrial fishery)	Mismatch
Stockwell et al. (2016)	Black parrot fish	*Scarus niger*	Philippines	Single genetic unit versus 1 management unit	Little (small-scale fishery)	Match

both simulation (Willing et al. 2012) and empirical studies already highlighted the higher resolution obtained from wide panels of SNP markers compared to a few microsatellites, for instance, in great scallop (*Pecten maximus*; Vendrami et al. 2017), American lobster (*Homarus americanus*; Benestan et al. 2015), sea cucumber (Xuereb et al. 2018), and harbor porpoise (*Phocoena phocoena*; Lal et al. 2016). While F_{ST} and $N_e m$ are still complex to interpret, these metrices could now more accurately be estimated using genome-wide panels of markers, which lead to narrow confidence intervals (e.g., 0.001) even around weak estimates of genetic differentiation as demonstrated by a simulated and empirical study (Willing et al. 2012; Vendrami et al. 2017). Vendrami et al. (2017) genotyped the same individuals for microsatellites and SNP markers in great scallop populations (*Pecten maximus*). They showed that a higher number of SNP markers could detect genetic structure where a standard panel of microsatellites fails. This gain of resolution substantially increases the accuracy and power of statistical tests of genetic differentiation and spatial patterns (Allendorf et al. 2010). These pieces of work suggest that confidence intervals and significance of F_{ST} values are now accurate enough to reject the null hypothesis of panmixia with less biases than from microsatellites. Yet, this test does not tell researchers and managers where the populations are along the continuum of genetic differentiation (Waples and Gaggiotti 2006). The significance of F_{ST} values is also highly sensitive to factors such as the numbers of genetic markers and individuals sampled; *P*-values tend to be more significant with a higher number of markers and individuals. Nevertheless, F_{ST} values are not always significant when thousands of SNP markers are genotyped on hundreds of individuals, which was the case for American eel (*Anguilla rostrata*; Pavey et al. 2015), European eel (*Anguilla anguilla*; Pujolar et al. 2014), and summer flounder (*Paralichthys dentatus*; Hoey and Pinsky 2018). These "negative control" studies suggest that a large number of markers and sampling size does not systematically lead to the rejection of panmixia and F_{ST} may still be a robust estimator. Yet, the interpretation of genetic structuring patterns observed in these species still requires a detailed understanding of their life histories and the environment in which they occur (Hansen and Hemmer-Hansen 2007; Riginos and Liggins 2013).

Box 2 Understanding Theory Underlying Population Genomics

Over the last 50 years, theory pertaining to population genetics did not change, whereas the potential of generating enormous amounts of genomic data has exponentially increased. This new data-driven discipline now needs a rethinking of the theory in order to be able to accurately interpret the data using appropriate tools (Allendorf et al. 2010; Benestan et al. 2016a, b). It is then important to remember that delineation of populations is based on the simple theoretical equilibrium relationship with the number of migrants exchanged between stocks and the estimation of genetic differentiation index ($N_e m = (1 - F_{ST}) / 4 F_{ST}$; Wright 1931). This relationship exists and has

(continued)

Box 2 (continued)
been theoretically demonstrated, supporting the idea that genetic connectivity – inferred from genetic differentiation index – depends on the number of migrants who successfully interbreed whereas demographic connectivity – which stands at the center of interest for managers – depends on the number of individuals that physically contribute to population abundance (Waples and Gaggiotti 2006). Genetic information is thus not of direct use for managers due to this contrasting paradigm (genetic versus demographic connectivity). Furthermore, defining a migration threshold below which populations should be independently managed is challenging, and this question remains open. Nevertheless, Hastings (1993) was the first to propose that the migration rate (m) should not exceed 0.1 to claim demographic independence between stocks. Since then, this threshold has been re-evaluated by Lowe and Allendorf (2010), who more carefully suggested that the amount of dispersal required for demographic connectivity depends on the context (e.g., conservation or harvest management), rather than being a scientific consensus. On the other hand, a very low genetic differentiation index could also result from non-equilibrium conditions between genetic drift and migration due to large effective population sizes and/or recent population divergence time rather than truly reflecting a high demographic connectivity among populations (Waples and Gaggiotti 2006). Inversely, a low but statistically significant level of genetic differentiation does not necessarily imply demographic independence (Waples and Gaggiotti 2006). This particular complexity of interpreting low levels of genetic structure (e.g., $F_{ST} < 0.01$) in marine populations compared to many other taxonomic groups largely pertains to their general (exceptions exist of course) life history characteristics such as large population sizes, high dispersal potential, and high fecundity (Nielsen et al. 2009).

3.2 Chromosomal Rearrangements

The mechanisms that maintain adaptive structure in the face of high gene flow are still little understood, whereas major advances and genomic analysis tools are now available to dissect genomic architecture underlying the demographic and adaptive processes of an exploited marine species, which is still one of the major challenges in marine genomics today (Hemmer-Hansen et al. 2014). In a high gene flow species, a simulation study by Yeaman and Whitlock (2011) highlighted that local adaptation can arise as long as selection acts on several closely linked divergent alleles rather than on many single loci. Evidence of these linked alleles promoting adaptive divergence has then been reported in studies addressing genomic architecture in Atlantic herring (Lamichhaney et al. 2012; Barrio et al. 2016) and Atlantic cod (Barney et al. 2017; Berg et al. 2015; Bradbury et al. 2013; Kirubakaran et al. 2016). In addition, other simulation studies showed that segregation of chromosomal

rearrangements at these linked alleles (i.e., recombination is suppressed, and important functional genes are inherited together) may lead to the acquisition of locally adaptive traits. This was empirically supported by the work of Barth et al. (2017), who identified haplotype blocks associated with adaptation to salinity in Atlantic cod populations inhabiting Scandinavian fjords. Genes critical for survival at low salinities were found to be located in a large inversion of 5 Mb, enabling ecotypes to be diagnostically distinguished (Barth et al. 2017). Using test crosses between migratory and stationary ecotypes of Atlantic cod, Kirubakaran et al. (2016) demonstrated the role of chromosomal inversions in the maintenance of genetic differentiation. Remarkably, the same chromosomal inversion was involved in the maintenance of genomic divergence between Atlantic cod ecotypes on both sides of the Atlantic Ocean. These examples emphasize the importance of taking genomic architecture into account when characterizing ecological adaptation, particularly for marine species, which are excellent models for studying the interaction between the homogenizing effects of gene flow and the diversifying effects of selection (Nielsen et al. 2009).

3.3 The Use of Outlier Markers for Inferring Genetic Connectivity

Recent technological developments have facilitated the detection of genomic regions underlying adaptive trait variation in natural populations (Allendorf et al. 2010). This genomic revolution has facilitated the simultaneous identification of neutral and fine-scale adaptive genetic differentiation patterns (Willette et al. 2014; Hemmer-Hansen et al. 2014) in species previously considered panmictic (i.e., showing a homogeneous genetic unit). Since adaptive diversity is essential to species persistence and stability, revealing adaptive genomic structure may help to delineate locally adapted stocks and refine the definition of conservation units (Funk et al. 2012). This avenue of research is promising since selection may act as a more efficient antagonist force – in opposing the homogenizing effect of migration in populations with large effective sizes – than genetic drift alone (Gagnaire et al. 2015). Several mechanisms could promote genetic divergence at a few genomic regions while most of the genome remains homogenized through gene flow. Marine species represent an excellent model for detecting adaptive genetic variation since genetic drift is reduced, the entire genome being homogenized under the effect of pronounced gene flow.

Furthermore, adaptive differences in the face of high gene flow may be more informative than neutral loci for delineating genetic clusters (Gagnaire and Gaggiotti 2016) such as for the Atlantic herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) populations, where outlier markers revealed additional barriers to gene flow, which were not apparent at neutral loci (Limborg et al. 2012; Bradbury et al. 2013). Remarkably, in the case of Atlantic cod, the adaptive structure uncovered by

Bradbury et al. (2013) resulted in a revision of the number of conservation units in this species in Canadian waters. Yet, outlier loci may also tell the same stories as neutral loci (Moore et al. 2014; Van Wyngaarden et al. 2017). In addition, a common issue of this approach is the actual identification of outliers, which is often inconsistent across methods (Rellstab et al. 2015). To define this adaptive genetic variation, it is possible to use methods based on population differentiation (population differentiation or PD) or environmental association (environmental association or EA) (Rellstab et al. 2015; François et al. 2016). These methods are used to identify potentially under-selected markers without a priori, in the case of PDs, and with a priori (i.e., environmental parameter values), for EA methods. The combination of these two methods makes it possible to limit type I errors by considering only the common set of genetic markers detected by the two methods or to avoid type II errors by considering the set of genetic markers detected by each of these methods. The joint use of PD and EA methods is therefore particularly relevant as it maximizes our chances of identifying any potential signatures of natural selection on the genome of marine species (Benestan et al. 2016b). Managing the stock structure revealed by these potential signatures of natural selection is then an important step toward ensuring that an exploited species retains adaptive traits or enough genetic variation to adapt to environmental change. Furthermore, quantifying and delimiting the influence of natural selection on potentially adaptive genetic variation is a key step in predicting how these species will respond to climate change (Savolainen et al. 2013). Indeed, climate change is currently driving important evolutionary changes on a contemporary time scale, and understanding the genetic basis of this evolutionary change is fundamental to prevent new fishery collapses.

4 Climate Change Influences Species Distribution and Local Adaptation Patterns

Climate change is shifting the productivity of fisheries, forcing thousands of marine species to move northward. Indeed, marine species have three ways to cope with global climate change: (1) move to a location with more suitable environmental conditions, (2) exhibit phenotypic plasticity (i.e., use existing genetic and epigenetic mechanisms to express different phenotypes in different environments), or (3) adapt through evolutionary changes (i.e., genetic change that occurs across generations; Hansen et al. 2012). Indeed, marine species are predicted to move northward as demonstrated by Stanley et al. (2018) who elegantly combined population genetics and genomics outcomes to habitat suitability models and climate forecast to show the distribution shift in five socioeconomically important marine species of the North Atlantic: Atlantic cod (*Gadus morhua*), American lobster (*Homarus americanus*), sea scallop (*Placopecten magellanicus*), northern shrimp (*Pandalus borealis*), and European green crab (*Carcinus maenas*). This prediction offers an important tool for fishery conservation and management since it may help to highlight species and

populations that are particularly vulnerable to climate change as well as to identify potential areas for the establishment of future fisheries. Species may also adapt to these warmer environments, and screening the potential signatures of selection in their genome may give insights about their persistence. Yet, it has proven very challenging to link this evidence to phenotypic changes and, thus, to distinguish between the proportions of phenotypic changes that are genetically based versus changes resulting from phenotypic plasticity. Indeed, the genetic underpinnings of most fitness-related phenotypic traits are still little documented (Savolainen et al. 2013). There are several explanations for this. First, progress toward addressing these questions has been methodologically hampered until recently because the technical and analytical resources necessary to investigate the genetic basis of adaptation lacked power (Pardo-Diaz et al. 2015). Second, the vast majority of studies have focused on measuring phenotypic responses potentially associated with increased individual fitness in new environments (e.g., growth, fecundity, physiology, and morphology), whereas the molecular pathways underlying rapid adaptive phenotypic responses and the changes in genetic variation have been less studied (Laikre et al. 2010) and would then require more investigation in the field. Indeed, one rapid phenotypic response of marine organisms to climate change is the incidence and prevalence of parasitic diseases worldwide. Investigating the host-parasite coevolution, such as for salmon (*Oncorhynchus* spp. and *Salmo* spp.) and their sea louse parasites (reviewed in Kreitzman et al. 2018), and finding genetic basis of resistance to parasitic disease are likely to be soon a priority for preventing the parasite dispersal and ensuring the fish productivity of the newly invaded ecosystem. On the other hand, another key objective of this epidemiology/fishery genomics field would be to define how genetic diversity may help to regulate parasite dispersal (Schwabl et al. 2017). Overall, preserving genetic diversity of marine-exploited populations through space and time will then be an important goal to pursue since it is the basis of the evolutionary potential of species and thus their response to climate changes.

5 Fishery Forensics: Identification of Geographic Origin for Transparency and Traceability

From the ocean to the fork, certainty about the origin and identity of the meal is crucial for consumer protection and regulatory enforcement. Illegal fishing, that is, harvesting activities conducted by vessels without fishing permission, may account for more than 15% of the world's total annual capture fisheries output and, as such, may contribute to the depletion of several marine species, especially in developing countries, where fishing vessel controls are expected to be less frequent (FAOSTAT 2015). Thus, illegal as well as unreported and unregulated (IUU) fisheries may have a major impact on the sustainability of many fisheries, which led to the development of international regulations and eco-certified labels. Yet, methods for verifying the

origin of the product are still scarce, and phenotypic tracing is not always possible. Indeed, the vast majority of the marine populations in the wild harbor similar phenotypes whereas they do not share the same status or management regulations. In this context, genomics may provide powerful tools for the traceability of marine products through the field of fisheries forensics, which aims to use scientific analyses to investigate illegal fishing and fish fraud. Tracking down illegal fishing, through the use of DNA tests, now lies at the core of fishery management programs of several exploited species as it encourages and supports the application of fishery management policies as well as limiting cost related to forensic investigation. This forensic investigation started in 2003, when a geneticist used a panel of microsatellites markers to show that a fishing vessel did not respect its quota and had caught too many Atlantic cod in the North Sea (Stokstad 2010). Then later, one of the most convincing applications of this genomics technology to industry was the FishPopTrace project (Martinsohn et al. 2009), which showed that only 13 and 32 markers are sufficient to successfully identify the origin of 98% of all the European populations of hake (*Merluccius merluccius*) and herring (*Clupea harengus*; Nielsen et al. 2012). Screening thousands of SNP markers in entire genomes of marine species increases our potential to detect such informative markers and then be able to discriminate geographic origin of an individual blindly. Genotyping thousands of individuals at these small sets of markers can provide a quick and efficient cost-effective tool to monitor populations of a wide range of a species (Table 2).

Table 2 Assignment tests studies on exploited marine species: author, species, Latin name, location, and assignment success

Author	Species	Latin name	Location	Assignment success
Benestan et al. (2015)	American lobster	*Homarus americanus*	West Atlantic	>90% between two regions
Larson et al. (2014)	Chinook salmon	*Oncorhynchus tshawytscha*	Alaska	>90% among five populations
Meek et al. (2016)	Chinook salmon	*Oncorhynchus tshawytscha*	West Pacific (California)	>90% among five populations
Martinsohn et al. (2009) (FishPopTrace)	European hake	*Merluccius merluccius*	North East Atlantic and Mediterranean Sea	97.5–99.5% between two populations
Martinsohn et al. (2009) (FishPopTrace)	Common sole	*Solea solea*	North East Atlantic	92–94% between two populations
Martinsohn et al. (2009) (FishPopTrace)	Atlantic cod	*Gadus morhua*	North East Atlantic	98–100% between two populations
Martinsohn et al. (2009) (FishPopTrace)	Atlantic herring	*Clupea harengus*	North East Atlantic	98–100% among three populations

Box 3 Dispersal Inferences with Population Assignments and Telemetric Data

Marine populations have a high potential to disperse at different scales, and tracking them, both at the adult and pelagic larval stages, is a laborious task to achieve through traditional and satellite telemetry because these methods are still expensive to perform and often suffer from a low sampling size (Pineda et al. 2007). Genomics represents an appealing alternative approach to, first, define biologically meaningful population units and, then, track and monitor movement of gene flow among these populations. Yet, to narrow the gap between genetic and demographic connectivity, several studies attempted to jointly analyze and interpret genomic and telemetry data. Demographic connectivity information may be collected from telemetric data, but tagging requires a large number of tags to adequately represent population patterns and in the worst case can modify organism behavior and fitness (Ropert-Coudert et al. 2000). Then, direct estimates of demographic connectivity obtained from telemetric studies are often hard to infer for many marine taxa. Yet, telemetry represents an appealing alternative and complementary approach, which facilitates interpretation of genomic data patterns as remarkably demonstrated by Knutsen et al. (2011). Indeed, the authors estimated a low but significant genetic structure (F_{ST} of 0.0037 on average) among Atlantic cod populations, consistent with capture-mark-recapture data showing that individuals tend to stay closer from their sampling location. Therefore, they clearly brought empirical evidence that even the subtle genetic structuring observed in Atlantic cod is biologically meaningful. Similarly, Moore et al. (2017) documented that a clear pattern of asymmetric gene flow matched dispersal movements among Arctic char populations in the Canadian Arctic using the powerful combination of population assignment test based on 6,136 SNP markers genotypes and acoustic telemetry data from 124 tracked individuals. Future studies in marine organisms should consider such research avenues for overcoming uncertainty related to the limitations of genomics tools as explicitly reviewed in Shafer et al. (2016).

6 Fisheries-Induced Evolution: Limiting the Impact of Fisheries on the Overall Fish Stock Productivity and Improving Resilience to Overfishing

Fisheries-induced evolution studies aim to assess the neutral and adaptive stability across a spatial and temporal scale. Fishing may erode genetic variation, leading to genetic changes that impact population productivity. In particular, harvesting may increase genetic drift since it reduces population size and may alter age structure, sex ratio, size, and maturity status depending on the target individuals (Kuparinen and Hutchings 2017). There is a burgeoning literature on fisheries-induced evolution showing that marine populations may be rapidly evolving in response to selection

pressures imposed by fishing (reviewed in Heino et al. 2015). In demographically isolated populations where new alleles are only generated though mutation, fishing may induce genetic erosion, which was documented in New Zealand snapper (*Pagrus auratus*; Hauser et al. 2002) and Atlantic cod (Hutchinson et al. 2003; Therkildsen et al. 2010). Evidence of fisheries-induced evolution in wild populations is still mostly based on experimental studies documenting phenotypic changes to infer genetic changes. The seminal example of fisheries-induced evolution was led by Conover and Munch (2002) who conducted an experiment on captive populations of an exploited marine fish, under a controlled and experimentally harvested environment. They demonstrated how size selective harvesting caused the population to grow slowly, which then resulted in quantitative genetic changes in body size, growth rate, and several life history traits. Marine populations may evolve to grow slowly due to fishing direct effects, for instance, escaping a fishing mortality that starts at a body size threshold (Conover and Munch 2002), or indirect effects such as investing more energy to early maturation. Phenotypic changes, such as body size in marine populations, have been reported worldwide, but it remains an open question how much is attributable to phenotypic plasticity versus genetic change. Indeed, temporal genetic changes of wild harvested populations have been reported in Atlantic cod (Therkildsen et al. 2013), but it remains challenging to link these changes to phenotypic traits under selection. Alternative methods, such as mutagenesis experiments, may help to further document the impact of these changes at the phenotypic level.

7 Conclusion

Despite the promising applications of new genomics tools to fishery management issues, their use has also been accompanied by an awareness of the biases associated with the development of such tools (Davey et al. 2013; Benestan et al. 2016a). These types of biases have forced the scientific community to first define and establish the bases of a methodological framework relevant to the analysis of genomic data in marine populations. Now, methodological challenges (e.g., interpreting the low population structure often observed for marine resources) are still there, but researchers need to move to the next step: stimulating cohesion between the field of population genomics and fisheries management (Fig. 4). For instance, fisheries scientists and managers are needed to increase the implementation of genetic stock structure information into management plans. In our work on American lobster, we documented how several thousands of SNPs can refine the definition of biologically meaningful management units in American lobster (Benestan et al. 2015). One important aspect of our study is that fishermen were not only involved in the sampling process but were also consulted for the sampling design of the study (Rochette et al. 2018). Indeed, sampling was made in areas of interest for the industry, where the results coming out of the study may have huge impact on the management plan and actions. Results were also communicated regularly to fishers, and interpretation of the population genomics results considered the fishers' "local

FISHERY MANAGEMENT ISSUES	GENOMIC OUTCOMES	MANAGEMENT APPLICATIONS
1. Fishery stock structure	*Highlighting stock structure*	*Redefining management units*
Define **sustainable quotas** and explain fishery productivity variation	**Delineate populations boundaries** and **identify conservation priorities** at the population level	Match **population boundaries with management units** and define catch limits or closed fishing areas
2. Climate change	*Delineating the influence of climate change*	*Redefining management priorities*
Predict the **shift in species distribution** and **reduce disease risk**	Investigate **local adaptation** and document the spatial distribution of **genetic diversity**	Identify **new areas for fishery management and conservation** and maintain the health of population at risk
3. Fishery forensics	*Using population assignment tests*	*Reinforcing management plan*
Develop **traceability tools** to support ecolabels and **track down illegal fishing**	Identify **the origin of a marine product** and assess the number of markers required	Develop a genotype assay for **population identification**
4. Fishery induced-evolution	*Genomic variation at spatial and temporal scale*	*Assessing the status of a fishery system*
Estimate the **spatial and temporal stability** of a stock and understand phenotypic changes observed at some areas	Consider spatial and temporal scale of **genomic variation** and identify **genes underlying phenotypic changes**	**Reduce fishing pressure**

Fig. 4 Summary of the four fishery genomics research axes identified in this chapter with a brief description of each axe regarding its main fishery management issues, genomic outcomes, and management applications

knowledge." This collaboration emphasizes the need to integrate the stakeholders at the beginning of the project, designing an integrative research plan with a realistic applied perspective, from the DNA to the fishers.

8 Future Perspectives

The ability to screen entire genomes has led to the development of new genomic resources for marine species that will enhance our ability to detect genetic markers putatively under divergent selection and subsequently increase our power to define stock structure with unique adaptive characteristics. Quantifying this putatively adaptive genetic variation among populations may also serve to better predict populations' loss or resilience to climate change, avoiding erroneous predictions and misplaced conservation efforts. A future avenue of research would be to test the effect of local climatic adaptations on the current and future distribution of marine resources by incorporating genomic information directly to forecasts of range changes. Additionally, these new genome-wide datasets may further serve as reference datasets for fisheries forensics. In fisheries forensics, the main challenge will be to develop a set of cheap, fast, and reliable markers to determine which particular local population or a sample – collected on board, from a fish market, or even off the plate of a restaurant – comes from. This easy to use DNA-based test could then be widely used by the fishery managers. Finally, improving our knowledge of the genetic basis of most traits in wild marine resources is critical for the field of fishery-induced evolution so that we can better assess how fishery practices may impact

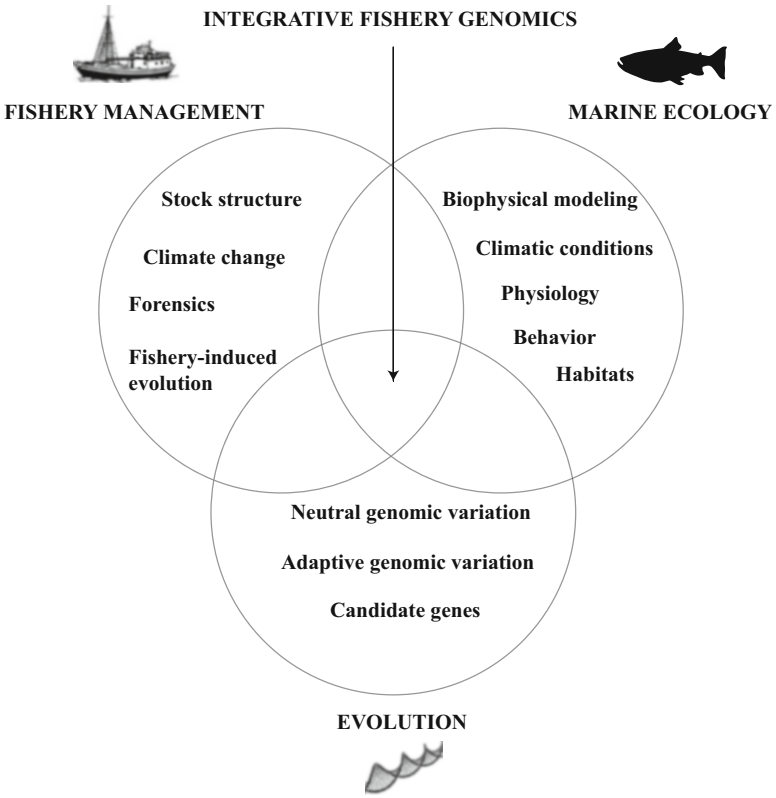

Fig. 5 Schematic representation of the three areas of research that are at the base of the emerging fishery genomics field. Connecting these areas would be the main challenge for the future of the field

these adaptive traits; we can then adapt the fishery practices to prevent or reduce this influence. More broadly, we are now entering in an era of multidisciplinary research. Therefore, building fishery genomics research that bridges fishery management, evolution, and marine ecology is the key for the future (Fig. 5).

References

Abdelrahman H, ElHady M, Alcivar-Warren A, Allen S, Al-Tobasei R, Bao L, et al. Aquaculture genomics, genetics and breeding in the United States: current status, challenges, and priorities for future research. BMC Genomics. 2017;18(1):191.

Allendorf FW, Hohenlohe PA, Luikart G. Genomics and the future of conservation genetics. Nat Rev Genet. 2010;11(10):697.

Barney BT, Munkholm C, Walt DR, Palumbi SR. Highly localized divergence within supergenes in Atlantic cod (Gadus morhua) within the Gulf of Maine. BMC Genomics. 2017;18(1):271.

Barrio AM, Lamichhaney S, Fan G, Rafati N, Pettersson M, Zhang H, et al. The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. Elife. 2016;5: e12081.

Barth JM, Berg PR, Jonsson PR, Bonanomi S, Corell H, Hemmer-Hansen J, et al. Genome architecture enables local adaptation of Atlantic cod despite high connectivity. Mol Ecol. 2017;26(17):4452–66.

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (Homarus americanus). Mol Ecol. 2015;24 (13):3299–315.

Benestan LM, Ferchaud AL, Hohenlohe PA, Garner BA, Naylor GJ, Baums IB, et al. Conservation genomics of natural and managed populations: building a conceptual and practical framework. Mol Ecol. 2016a;25(13):2967–77.

Benestan L, Quinn BK, Maaroufi H, Laporte M, Clark FK, Greenwood SJ, et al. Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (Homarus americanus). Mol Ecol. 2016b;25(20):5073–92.

Berg PR, Jentoft S, Star B, Ring KH, Knutsen H, Lien S, et al. Adaptation to low salinity promotes genomic divergence in Atlantic cod (Gadus morhua L.). Genome Biol Evol. 2015;7 (6):1644–63.

Bernatchez L, Wellenreuther M, Araneda C, Ashton DT, Barth JM, Beacham TD, et al. Harnessing the power of genomics to secure the future of seafood. Trends Ecol Evol. 2017;32(9):665–80.

Bowler DE, Benton TG. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biol Rev. 2005;80(2):205–25.

Bradbury IR, Hubert S, Higgins B, Bowman S, Borza T, Paterson IG, et al. Genomic islands of divergence and their consequences for the resolution of spatial structure in an exploited marine fish. Evol Appl. 2013;6(3):450–61.

Carreras C, Ordóñez V, Zane L, Kruschel C, Nasto I, Macpherson E, Pascual M. Population genomics of an endemic Mediterranean fish: differentiation by fine scale dispersal and adaptation. Sci Rep. 2017;7:43417.

Conover DO, Munch SB. Sustaining fisheries yields over evolutionary time scales. Science. 2002;297(5578):94–6.

Davey JW, Cezard T, Fuentes-Utrilla P, Eland C, Gharbi K, Blaxter ML. Special features of RAD sequencing data: implications for genotyping. Mol Ecol. 2013;22(11):3151–64.

Essington TE, Beaudreau AH, Wiedenmann J. Fishing through marine food webs. Proc Natl Acad Sci. 2006;103(9):3171–5.

FAOSTAT. Fish and fishery products – world apparent consumption statistics based on food balance sheets (1961-). In: FAO yearbook. Fishery and aquaculture statistics (FAO annuaire. Statistiques des pêches et de l'aquaculture/FAO anuario. Estadísticas de pesca y acuicultura). Rome; 2015.

François O, Martins H, Caye K, Schoville SD. Controlling false discoveries in genome scans for selection. Mol Ecol. 2016;25(2):454–69.

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW. Harnessing genomics for delineating conservation units. Trends Ecol Evol. 2012;27(9):489–96.

Gagnaire PA, Gaggiotti OE. Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. Curr Zool. 2016;62(6):603–16.

Gagnaire PA, Broquet T, Aurelle D, Viard F, Souissi A, Bonhomme F, et al. Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. Evol Appl. 2015;8(8):769–86.

Garner BA, Hand BK, Amish SJ, Bernatchez L, Foster JT, Miller KM, et al. Genomics in conservation: case studies and bridging the gap between data and application. Trends Ecol Evol. 2016;31(2):81–3.

Hansen MM, Hemmer-Hansen J. Landscape genetics goes to sea. J Biol. 2007;6(3):6.
Hansen MM, Olivieri I, Waller DM, Nielsen EE, GeM Working Group. Monitoring adaptive genetic responses to environmental change. Mol Ecol. 2012;21(6):1311–29.
Hastings A. Complex interactions between dispersal and dynamics: lessons from coupled logistic equations. Ecology. 1993;74(5):1362–72.
Hauser L, Adcock GJ, Smith PJ, Ramírez JHB, Carvalho GR. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (Pagrus auratus). Proc Natl Acad Sci. 2002;99(18):11742–7.
Heino M, Pauli BD, Dieckmann U. Fisheries-induced evolution. Annu Rev Ecol Evol Syst. 2015;46:461–80.
Hemmer-Hansen J, Therkildsen NO, Pujolar JM. Population genomics of marine fishes: next-generation prospects and challenges. Biol Bull. 2014;227(2):117–32.
Hoey JA, Pinsky ML. Genomic signatures of environmental selection despite near-panmixia in summer flounder. Evol Appl. 2018;11(9):1732–47.
Hutchinson WF, van Oosterhout C, Rogers SI, Carvalho GR. Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (Gadus morhua). Proc R Soc Lond B Biol Sci. 2003;270(1529):2125–32.
Jackson AM, Semmens BX, De Mitcheson YS, Nemeth RS, Heppell SA, Bush PG, et al. Population structure and phylogeography in Nassau grouper (Epinephelus striatus), a mass-aggregating marine fish. PLoS One. 2014;9(5):e97508.
Kelley JL, Brown AP, Therkildsen NO, Foote AD. The life aquatic: advances in marine vertebrate genomics. Nat Rev Genet. 2016;17(9):523.
Kirubakaran TG, Grove H, Kent MP, Sandve SR, Baranski M, Nome T, et al. Two adjacent inversions maintain genomic differentiation between migratory and stationary ecotypes of Atlantic cod. Mol Ecol. 2016;25(10):2130–43.
Knutsen H, Olsen EM, Jorde PE, Espeland SH, André C, Stenseth NC. Are low but statistically significant levels of genetic differentiation in marine fishes 'biologically meaningful'? A case study of coastal Atlantic cod. Mol Ecol. 2011;20(4):768–83.
Kreitzman M, Ashander J, Driscoll J, Bateman AW, Chan KM, Lewis MA, Krkosek M. Wild salmon sustain the effectiveness of parasite control on salmon farms: conservation implications from an evolutionary ecosystem service. Conserv Lett. 2018;11(2):e12395.
Kuparinen A, Hutchings JA. Genetic architecture of age at maturity can generate divergent and disruptive harvest-induced evolution. Phil Trans R Soc B. 2017;372(1712):20160035.
Laikre L, Allendorf FW, Aroner LC, Baker CS, Gregovich DP, Hansen MM, et al. Neglect of genetic diversity in implementation of the convention on biological diversity. Conserv Biol. 2010;24(1):86–8.
Lal MM, Southgate PC, Jerry DR, Zenger KR. Fishing for divergence in a sea of connectivity: the utility of ddRADseq genotyping in a marine invertebrate, the black-lip pearl oyster Pinctada margaritifera. Mar Genomics. 2016;25:57–68.
Lal MM, Southgate PC, Jerry DR, Bosserelle C, Zenger KR. Swept away: ocean currents and seascape features influence genetic structure across the 18,000 km Indo-Pacific distribution of a marine invertebrate, the black-lip pearl oyster Pinctada margaritifera. BMC Genomics. 2017;18 (1):66.
Lamichhaney S, Barrio AM, Rafati N, Sundström G, Rubin CJ, Gilbert ER, et al. Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. Proc Natl Acad Sci. 2012;109(47):19345–50.
Larson WA, Seeb LW, Everett MV, Waples RK, Templin WD, Seeb JE. Genotyping by sequencing resolves shallow population structure to inform conservation of Chinook salmon (Oncorhynchus tshawytscha). Evol Appl. 2014;7(3):355–69.
Le Moan A, Gagnaire PA, Bonhomme F. Parallel genetic divergence among coastal–marine ecotype pairs of European anchovy explained by differential introgression after secondary contact. Mol Ecol. 2016;25(13):3187–202.

Limborg MT, Helyar SJ, De Bruyn M, Taylor MI, Nielsen EE, Ogden ROB, et al. Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (Clupea harengus). Mol Ecol. 2012;21(15):3686–703.

Lowe WH, Allendorf FW. What can genetics tell us about population connectivity? Mol Ecol. 2010;19(15):3038–51.

Martinsohn JT, Ogden R, FishPopTrace Consortium. FishPopTrace – developing SNP-based population genetic assignment methods to investigate illegal fishing. Forensic Sci Int Genet. 2009;2(1):294–6. Supplement Series.

McCauley DJ, Pinsky ML, Palumbi SR, Estes JA, Joyce FH, Warner RR. Marine defaunation: animal loss in the global ocean. Science. 2015;347(6219):1255641.

Meek MH, Baerwald MR, Stephens MR, Goodbla A, Miller MR, Tomalty KM, May B. Sequencing improves our ability to study threatened migratory species: genetic population assignment in California's Central Valley Chinook salmon. Ecol Evol. 2016;6(21):7706–16.

Miller AD, van Rooyen A, Rašić G, Ierodiaconou DA, Gorfine HK, Day R, et al. Contrasting patterns of population connectivity between regions in a commercially important mollusc Haliotis rubra: integrating population genetics, genomics and marine LiDAR data. Mol Ecol. 2016;25(16):3845–64.

Moore JS, Bourret V, Dionne M, Bradbury I, O'Reilly P, Kent M, et al. Conservation genomics of anadromous Atlantic salmon across its North American range: outlier loci identify the same patterns of population structure as neutral loci. Mol Ecol. 2014;23(23):5680–97.

Moore JS, Harris LN, Le Luyer J, Sutherland BJ, Rougemont Q, Tallman RF, et al. Genomics and telemetry suggest a role for migration harshness in determining overwintering habitat choice, but not gene flow, in anadromous Arctic Char. Mol Ecol. 2017;26(24):6784–800.

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D. Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol. 2009;18(15):3128–50.

Nielsen EE, Cariani A, Mac Aoidh E, Maes GE, Milano I, Ogden R, et al. Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. Nat Commun. 2012;3:851.

Ovenden JR, Berry O, Welch DJ, Buckworth RC, Dichmont CM. Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. Fish Fish. 2015;16(1):125–59.

Palsbøll PJ, Berube M, Allendorf FW. Identification of management units using population genetic data. Trends Ecol Evol. 2007;22(1):11–6.

Pardo-Diaz C, Salazar C, Jiggins CD. Towards the identification of the loci of adaptive evolution. Methods Ecol Evol. 2015;6(4):445–64.

Pauly D, Zeller D. Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. Nat Commun. 2016;7:ncomms10244.

Pauly D, Christensen V, Dalsgaard J, Froese R, Torres F. Fishing down marine food webs. Science. 1998;279(5352):860–3.

Pavey SA, Gaudin J, Normandeau E, Dionne M, Castonguay M, Audet C, Bernatchez L. RAD sequencing highlights polygenic discrimination of habitat ecotypes in the panmictic American eel. Curr Biol. 2015;25(12):1666–71.

Pecoraro C, Babbucci M, Villamor A, Franch R, Papetti C, Leroy B, et al. Methodological assessment of 2b-RAD genotyping technique for population structure inferences in yellowfin tuna (Thunnus albacares). Mar Genomics. 2016;25:43–8.

Pineda J, Hare JA, Sponaugle SU. Larval transport and dispersal in the coastal ocean and consequences for population connectivity. Oceanography. 2007;20(3):22–39.

Pinsky ML, Palumbi SR. Meta-analysis reveals lower genetic diversity in overfished populations. Mol Ecol. 2014;23(1):29–39.

Poćwierz-Kotus A, Kijewska A, Petereit C, Bernaś R, Więcaszek B, Arnyasi M, et al. Genetic differentiation of brackish water populations of cod Gadus morhua in the southern Baltic, inferred from genotyping using SNP-arrays. Mar Genomics. 2015;19:17–22.

Pujolar JM, Jacobsen MW, Als TD, Frydenberg J, Munch K, Jónsson B, et al. Genome-wide single-generation signatures of local selection in the panmictic European eel. Mol Ecol. 2014;23 (10):2514–28.

Reiss H, Hoarau G, Dickey-Collas M, Wolff WJ. Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish Fish. 2009;10(4):361–95.
Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. A practical guide to environmental association analysis in landscape genomics. Mol Ecol. 2015;24(17):4348–70.
Riginos C, Liggins L. Seascape genetics: populations, individuals, and genes marooned and adrift. Geogr Compass. 2013;7(3):197–216.
Rochette R, Sainte-Marie B, Allain M, Baker J, Bernatchez L, Boudreau V, et al. The Lobster Node of the CFRN: co-constructed and collaborative research on productivity, stock structure, and connectivity in the American lobster (Homarus americanus). Can J Fish Aquat Sci. 2018;75:813–24.
Rodríguez-Ezpeleta N, Bradbury IR, Mendibil I, Álvarez P, Cotano U, Irigoien X. Population structure of Atlantic mackerel inferred from RAD-seq-derived SNP markers: effects of sequence clustering parameters and hierarchical SNP selection. Mol Ecol Resour. 2016;16(4):991–1001.
Ropert-Coudert Y, Bost CA, Handrich Y, Bevan RM, Butler PJ, Woakes AJ, Le Maho Y. Impact of externally attached loggers on the diving behaviour of the king penguin. Physiol Biochem Zool. 2000;73(4):438–44.
Savolainen O, Lascoux M, Merilä J. Ecological genomics of local adaptation. Nat Rev Genet. 2013;14(11):807.
Scheffer M, Carpenter S, de Young B. Cascading effects of overfishing marine systems. Trends Ecol Evol. 2005;20(11):579–81.
Schwabl P, Llewellyn MS, Landguth EL, Andersson B, Kitron U, Costales JA, et al. Prediction and prevention of parasitic diseases using a landscape genomics framework. Trends Parasitol. 2017;33(4):264–75.
Shafer AB, Wolf JB, Alves PC, Bergström L, Bruford MW, Brännström I, et al. Genomics and the challenging translation into conservation practice. Trends Ecol Evol. 2015;30(2):78–87.
Shafer AB, Northrup JM, Wikelski M, Wittemyer G, Wolf JB. Forecasting ecological genomics: high-tech animal instrumentation meets high-throughput sequencing. PLoS Biol. 2016;14(1): e1002350.
Stanley RR, DiBacco C, Lowen B, Beiko RG, Jeffery NW, Van Wyngaarden M, et al. A climate-associated multispecies cryptic cline in the northwest Atlantic. Sci Adv. 2018;4(3):eaaq0929.
Stockwell BL, Larson WA, Waples RK, Abesamis RA, Seeb LW, Carpenter KE. The application of genomics to inform conservation of a functionally important reef fish (Scarus niger) in the Philippines. Conserv Genet. 2016;17(1):239–49.
Stokstad E. To fight illegal fishing, forensic DNA gets local. Science. 2010;330:1468–9.
Therkildsen NO, Nielsen EE, Swain DP, Pedersen JS. Large effective population size and temporal genetic stability in Atlantic cod (Gadus morhua) in the southern Gulf of St. Lawrence. Can J Fish Aquat Sci. 2010;67(10):1585–95.
Therkildsen NO, Hemmer-Hansen J, Als TD, Swain DP, Morgan MJ, Trippel EA, et al. Micro-evolution in time and space: SNP analysis of historical DNA reveals dynamic signatures of selection in Atlantic cod. Mol Ecol. 2013;22(9):2424–40.
Valenzuela-Quiñonez F. How fisheries management can benefit from genomics? Brief Funct Genomics. 2016;15(5):352–7.
Van Wyngaarden M, Snelgrove PV, DiBacco C, Hamilton LC, Rodríguez-Ezpeleta N, Jeffery NW, et al. Identifying patterns of dispersal, connectivity and selection in the sea scallop, Placopecten magellanicus, using RAD seq-derived SNP s. Evol Appl. 2017;10(1):102–17.
Vendrami DL, Telesca L, Weigand H, Weiss M, Fawcett K, Lehman K, et al. RAD sequencing resolves fine-scale population structure in a benthic invertebrate: implications for understanding phenotypic plasticity. R Soc Open Sci. 2017;4(2):160548.
Waples RS. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J Hered. 1998;89(5):438–50.
Waples RS, Gaggiotti O. INVITED REVIEW: What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol. 2006;15(6):1419–39.

Waples RS, Naish KA. Genetic and evolutionary considerations in fishery management: research needs for the future. In: The future of fisheries science in North America. Dordrecht: Springer; 2009. p. 427–51.

Waples RS, Pess GR, Beechie T. Evolutionary history of Pacific salmon in dynamic environments. Evol Appl. 2008;1(2):189–206.

Ward RD. Genetics in fisheries management. Hydrobiologia. 2000;420(1):191–201.

Whitlock MC, Mccauley DE. Indirect measures of gene flow and migration: FST$\neq$ 1/(4Nm+ 1). Heredity. 1999;82(2):117–25.

Willette DA, Allendorf FW, Barber PH, Barshis DJ, Carpenter KE, Crandall ED, et al. So, you want to use next-generation sequencing in marine systems? Insight from the Pan-Pacific Advanced Studies Institute. Bull Mar Sci. 2014;90(1):79–122.

Willing EM, Dreyer C, Van Oosterhout C. Estimates of genetic differentiation measured by FST do not necessarily require large sample sizes when using many SNP markers. PLoS One. 2012;7(8):e42649.

Wright S. Evolution in Mendelian populations. Genetics. 1931;16(2):97.

Xuereb A, Benestan L, Normandeau E, Daigle RM, Curtis JM, Bernatchez L, Fortin MJ. Asymmetric oceanographic processes mediate connectivity and population genetic structure, as revealed by RAD seq, in a highly dispersive marine invertebrate (Parastichopus californicus). Mol Ecol. 2018;27(10):2347–64.

Yeaman S, Whitlock MC. The genetic architecture of adaptation under migration–selection balance. Evolution. 2011;65(7):1897–911.

Marine Conservation and Marine Protected Areas

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Abstract Marine protected areas are important tools for the conservation of marine biodiversity, providing refuge for harvested species and mitigating the negative impacts of human activities in marine ecosystems. However, delineating sites for protection within effective MPA networks is a formidable challenge. A primary

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objective of MPA planning is to optimize connectivity among reserve sites, such that immigration from distant sites or populations sustains biodiversity, both within MPAs and in adjacent unprotected areas. Additionally, as climate change further threatens marine biodiversity, adaptation to novel climatic and environmental conditions also has important consequences for the persistence of marine populations. Inferences from population genomics can provide valuable insight into the design of MPA networks, both for ensuring connectivity and preserving adaptive potential for future environmental change. However, genetic and genomic data are rarely used to inform marine spatial planning. Effective dissemination of primary research to practitioners will be key to the successful integration of these valuable data into MPA network designs.

Keywords Climate change · Conservation genomics · Local adaptation · Marine connectivity · Marine protected area networks · Marine spatial planning

1 Marine Protected Areas: Tools for Marine Conservation

Anthropogenic activities in coastal and marine environments have considerable effects on ocean health and ecosystem functioning (Halpern et al. 2015). Beyond the impacts of exploitation, the escalating effects of climate change also threaten the long-term persistence of many marine species (Stuart-Smith et al. 2015). Marine protected areas (MPAs) are considered to be effective tools for mitigating the pervasive human impacts on marine biodiversity by limiting resource use and extraction within MPA boundaries and enhancing ecosystem resilience (Levin and Lubchenco 2008; Gaines et al. 2010; Edgar et al. 2014; Krueck et al. 2017). The definition of a MPA can vary with respect to the level of protection, ranging from multiple-use MPAs, where some degree of extraction is permitted, to highly protected no-take marine reserves (Day et al. 2012; Costello and Ballantine 2015). Studies have shown that MPAs, in particular those with well-enforced no-take policies, are indeed effective tools for enhancing marine biomass and ecosystem resilience in the face of climate change (Micheli et al. 2012; Edgar et al. 2014; Mellin et al. 2016; Gill et al. 2017; Sala and Giakoumi 2018). However, recent estimates suggest that worldwide, only 3.6% of the ocean is protected within MPAs, and only 2% is fully protected under no-take marine reserves (Sala et al. 2018). Efforts

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to improve the protection of our oceans are ongoing, with global commitments to increase ocean protection to 10% by 2020 (United Nations' Convention on Biological Diversity (CBD) Aichi Target 11). In light of efforts to enhance protection of the world's oceans, various innovative approaches to inform effective MPA planning are being explored, including approaches based on genetic and genomic data.

2 Using Genetic and Genomic Data to Inform Conservation and Management

Genetic methods provide a valuable means for answering a wide range of questions related to the conservation and protection of biodiversity (Frankham 2010). Generally speaking, conservation genetics as a field aims to preserve genetic diversity and maintain evolutionary potential in wild populations. The application of genetic techniques for conservation purposes has been successful across systems and diverse types of issues, including mitigating effects of inbreeding depression in small populations (Frankham et al. 2014; Frankham 2015), delineating units for management (Palsbøll et al. 2007), and informing and monitoring reintroductions of endangered or extirpated species into the wild (Frankham 2008; Koelewijn et al. 2010; Çilingir et al. 2017).

With recent advances in molecular technologies, the ability to ascertain hundreds or thousands of single-nucleotide polymorphisms (SNPs) across the genomes of non-model organisms (Andrews et al. 2016) has opened doors for addressing several challenges in conservation genetics by enhancing the number of genetic markers available and enabling the detection of loci underlying functionally important traits (Allendorf et al. 2010; Benestan et al. 2016a). The usefulness of genomic data for biodiversity conservation has been acknowledged in the context of spatial planning (Funk et al. 2012; Nielsen et al. 2017; Barbosa et al. 2018), biosecurity and aquaculture (Bernatchez et al. 2017), and predicting evolutionary responses to climate change (Bay et al. 2017). One particular advantage of genomic data in the context of conservation is that they offer the opportunity to discriminate between putatively *neutral* and *adaptive* markers of genetic variation (Allendorf et al. 2010; Manel et al. 2010). Frameworks for incorporating genetic data into marine conservation planning have generally focused primarily on neutral markers, which are suitable for calculating metrics such as genetic diversity, uniqueness, and connectivity (e.g. Beger et al. 2014). However, neutral markers do not provide any information about adaptive evolutionary processes, and questions regarding the effects of natural selection and potential for adaptation thus rely on the characterization of adaptive loci (Holderegger et al. 2006; Allendorf et al. 2010; Manel and Holderegger 2013). Yet, until recently, detecting adaptive genetic variation had been largely confined to model organisms, due in

large part to the lack of genomic resources for non-model species (Manel et al. 2016) and the experimental tractability needed to identify loci underlying ecologically important traits under selection (Savolainen et al. 2013). The ability to incorporate inferences regarding both neutral and adaptive genetic variation into conservation objectives presents an opportunity to create a unified framework that addresses both demographic and evolutionary processes. The subsequent sections of this chapter elaborate on the genetic and genomic approaches that can be used to inform marine conservation objectives, particularly with respect to MPA planning.

3 MPA Network Connectivity

The spatial arrangement of individual MPAs has important consequences for the ecological and evolutionary dynamics of populations, and it is widely acknowledged that implementing *networks* of MPAs is more effective than delineating single reserve sites (Gaines et al. 2010; Kininmonth et al. 2011). MPA networks rely on connectivity (Box 1) between reserve sites through dispersal of larvae or adults. Appropriate spatial configuration (e.g. size and spacing) of protected sites is thought to lead to self-sustaining MPA networks that can meet fisheries and conservation goals by supporting recruitment within and between MPAs while simultaneously increasing recruitment to unprotected sites via spillover (Gaines et al. 2010; Lubchenco and Grorud-Colvert 2015; Andrello et al. 2017) (Fig. 1). As such, connectivity has become an integral principle in marine spatial planning (Lagabrielle et al. 2008; Green et al. 2015; Daigle et al. 2018; Magris et al. 2018). This emphasis on ensuring connectivity has gained momentum in the MPA and marine reserve science literature, with the number of publications citing connectivity as an essential criterion for effective reserve network design increasing over the last 15 years (Fig. 2).

Box 1 Definitions of Connectivity

In natural systems, connectivity is broadly defined as the degree to which spatially subdivided populations or patches are able to exchange individuals (Taylor et al. 1993; Cowen and Sponagule 2009). However, connectivity can be measured in many different ways. Here, we define some of the most commonly used measurements of connectivity:

Structural connectivity: connectivity is measured based on the structure of the landscape without considering behavior or movement capabilities of any organism (Tischendorf and Fahrig 2000). For example, the spatial

(continued)

Box 1 (continued)
arrangement, location of barriers, and distance between habitat patches influence the likelihood that organisms will move between them. In terrestrial environments, corridors are important structural components that facilitate movement and connectivity. In the marine environment, physical ocean circulation dynamics (e.g. directional surface currents, circular eddies or gyres) can influence the probability of movement between populations.

Functional connectivity: refers to the actual movement of organisms and can be influenced by dispersal rates, behavioral responses, and mortality (Tischendorf and Fahrig 2000). In the marine environment, larval behaviors such as active swimming and natal homing can influence the degree of functional connectivity between populations or habitat patches.

Demographic connectivity: the degree to which dispersal/immigration contributes to population growth and vital rates (e.g. survival and birth rates) relative to local recruitment (Lowe and Allendorf 2010).

Genetic connectivity: the degree to which gene flow (i.e. the exchange of genetic material between populations) influences evolutionary processes (Lowe and Allendorf 2010). The level of gene flow can be assessed based on estimates of genetic differentiation between populations.

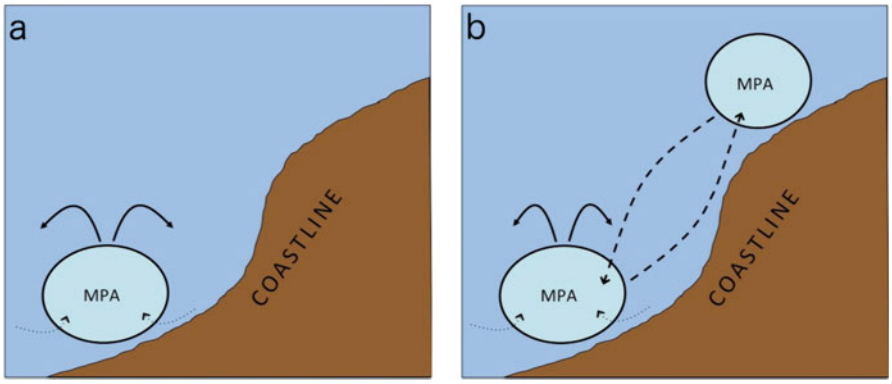

Fig. 1 (**a**) Dispersal of individuals out of a marine protected area (MPA) can benefit adjacent sites or populations via spillover (solid arrows). However, if immigration into MPAs is low due to declining populations in harvested sites (dotted arrows), the population within the MPA may not be self-sustaining. (**b**) Nearby and/or well-connected MPAs can enhance the stability of both protected and fished populations by increasing recruitment into MPAs (dashed arrows) while maintaining a supply of individuals for the fishery via spillover (solid arrows). Figure adapted from Gaines et al. (2010)

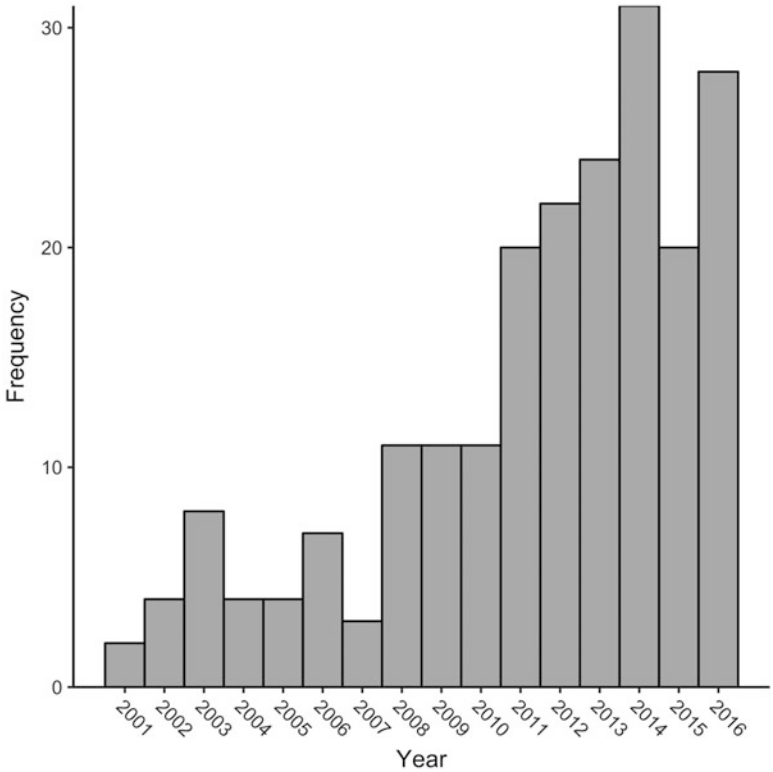

Fig. 2 Frequency distribution of studies published between 2001 and 2016 that emphasize the importance of integrating connectivity in marine reserve or marine protected area networks. An initial raw list of 335 publications was generated from a Web of Science literature search. Book chapters and conference symposia were excluded, while all research articles, reviews, and letters were retained. Articles that did not meet any of the following criteria were also excluded: (1) those that discussed measuring connectivity for the purpose of MPA network design or selecting reserve sites using connectivity metrics; (2) those that measured connectivity among existing MPAs to evaluate performance; and (3) those that emphasized the need for connectivity in effective MPA network designs

3.1 Measuring Marine Connectivity

Despite the asserted benefits of optimizing connectivity in MPA networks, measuring and integrating marine connectivity into conservation plans is not an easy task. Perhaps one of the most challenging aspects of obtaining accurate estimates of marine connectivity is related to the fact that most marine species disperse as tiny pelagic larvae, making direct observations of dispersal in coastal and marine systems challenging (Cowen and Sponagule 2009). While artificial tagging methods have been used to track movements of dispersing larvae in some case studies, this

approach is most appropriate for estimating larval retention in local populations at rather fine spatial scales, as opposed to quantifying broad scale connectivity (Thorrold et al. 2007; Almany et al. 2007). Genetic approaches thus provide a valuable set of tools for evaluating connectivity across a range of spatial scales (Manel et al. 2003; Hedgecock et al. 2007). For example, parentage analyses and assignment tests are two commonly used methods for assessing connectivity using genotype information to identify putative migrants from recent dispersal events (Manel et al. 2005; Saenz-Agudelo et al. 2009; Harrison et al. 2012; Christie et al. 2017). Assignment tests allocate individuals to their subpopulation of origin, while parentage analyses identify parent-offspring relationships to determine single-generation dispersal distances. These individual-based analyses can provide robust estimates of marine connectivity and the proportion of migrants relative to local recruits within a population, but may be limited in terms of sample size and overall strength of genetic differentiation. Parentage analyses in particular require exhaustive sampling to accurately detect immigrants and thus may not be feasible for many species. Though assignment tests generally do not perform well in cases of low population divergence and high gene flow (a typical scenario in marine populations), increasing the number of genetic markers, especially to the extent of modern genomic data sets with hundreds or thousands of loci, has been shown to improve the accuracy of assignment tests even when overall divergence is low (Patkeau et al. 2004; Benestan et al. 2015). Additionally, individual-based clustering approaches are commonly used to delineate populations based on membership coefficients to distinct genetic clusters generated from individual genotypes (e.g. STRUCTURE – Pritchard et al. 2000). In this way, information on the probability of an individual's assignment to each inferred genetic cluster can be used to understand not just how populations are structured but also the extent to which given populations are connected to one another by gene flow (Manel et al. 2005).

Analyses of population genetic structure also provide indirect methods for assessing *genetic* connectivity (see Box 1) based on differentiation (e.g. using F_{ST}) between subpopulations (Lowe and Allendorf 2010). It is important to note that inferences about *demographic* connectivity based on population genetic structure must be made with caution since a very small number of migrants per generation can lead to relatively homogenous populations in some cases (Whitlock and McCauley 1999; Lowe and Allendorf 2010), meaning that the amount of individual exchange is actually quite low despite high genetic similarity. However, significant levels of genetic differentiation can indicate a lack of panmixia and therefore the potential presence of barriers to gene flow and restricted connectivity, although disentangling historical drivers of population genetic differentiation from contemporary patterns of gene flow can be challenging (Hedgecock et al. 2007). Consequently, biophysical modelling approaches that integrate hydrodynamic models and biological information to simulate dispersal can be combined with assessments of genetic structure to help shed light upon the potential determinants of gene flow and population connectivity in marine environments (Selkoe et al. 2016; Riginos et al. 2016). Another promising approach uses a framework of isolation by distance (IBD) to describe population connectivity, whereby populations that are closer together

experience a higher degree of gene flow compared to more distant populations, and genetic differentiation thus increases as the spatial distance between populations increases (Wright 1943; Palumbi 2003). Theoretical work suggests that the slope of the IBD relationship can be used to estimate dispersal distances (Rousset 1997), and empirical studies have demonstrated the application of IBD models to infer the spatial scale of connectivity in coral reef fish populations (Pinsky et al. 2010; Puebla et al. 2012).

3.2 Applying Connectivity Estimates to MPA Network Design

Understanding variability in the scale of dispersal for multiple species is important for ensuring the appropriate spatial configuration of MPA networks. For example, using a genetic parentage analysis combined with biophysical larval dispersal models, Nanninga et al. (2015) found evidence for very low self-recruitment in an anemone fish (*Amphiprion bicinctus*) in the Red Sea, in contrast to previous studies that demonstrated high levels of larval retention in other reef species (e.g. James et al. 2002; Cowen et al. 2006; Gerlach et al. 2007; Patterson and Swearer 2007). These findings are critical for the design of MPA networks as they caution against assumptions that self-replenishment will always be sufficient to sustain small, isolated MPAs. Other studies show varying estimates of mean dispersal distances across species. For example, D'Aloia et al. (2015) performed a large-scale parentage analysis to fit a dispersal kernel for the neon goby, *Elacatinus lori*, in the Belize Barrier Reef, and identified a relatively short mean dispersal distance for this species (1.8 km). In contrast, using a similar approach, a study by Almany et al. (2017) demonstrated much longer mean dispersal distances for the orange clownfish, *Amphiprion percula* (>10 km), and the vagabond butterflyfish, *Chaetodon vagabundus* (>100 km), in Papua New Guinea. Importantly, these studies show that the optimal size and spacing between MPAs to maintain connectivity or replenish harvested populations can differ between species. Though parentage analyses can provide accurate estimates of the scale of dispersal for marine species, the extensive sampling effort required may be prohibitive for many species, especially for those with relatively large ranges, high fecundity, and/or a strong dispersal capacity. However, a study by Pinsky et al. (2017) highlighted the potential for approaches based on IBD models for accurate assessments of dispersal distances, revealing a strong agreement in dispersal distances estimated from the slope of IBD relationships compared to direct parent-offspring dispersal patterns in *A. percula*.

Spatial patterns of population genetic structure and gene flow also have important implications for selecting and prioritizing sites or populations for protection within MPAs. Some studies have used genetic and genomic data to identify particular regions that serve as key connectivity nodes, lending support to prioritizing these areas in order to link distant sites within a larger metapopulation (e.g. Rozenfeld et al. 2008; Almany et al. 2017; Jahnke et al. 2018). In one study, Xuereb et al. (2018) used a population graph approach based on genetic covariance (Dyer et al.

2010) to demonstrate that the Central Coast region of British Columbia, Canada, was an important stepping-stone for connectivity between north and south regional genetic groups of the giant California sea cucumber (*Parastichopus californicus*) (see Box 2). Indeed, this study supported earlier findings based on biophysical modelling that the British Columbia Central Coast may be an intermediate site for gene flow across a similar genetic boundary in the bat star, *Patiria miniata* (Sunday et al. 2014). Taken together, these studies suggest that this region could facilitate connectivity within a broader MPA network in coastal British Columbia for co-distributed species. Moreover, asymmetric ocean circulation patterns have been implicated as an important predictor of spatial patterns of genetic differentiation in marine organisms (e.g. Benestan et al. 2016b; Dalongeville et al. 2018a; Xuereb et al. 2018), and the use of biophysical modelling to supplement population genetic structure analyses therefore provides insight about the direction of gene flow and the location of key source populations (Cowen et al. 2007; Jenkins and Stevens 2018).

Box 2 Identifying Key Areas for Connectivity Using Population Graphs

In coastal habitats and complex seascapes, specific sites may serve as key intermediate sites, or stepping-stones, for maintaining connectivity within a broader metapopulation. These sites are important in the context of MPA networks, as their protection can facilitate dispersal and gene flow between distant and otherwise disconnected populations. The development of population graphs based on genetic covariance among populations (Dyer and Nason 2004; Dyer et al. 2010) can illuminate the potential for certain locations to serve as critical connectivity nodes within a network. First, a population graph, or network, is constructed based on genetic covariance relationships among all sampling locations. Then, various metrics can be used to evaluate the contribution of individual nodes to the overall structure of the network; in other words, how important is a particular node for maintaining connections among other nodes within the entire network? In one example, Xuereb et al. (2018) used such an approach to investigate population connectivity of the giant California sea cucumber (Fig. 3a) from 24 sampling locations in coastal British Columbia (Fig. 3b). A population graph was constructed based on genetic covariance among these sampling locations that highlighted limited connectivity between north and south regional groups (Fig. 3c). The authors evaluated the relative importance of each node to the overall structure of the network using the metric *betweenness centrality*, which quantifies the number of shortest paths through the entire graph that pass through a given node. Based on this metric, it was determined that Calvert Island ('CAL' in Fig. 3b) had the highest betweenness centrality, which suggests that this region in the Central Coast of British Columbia might be important for facilitating connectivity between northern and southern coastal regions.

(continued)

Box 2 (continued)

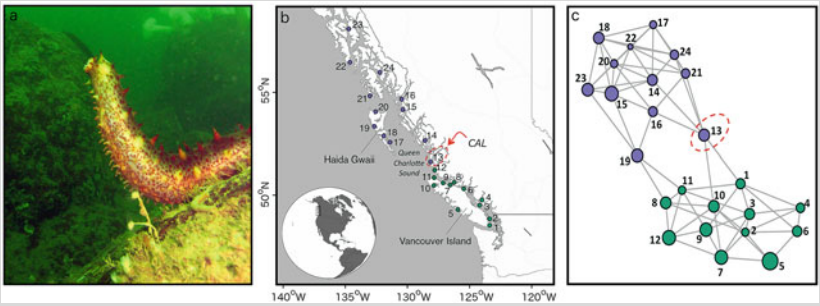

Fig. 3 (**a**) Photo of the giant California sea cucumber (*Parastichopus californicus*) (Photo: Isabelle Côté). (**b**) Map of sampling locations from Xuereb et al. (2018) showing the north (purple) and south (green) regional clusters. Calvert Island (*CAL*) is circled to highlight the sampling location in the Central Coast of British Columbia, Canada, which was determined to be an important connectivity node based on the population graph in (**c**). Here, the circles (nodes) represent sample locations, and the edges (lines) represent genetic covariance between a given pair of locations. The nodes are colored according to the regional clusters (purple = north; green = south). Only edges that adequately contribute to explaining the overall genetic covariance structure are retained. The size of the nodes indicates the relative amount of genetic diversity, and the edge length is inversely proportional to genetic covariance. Figure adapted from Xuereb et al. (2018)

Source populations are important in the context of MPA networks because their protection can facilitate recruitment into populations that are not self-sustaining (e.g. populations with limited larval retention) or into populations at risk of declining to extinction (e.g. harvested populations outside of MPAs). For this reason, source populations that are large and consist of high levels of genetic diversity should be protected to ensure the potential spread of genetic variation into recipient populations (Beger et al. 2014). This is especially critical when source populations contain unique genetic diversity. For example, Jenkins and Stevens (2018) highlighted a recent case study on population genetic structure in the pink sea fan (*Eunicella verrucosa*), a species of conservation concern in England and Wales (Holland et al. 2017), and demonstrated how their results could inform the planning and evaluation of MPA networks. The original study found strong genetic similarity among sites in southwest Britain where reserves have been designated to protect *E. verrucosa*, indicating a high level of gene flow and connectivity among established reserve sites in this region (Holland et al. 2017). In contrast, genetic differentiation between sites in southwest Britain and others in northwest France, Portugal, and Ireland indicates the presence of unique genetic variation that may warrant protection within MPAs to ensure the preservation of intraspecies genetic diversity (Jenkins and Stevens 2018). Numerous published population genetic studies have identified important spatial patterns of genetic differentiation and

gene flow across marine taxa with a range of dispersal abilities, including both vertebrates (e.g. Andrews et al. 2010; Schunter et al. 2011; Siegle et al. 2013; Larson et al. 2014; Liggins et al. 2016; Dalongeville et al. 2018a) and invertebrates (e.g. Thomas and Bell 2013; Schiavina et al. 2014; Sunday et al. 2014; Jorde et al. 2015; Thomas et al. 2015; Benestan et al. 2016b; Iacchei et al. 2016; Cros et al. 2017; Lal et al. 2017; Truelove et al. 2017; Xuereb et al. 2018). These and many other studies provide a wealth of knowledge for informing marine policy and addressing conservation issues, especially with respect to selecting sites for MPA designation that support network connectivity and capture genetic diversity for the long-term persistence of marine biodiversity.

4 Evolutionary Perspectives for MPA Networks in the Face of Climate Change

In the face of climate change and other anthropogenic stressors, populations and species will need to acclimate or adapt to novel environmental conditions to persist in their current geographic range. For some species, plasticity in tolerance to stressors such as warming ocean temperatures and acidification might allow individuals to endure environmental shifts (Calosi et al. 2016). Experimental studies have demonstrated the ability for trans-generational plasticity to enhance the acclimatization potential of individuals under environmental stress (Veilleux et al. 2015; Ryu et al. 2018). However, the extent to which trans-generational effects can improve resilience to climate change over time is unknown, and adaptive evolution might be essential in the longer term, especially under extreme environmental change (Gunderson and Stillman 2015). Regardless of the potential for acclimation in the short term, improved knowledge of the spatial patterns of adaptive genetic variation associated with environmental tolerance is important for safeguarding the evolutionary potential of marine populations in the future.

4.1 Local Adaptation in the Sea

The potential for adaptation largely depends on (1) the magnitude and patterns of gene flow and (2) the amount of standing genetic variation. Marine organisms are typically characterized by long-distance dispersal abilities, and because of this, gene flow is expected to be high, potentially counteracting selective forces driving local adaptation (Kawecki and Ebert 2004) and leading to an early assumption that local adaptation is rare in marine populations. However, marine populations often have large effective population sizes, which can increase the efficiency of selection and maintain standing genetic variation (Kimura and Ohta 1969). Recent studies have demonstrated the capacity for rapid adaptation to environmental changes in marine species as a result of selection on standing genetic variation (e.g. Pespeni et al.

2013). Moreover, spatially varying selection, a form of balancing selection, can maintain genetic polymorphisms and support adaptive differentiation and local adaptation despite high gene flow (Bernatchez 2016; Sanford and Kelly 2011). Empirical work has supported this hypothesis. For example, Gagnaire et al. (2012) and Babin et al. (2017) found evidence of spatially varying selection maintaining balanced polymorphisms in the American eel (*Anguilla rostrata*), a classic case of a panmictic species with an absence of within-species population structure (Côté et al. 2013). Therefore, local adaptation in marine populations is not a rare phenomenon as it was once perceived, and the ability to detect and measure adaptive differentiation has important implications for marine conservation.

4.2 Adaptive Genetic Variation Applied to Marine Conservation

The ability to now include adaptive genetic variation in conservation plans has the potential to achieve conservation objectives that aim to preserve evolutionary resilience and adaptive potential (Allendorf et al. 2010; Sgrò et al. 2011; Funk et al. 2012; Flanagan et al. 2017; von der Heyden 2017), with important consequences for the survival of species and populations in the face of climate change. Identifying and monitoring locally adaptive genetic variation can provide insight into the prevalence of population divergence as a consequence of selection by environmental factors and can uncover fine-scale spatial genetic structure when population differentiation as measured using neutral loci is weak (Bay and Palumbi 2014; Babin et al. 2017; Gagnaire et al. 2012). Funk et al. (2012) outlined a useful approach for delineating conservation units, including evolutionary significant units (ESUs) using both neutral and adaptive genomic markers to maximize evolutionary potential, as well as management units (MUs) that maintain adaptive genetic differentiation within a species. Identification of loci underlying adaptation to environmental conditions can also improve translocation efforts and assisted gene flow (AGF) to introduce beneficial genetic variation into recipient populations that are experiencing (or will likely experience) climates similar to historical conditions in source populations (Aitken and Whitlock 2013). AGF has been proposed as a potential strategy for managing coral reefs by introducing genotypes from populations that have survived devastating bleaching events into populations that will likely experience similar extreme climatic disturbances in the future (Riegl et al. 2011).

From an MPA network planning perspective, characterizing the spatial pattern of putatively adaptive genetic variation has important implications for selecting sites or populations for protection to maintain adaptive potential under environmental changes. Natural populations that are already locally adapted to stressful or extreme environmental conditions might be important sources of 'preadapted' alleles that can enhance the resistance of other populations to future environmental change. Gene flow from these populations containing pre-existing adaptive genetic variation can increase the fitness of other populations by introducing novel (beneficial) alleles into

the gene pool (i.e. genetic rescue) (Whiteley et al. 2015). The related concept of evolutionary rescue (Carlson et al. 2014) occurs when environmental change initially reduces population growth, but the population avoids extinction by adapting to the new conditions (typically via selection on standing genetic variation since selection on new mutations is not likely to be fast enough relative to the pace of environmental change). As environmental stressors intensify with climate change, populations harboring pre-existing adaptive genetic variation may merit conservation prioritization and integration within an MPA network to ensure the preservation of genetic variation that can enhance the evolutionary resilience of other populations in the future (von der Heyden 2017). For example, Bay and Palumbi (2014) identified a population of the coral *Acropora hyacinthus* containing alleles associated with heat resistance that are maintained by spatially varying selection. In another study, Golbuu et al. (2016) demonstrated that some coral populations thrive at considerably lower pH compared to other populations and have survived bleaching events that decimated nearby reefs (Golbuu et al. 2016). Contemporary conditions recorded in these low pH areas are already at levels that other reefs are expected to face in the future under climate change projections. These examples highlight the potential for populations that are locally adapted to extreme conditions to serve as reservoirs of preadapted alleles that could benefit other populations via genetic and/or evolutionary rescue. Designs of MPA networks that do not incorporate assessments of adaptive genetic variation may risk losing 'genetic insurance' for adaptation in the longer term.

5 An Integrative Approach: Connectivity and Adaptation in MPA Networks

Spatial networks are often conceptualized as a series of *nodes*, which refer to habitat patches or sites, and *links*, which refer to the connections between patches (Dale and Fortin 2014). Similarly, MPA networks can be represented by nodes and links, where individual MPAs comprise the nodes and dispersal and gene flow between MPAs are the links. Together, neutral and adaptive genetic differentiation provide information about the processes occurring at both of these levels (Wagner and Fortin 2013), where assessments of neutral genetic variation can be used to evaluate *between-site* processes that facilitate or impede gene flow and genetic connectivity between MPAs (i.e. links), while assessment of adaptive genetic variation can provide insights into the *at-site* characteristics of the environment or climate that might be important drivers of natural selection and local adaptation (i.e. nodes) (Box 3). Understanding both at-site and between-site processes has important implications for selecting protected sites within MPA networks since opposing processes of natural selection and connectivity can affect the probability of rapid adaptation. On one hand, a design that supports dispersal and gene flow can lead to increased population sizes and levels of genetic variation within MPAs due to an increase in larval export from other protected sites (Fig. 4a). However, while

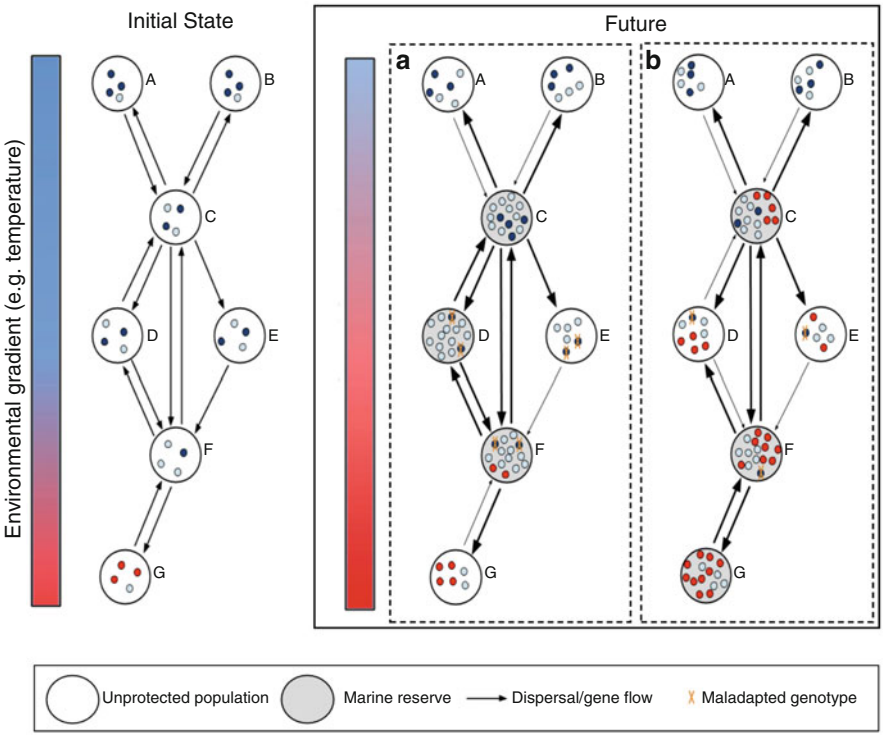

Fig. 4 A schematic representation of marine populations along an environmental gradient (e.g. temperature, increasing downwards towards the red color on the bar) and site prioritization for designating MPAs (grey-shaded circles) under a scenario of environmental change (e.g. warming ocean temperature) based on (**a**) selecting the most well-connected populations and (**b**) considering both connectivity and adaptive genetic variation. Small colored circles represent individual genotypes: dark blue genotypes are favored in low temperatures; light blue genotypes are favored in intermediate temperatures; and red genotypes are favored in the highest temperature. Arrows indicate the direction of dispersal/gene flow between populations. Gene flow is higher out of MPAs (thicker arrows) due to increased larval export as a result of increased population sizes within MPAs and lower out of unprotected sites (thinner arrows) due to declining population sizes as a result of fishing. High connectivity between MPAs homogenizes genetic variation across the whole network, potentially swamping the locally adapted red genotype in panel (**a**). Protection of population 'G' in panel (**b**) allows the beneficial red genotype to increase in frequency and spread to populations 'C', 'D', 'E', and 'F', which might be vulnerable to warming temperatures in the future

environmental heterogeneity can maintain genetic polymorphisms in the presence of gene flow, dispersal between adaptively divergent populations might in some cases lead to migration load, whereby population fitness is lowered due to an influx of maladapted genotypes, or to selection against maladapted migrants, thus reducing recruitment rates and population growth (Carlson et al. 2014). Furthermore, where local adaptation occurs in isolated populations in the absence of gene flow, MPA network designs that prioritize well-connected sites might inadvertently exclude populations containing standing genetic variation that can aid rapid adaptation of other populations in the future (Fig. 4a).

Box 3 Summary of Link-Level and Node-Level Analyses for the Design of MPA Networks

A spatial network framework borrows terminology and concepts from graph theory. A *graph* (i.e. network) is defined by a set of *nodes* that occur at some location in space and a set of *edges* or *links*, which reflect processes that connect nodes (e.g. gene flow). Graph theory has been adopted in landscape genetics to evaluate connectivity between populations and individuals across landscapes (see Box 2 in Manel and Holderegger 2013). Genomic data sets that can be divided into groups of neutral and adaptive loci allow the investigation of processes acting on genetic variation at both the link and node level (Fig. 5).

Link (edge) level: Link-based analyses address questions about *between-site* processes, such as the movement of individuals or genes between patches or populations (Wagner and Fortin 2013). These analyses rely on neutral genetic markers, such that connectivity can be estimated independently of selective forces (Holderegger et al. 2006), and may be performed at the individual or population level. Individual-based methods include parentage analyses and assignment tests, which detect recent dispersal events based on

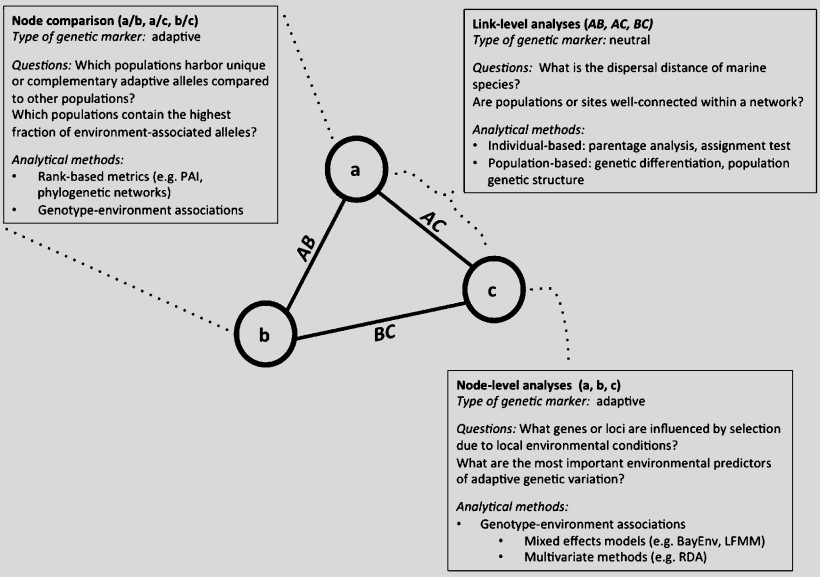

Fig. 5 Summary of questions and analysis types for assessing multi-level processes within a spatial network with genomic data. Circles (a, b, c) represent nodes (i.e. sites or populations); lines connecting nodes (AB, AC, BC) represent links, defined by dispersal/gene flow between sites

(continued)

Box 3 (continued)
assigning individuals to their natal site. Population-based methods that assess genetic differentiation and patterns of population genetic structure can be used to infer the scale and extent of gene flow between subpopulations. When combined with oceanographic information (e.g. biophysical models), population-based approaches provide insight into the potential factors driving contemporary connectivity. Collectively, analyses performed at the link level may be used to optimize the spatial arrangement of MPAs to maintain network connectivity and sustainability.

Node level: Analyses at the node level can incorporate adaptive genetic markers to investigate the influence of local, or *at-site*, conditions on the abundance and distribution of genetic variation (Wagner and Fortin 2013). Genotype-environment association (GEA) approaches are useful at this level of analysis because they not only detect the presence of putatively adaptive loci, but they also identify important environmental factors that might drive local adaptation. Many different GEA methods have been developed, including mixed-effects models that control for neutral population structure and multivariate approaches that consider the polygenic nature of adaptation (see review by Rellstab et al. 2015). In the context of MPA networks, analyses at the node level can be used to prioritize populations that harbor high levels of adaptive genetic variation, especially those that contain genetic variants adapted to environmental variables associated with climate change.

Several indices have been derived to assist the prioritization of populations for conservation planning by comparing node-level characteristics. For example, the population adaptive index (PAI; Bonin et al. 2007) identifies the proportion of adaptive loci in a population with significantly different allele frequencies compared to other populations. Phylogenetic network approaches have been proposed to rank populations based on the genetic isolation and expected evolutionary contribution to future networks (Volkmann et al. 2014). Another recent study developed an approach to measure the current potential of a population to adapt in response to changing environmental conditions based on the fraction of adaptive alleles associated with environmental variables (Manel et al. 2018). The most appropriate type of index used to prioritize sites will depend on the conservation goal and whether adaptive genetic differentiation can be attributed to key environmental drivers of natural selection.

By taking into account patterns of both neutral and adaptive genetic variation, conservation and management decisions can aim to achieve an optimal balance between maintaining connectivity and protecting adaptive potential. While inferences about local adaptation per se are best made using well-designed experimental studies (e.g. common garden or reciprocal transplants; Kawecki and Ebert 2004), the methods outlined in Box 3 may be used as a starting point to identify the presence of adaptive differentiation and potential sources of beneficial alleles for adaptation to changing conditions, especially when experimental tests of local adaptation are

logistically infeasible. Genotype-environment association methods can be applied to identify populations showing strong associations between potentially adaptive loci and environmental variables. Populations containing alleles associated with climatic or environmental variables might warrant protection so as to maintain adaptive genetic variation within the network while limiting the potential for genetic homogenization across MPAs (Fig. 4b). To prioritize sites for protection, indices that rank populations based on the level and distinctiveness of adaptive genetic diversity (e.g. Bonin et al. 2007; Volkmann et al. 2014) and associations with important climatic and environmental variables (e.g. Manel et al. 2018) can be applied to select sites that maximize the range of adaptive alleles and minimize genetic redundancy across MPAs (see Box 3). Estimates of connectivity can then be used to determine whether natural gene flow is sufficient to introduce migrants from potentially locally adapted populations into populations that are (or are projected to be) experiencing similar environmental stressors or whether AGF might be considered a viable option. When environmental heterogeneity is low and adaptive genetic differentiation is weak due to similar selection pressures across space, prioritizing well-connected MPAs might instead be favored in order to replenish protected populations and adjacent unprotected sites.

6 Concluding Remarks

The increasing availability of genomic data sets for non-model species, and especially for species of conservation concern, has the potential to revolutionize conservation planning and decision-making. For marine species in particular, genomic data sets have considerably improved the resolution for detecting fine-scale patterns of population genetic structure, challenging the notion that marine populations are characterized by widespread gene flow. Moreover, the ability to identify putatively adaptive genetic variation has provided mounting evidence in support of the presence of local adaptation in marine populations (e.g. De Wit and Palumbi 2013; Bay and Palumbi 2014; Benestan et al. 2016b; Van Wyngaarden et al. 2017; Stanley et al. 2018; Dalongeville et al. 2018b). Despite the plethora of studies highlighting the usefulness of genetic and genomic data to inform marine conservation and management objectives (Beger et al. 2014; von der Heyden 2017; Nielsen et al. 2017; Jenkins and Stevens 2018), they are rarely integrated into marine conservation plans in practice. This is likely due to a number of reasons, including the perception that genomic studies are too expensive, ineffective communication between researchers and policymakers (Bernatchez et al. 2017; Shafer et al. 2015), and the relative paucity of multispecies data sets (but see Deck et al. 2017). Though costs of obtaining samples and generating genomic data can be prohibitive for local conservation efforts, many scientists are generating these data for diverse marine species all over the world. However, the results of these studies may not be effectively disseminated to practitioners or translated into practical objectives. As a result, strong collaborations and communication between those conducting the primary

research and those making conservation decisions are key (Shafer et al. 2015; Ovenden et al. 2015; Garner et al. 2016). In addition, online platforms for sharing genomic and geographic data hold promise for the widespread accessibility and integration of genomic data for multiple species into MPA planning. Ongoing efforts to share genomic data in a user-friendly format include the new Genomic Observatories Metadatabase (GeOMe; Deck et al. 2017), which already contains more than 8,000 entries of sequence data for approximately 80 marine species across 4 phyla (Chordata, Echinodermata, Mollusca, and Arthropoda) collected from 29 countries (currently focused in the Indo-Pacific region) along with associated metadata (e.g. geographic coordinates, date of collection, tissue type). Additionally, geospatial genetic data available online (e.g. Geospatial Genetics SeaSketch projects; https://www.seasketch.org/) provide maps and GIS data layers to facilitate visualization and spatial prioritization. Data from two pilot SeaSketch projects are currently available for the identification of important marine mammal areas, specifically for spinner dolphins (Andrews et al. 2010) and humpback whales (Kershaw et al. 2017). As studies have shown the importance of integrating information across multiple species for effective MPA designs (e.g. Nielsen et al. 2017), these databases will likely be a crucial resource for informing multispecies genetic conservation objectives.

As climate change continues to threaten marine population persistence, designing MPA networks that will maintain evolutionary and adaptive potential in the future is critical. There is therefore a need to expand upon existing frameworks (e.g. Beger et al. 2014) by incorporating assessments of both neutral and adaptive genetic variation into conservation planning (Funk et al. 2012; Flanagan et al. 2017). Under a spatial network approach, evaluating the distribution of neutral and adaptive genetic information across seascapes provides insight into (1) the degree of gene flow among populations, allowing optimization of connectivity between MPAs, and (2) identifying and protecting local sites that contain adaptive variation to preserve evolutionary resilience to environmental disturbances. Thus, the inclusion of inferences from population genomics has the potential to produce robust designs of MPA networks that consider both connectivity and adaptive evolution for the persistence of marine biodiversity, both today and in the future.

References

Aitken SN, Whitlock MC. Assisted gene flow to facilitate local adaptation to climate change. Annu Rev Ecol Evol Syst. 2013;44:367–88.

Allendorf FW, Hohenlohe PA, Luikart G. Genomics and the future of conservation genetics. Nat Rev Genet. 2010;11:697–709.

Almany GR, Berumen ML, Thorrold SR, Planes S, Jones GP. Local replenishment of coral reef fish populations in a marine reserve. Science. 2007;316:742–4.

Almany GR, Planes S, Thorrold SR, Berumen ML, Bode M, Saenz-Agudelo P, Bonin MC, Frisch AJ, Harrison HB, Messmer V, Nanninga GB, Priest MA, Srinivasan M, Sinclair-Taylor T, Williamson DH, Jones GP. Larval fish dispersal in a coral-reef seascape. Nat Ecol Evol. 2017;1(6):148.

Andrello M, Guilhaumon F, Albouy C, Parravicini V, Scholtens J, Verley P, Barange M, Sumaila UR, Manel S, Mouillot D. Global mismatch between fishing dependency and larval supply from marine reserves. Nat Commun. 2017;8:16039.

Andrews KR, Karczmarski L, Au WWL, Rickards SH, Vanderlip CA, Bowen BW, Grau EG, Toonen RJ. Rolling stones and stable homes: social structure, habitat diversity and population genetics of the Hawaiian spinner dolphin (*Stenella longirostris*). Mol Ecol. 2010;19:732–48.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. Harnessing the power of RADseq for ecological and evolutionary genomics. Nat Rev Genet. 2016;17:81–92.

Babin C, Gagnaire P-A, Pavey SA, Bernatchez L. RAD-seq reveals patterns of additive polygenic variation caused by spatially-varying selection in the American eel (*Anguilla rostrata*). Genome Biol Evol. 2017;9:2974–86.

Barbosa S, Mestre F, White TA, Paupério J, Alves PC, Searle JB. Integrative approaches to guide conservation decisions: using genomics to define conservation units and functional corridors. Mol Ecol. 2018;27:3452–65.

Bay RA, Palumbi SR. Multilocus adaptation associated with heat resistance in reef-building corals. Curr Biol. 2014;24:2952–6.

Bay RA, Rose N, Barrett R, Bernatchez L, Ghalambor CK, Lasky JR, Brem RB, Palumbi SR, Ralph P. Predicting evolutionary responses to contemporary environmental change using population genomic data. Am Nat. 2017;189:463–73.

Beger M, Selkoe KA, Treml E, Barber PH, Crandall ED, Toonen RJ, Riginos C. Evolving coral reef conservation with genetic information. Bull Mar Sci. 2014;90:159–85.

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). Mol Ecol. 2015;24:3299–315.

Benestan LM, Ferchaud A-L, Hohenlohe PA, Garner BA, Naylor GJP, Baums IB, Schwartz MK, Kelley JL, Luikart G. Conservation genomics of natural and managed populations: building a conceptual and practical framework. Mol Ecol. 2016a;25:2967–77.

Benestan L, Quinn B, Laporte M, Maaroufi H, Rochette R, Bernatchez L. Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (*Homarus americanus*). Mol Ecol. 2016b;25:5073–92.

Bernatchez L. On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. J Fish Biol. 2016;89:2519–56.

Bernatchez L, Wellenreuther M, Araneda C, Ashton DT, Barth JMI, Beacham TD, Maes GE, Martinsohn JT, Miller KM, Naish KA, Ovenden JR, Primmer CR, Suk HY, Therkildsen NO, Withler RE. Harnessing the power of genomics to secure the future of seafood. Trends Ecol Evol. 2017;32:665–80.

Bonin A, Nicole F, Pompanon F, Miaud C, Taberlet P. Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conserv Biol. 2007;21:697–708.

Calosi P, De Wit P, Thor P, Dupont S. Will life find a way? Evolution of marine species under global change. Evol Appl. 2016;9:1035–42.

Carlson SM, Cunningham CJ, Westley PAH. Evolutionary rescue in a changing world. Trends Ecol Evol. 2014;29:521–30.

Christie MR, Meirmans PG, Gaggiotti OE, Toonen RJ, White C. Disentangling the relative merits and disadvantages of parentage analysis and assignment tests for inferring population connectivity. ICES J Mar Sci. 2017;74:1749–62.

Çilingir FG, Rheindt FE, Garg KM, Platt K, Platt SG, Bickford DP. Conservation genomics of the endangered Burmese roofed turtle. Conserv Biol. 2017;31:1469–76.

Costello MJ, Ballantine B. Biodiversity conservation should focus on no-take marine reserves: 94% of marine protected areas allow fishing. Trends Ecol Evol. 2015;30:507–9.

Côté CL, Gagnaire P-A, Bourret V, Verreault G, Castonguay M, Bernatchez L. Population genetics of the American eel (Anguilla rostrata): FST = 0 and North Atlantic Oscillation effects on demographic fluctuations in panmictic species. Mol Ecol. 2013;22:1763–76.

Cowen RK, Sponagule S. Larval dispersal and marine population connectivity. Annu Rev Mar Sci. 2009;1:443–66.

Cowen RK, Paris CB, Srinivasan A. Scaling of connectivity in marine populations. Science. 2006;311:522–7.

Cowen RK, Gawarkiewicz G, Pineda J, Thorrold SR, Werner FE. Population connectivity in marine systems: an overview. Oceanography. 2007;20:14–21.

Cros A, Toonen RJ, Donahue MJ, Karl SA. Connecting Palau's marine protected areas: a population genetic approach to conservation. Coral Reefs. 2017;36:735–48.

D'Aloia CC, Bogdanowicz SM, Francis RK, Majoris JE, Harrison RG, Buston PM. Patterns, causes, and consequences of marine larval dispersal. Proc Natl Acad Sci U S A. 2015;112:13940–5.

Daigle RM, Metaxas A, Balbar A, McGowan J, Treml EA, Kuempel CD, Possingham HP, Beger M. Operationalizing ecological connectivity in spatial conservation planning with Marxan Connect. bioRxiv. 2018;315424.

Dale MRT, Fortin M-J. Spatial analysis: a guide for ecologists. 2nd ed. Cambridge: Cambridge University Press; 2014.

Dalongeville A, Andrello M, Mouillot D, Lobreaux S, Forti M-J, Lasram F, Belmaker J, Rocklin D, Manel S. Geographic isolation and larval dispersal shape seascape genetic patterns differently according to spatial scale. Evol Appl. 2018a;11:1437–47.

Dalongeville A, Benestan L, Mouillot D, Lobreaux S, Manel S. Combining six genome scan methods to detect candidate genes to salinity in the Mediterranean striped red mullet (*Mullus surmuletus*). BMC Genomics. 2018b;19:217.

Day J, Dudley N, Hockings M, Holmes G, Laffoley D, Stolton S, Wells S. Guidelines for applying the IUCN protected area management categories to marine protected areas. Gland: IUCN; 2012. p. 36.

De Wit P, Palumbi SR. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Mol Ecol. 2013;22:2884–97.

Deck J, Gaither MR, Ewing R, Bird CE, Davies N, Meyer C, Riginos C, Toonen RJ, Crandall ED. The Genomic Observatories Metadatabase (GeOMe): a new repository for field and sampling event metadata associated with genetic samples. PLoS Biol. 2017;15:e2002925.

Dyer RJ, Nason JD. Population graphs: the graph theoretic shape of genetic structure. Mol Ecol. 2004;13:1713–27.

Dyer RJ, Nason JD, Garrick RC. Landscape modelling of gene flow: improved power using conditional genetic distance derived from the topology of population networks. Mol Ecol. 2010;19:3746–59.

Edgar GJ, Stuart-Smith RD, Willis TJ, Kininmonth S, Baker SC, Banks S, Barrett NS, Becerro MA, Bernard ATF, Berkhout J, Buxton CD, Campbell SJ, Cooper AT, Davey M, Edgar SC, Försterra G, Galván DE, Irigoyen AJ, Kushner DJ, Moura R, Parnell PE, Shears NT, Soler G, Strain EMA, Thomson RJ. Global conservation outcomes depend on marine protected areas with five key features. Nature. 2014;506:216–20.

Flanagan SP, Forester BR, Latch EK, Aitken SN, Hoban S. Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. Evol Appl. 2017;11:1035–52.

Frankham R. Genetic adaptation to captivity in species conservation. Mol Ecol. 2008;17:325–33.

Frankham R. Where are we in conservation genetics and where do we need to go? Conserv Genet. 2010;11:661–3.

Frankham R. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol Ecol. 2015;24:2610–8.

Frankham R, Bradshaw CJA, Brook BW. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol Conserv. 2014;170:56–63.

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW. Harnessing genomics for delineating conservation units. Trends Ecol Evol. 2012;27:489–96.

Gagnaire P-A, Normandeau E, Côté C, Hansen MM, Bernatchez L. The genetic consequences of spatially varying selection in the panmictic American eel (*Anguilla rostrata*). Genetics. 2012;190:725–36.

Gaines SD, White C, Carr MH, Palumbi SR. Designing marine reserve networks for both conservation and fisheries management. Proc Natl Acad Sci U S A. 2010;107:18286–93.

Garner BA, Hand BK, Amish SJ, Bernatchez L, Foster JT, Miller KM, Morin PA, Narum SR, O'Brien SJ, Roffler G, Templin WD, Sunnucks P, Strait J, Warheit KI, Seamons TR, Wenburg J, Olsen J, Luikart G. Genomics in conservation: case studies and bridging the gap between data and application. Trends Ecol Evol. 2016;31:81–3.

Gerlach G, Atema J, Kingsford MJ, Black KP, Millers-Sims V. Smelling home can prevent dispersal of reef fish larvae. Proc Natl Acad Sci. 2007;104:858–63.

Gill DA, Mascia MB, Ahmadia GN, Glew L, Lester SE, Barnes M, Craigie I, Darling E, Free CM, Geldmann J, Holst S, Jensen OP, White AT, Basurto X, Coad L, Gates RD, Guannel G, Mumby PJ, Thomas H, Whitmee S, Woodley S, Fox HE. Capacity shortfalls hinder the performance of marine protected areas globally. Nature. 2017;543:665–9.

Golbuu Y, Gouezo M, Kurihara H, Rehm L, Wolanski E. Long-term isolation and local adaptation in Palau's Nikko Bay help corals thrive in acidic waters. Coral Reefs. 2016;35:909–18.

Green AL, Maypa AP, Almany GR, Rhodes KL, Weeks R, Abesamis RA, Gleason MG, Mumby PJ, White AT. Larval dispersal and movement patterns of coral reef fishes, and implications for marine reserve network design. Biol Rev. 2015;90:1215–47.

Gunderson AR, Stillman JH. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc Biol Sci. 2015;282:20150401.

Halpern BS, Frazier M, Potapenko J, Casey KS, Koenig K, Longo C, Lowndes JS, Rockwood RC, Selig ER, Selkoe KA, Walbridge S. Spatial and temporal changes in cumulative human impacts on the world's ocean. Nat Commun. 2015;6:1–7.

Harrison HB, Williamson DH, Evans RD, Almany GR, Thorrold SR, Russ GR, Feldheim KA, van Herwerden L, Planes S, Srinivasan M, Berumen ML, Jones GP. Larval export from marine reserves and the recruitment benefit for fish and fisheries. Curr Biol. 2012;22:1023–8.

Hedgecock D, Barber PH, Edmands S. Genetic approaches to measuring connectivity. Oceanography. 2007;20:70–9.

Holderegger R, Kamm U, Gugerli F. Adaptive vs. neutral genetic diversity: implications for landscape genetics. Landsc Ecol. 2006;21:797–807.

Holland LP, Jenkins TL, Stevens JR. Contrasting patterns of population structure and gene flow facilitate exploration of connectivity in two widely distributed temperate octocorals. Heredity. 2017;119:35–48.

Iacchei M, Gaither MR, Bowen BW, Toonen RJ. Testing dispersal limits in the sea: range-wide phylogeography of the pronghorn spiny lobster *Panulirus penicillatus*. J Biogeogr. 2016;43:1032–44.

Jahnke M, Jonsson PR, Moksnes P-O, Loo L-O, Jacobi MN, Olsen JL. Seascape genetics and biophysical connectivity modelling support conservation of the seagrass *Zostera marina* in the Skagerrak-Kattegat region of the eastern North Sea. Evol Appl. 2018;11:645–61.

James MK, Armsworth PR, Mason LB, Bode L. The structure of reef fish metapopulations: modeling larval dispersal and retention patterns. Proc Biol Sci. 2002;269:2079–86.

Jenkins TL, Stevens JR. Assessing connectivity between MPAs: selecting taxa and translating genetic data to inform policy. Mar Policy. 2018;94:164–73.

Jorde PE, Søvik G, Westgaard J-I, Albretsen J, André C, Hvingel C, Johansen T, Sandvik AD, Kingsley M, Jørstad KE. Genetically distinct populations of northern shrimp, *Pandalus borealis*, in the North Atlantic: adaptation to different temperatures as an isolation factor. Mol Ecol. 2015;24:1742–57.

Kawecki TJ, Ebert D. Conceptual issues in local adaptation. Ecol Lett. 2004;7:1225–41.

Kershaw F, Carvalho I, Loo J, Pomilla C, Best PR, Findlay KP, Cerchio S, Collins T, Engel MH, Minton G, Ersts P, Barendse J, Kotze PGH, Razafindrakoto Y, Ngouessono S, Meÿer M, Thornton M, Rosenbaum HC. Multiple processes drive genetic structure of humpback whale (*Megaptera novaeangliae*) populations across spatial scales. Mol Ecol. 2017;26:977–94.

Kimura M, Ohta T. The average number of generations until fixation of a mutant gene in a finite population. Science. 1969;61:763–71.

Kininmonth S, Beger M, Bode M, Peterson E, Adams VM, Dorfman D, Brumbaugh DR, Possingham HP. Dispersal connectivity and reserve selection for marine conservation. Ecol Model. 2011;222:1272–82.

Koelewijn HP, Pérez-Haro M, Jansman HAH, Boerwinkel MC, Bovenschen J, Lammertsma DR, Niewold FJJ, Kuiters AT. The reintroduction of the Eurasian otter (*Lutra lutra*) into the Netherlands: hidden life revealed by noninvasive genetic monitoring. Conserv Genet. 2010;11:601–14.

Krueck NC, Ahmadia GN, Possingham HP, Riginos C, Treml EA, Mumby PJ. Marine reserve targets to sustain and rebuild unregulated fisheries. PLoS Biol. 2017;15:1–20.

Lagabrielle E, Crochelet E, Andrello M, Schill SR, Arnaud-Haond S, Alloncle N, Ponge B. Connecting MPAs – eight challenges for science and management. Aquat Conserv. 2008;24:94–110.

Lal MM, Southgate PC, Jerry DR, Bosserelle C, Zenger KR. Swept away: ocean currents and seascape features influence genetic structure across the 18,000 km Indo-Pacific distribution of a marine invertebrate, the black-lip pearl oyster *Pinctada margaritifera*. BMC Genomics. 2017;18:66.

Larson WA, Seeb LW, Everett MV, Waples RK, Templin WD, Seeb JE. Genotyping by sequencing resolves shallow population structure to inform conservation of Chinook salmon (*Oncorhynchus tshawytscha*). Evol Appl. 2014;7:355–69.

Levin SA, Lubchenco J. Resilience, robustness, and marine ecosystem-based management. AIBS Bull. 2008;58:27–32.

Liggins L, Treml EA, Possingham HP, Riginos C. Seascape features, rather than dispersal traits, predict spatial genetic patterns of co-distributed reef fishes. J Biogeogr. 2016;43:256–67.

Lowe WH, Allendorf FW. What can genetics tell us about population connectivity? Mol Ecol. 2010;19:3038–51.

Lubchenco J, Grorud-Colvert K. Making waves: the sciences and politics of ocean protection. Science. 2015;350:382–3.

Magris RA, Andrello M, Pressey RL, Mouillot D, Dalongeville A, Jacobi MN, Manel S. Biologically representative and well-connected marine reserves enhance biodiversity persistence in conservation planning. Conserv Lett. 2018;11:e12439.

Manel S, Holderegger R. Ten years of landscape genetics. Trends Ecol Evol. 2013;28:614–21.

Manel S, Schwartz MK, Luikart G, Taberlet P. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol. 2003;18:189–97.

Manel S, Gaggiotti OE, Waples RS. Assignment methods: matching biological questions with appropriate techniques. Trends Ecol Evol. 2005;20:136–42.

Manel S, Joost S, Epperson BK, Holderegger R, Storfer A, Rosenberg MS, Scribner KT, Bonin A, Fortin M-J. Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. Mol Ecol. 2010;19:3760–72.

Manel S, Perrier C, Pratlong M, Abi-Rached L, Paganini J, Pontarotti P, Aurelle D. Genomic resources and their influence on the detection of the signal of positive selection in genome scans. Mol Ecol. 2016;25:170–84.

Manel S, Andrello M, Henry K, Verdelet D, Darracq A, Guerin P-E, Desprez B, Devaux P. Predicting genotype environmental range from genome-environment associations. Mol Ecol. 2018;27:2823–33.

Mellin C, MacNeil MA, Cheal AJ, Emslie MJ, Caley JM. Marine protected areas increase resilience among coral reef communities. Ecol Lett. 2016;19:629–37.

Micheli F, Saenz-Arroyo A, Greenley A, Vazquez L, Espinoza Montes JA, Rossetto M, De Leo GA. Evidence that marine reserves enhance resilience to climatic impacts. PLoS One. 2012;7:e40832.

Nanninga GB, Saenz-Agudelo P, Zhan P, Hoteit I, Berumen ML. Not finding Nemo: limited reef-scale retention in a coral reef fish. Coral Reefs. 2015;34:383–92.

Nielsen ES, Beger M, Henriques R, Selkoe KA, von der Heyden S. Multispecies genetic objectives in spatial conservation planning. Conserv Biol. 2017;31:872–82.

Ovenden JR, Berry O, Welch DJ, Buckworth RC, Dichmont CM. Ocean's eleven: a critical evaluation of the role of population, evolutionary, and molecular genetics in the management of wild fisheries. Fish Fish. 2015;16:125–59.

Palsbøll PJ, Bérubé M, Allendorf FW. Identification of management units using population genetic data. Trends Ecol Evol. 2007;22:11–6.

Palumbi SR. Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl. 2003;13:S146–58.
Patkeau D, Slade R, Burden M, Estoup A. Genetic assignment methods for the direct real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol. 2004;13:55–65.
Patterson HM, Swearer SE. Long-distance dispersal and local retention of larvae as mechanisms of recruitment in an island population of a coral reef fish. Austral Ecol. 2007;32:122–30.
Pespeni MH, Sanford E, Gaylord B, Hill TM, Hosfelt JD, Jaris HK, LaVigne M, Lenz EA, Russell AD, Young MK, Palumbi SR. Evolutionary change during experimental ocean acidification. Proc Natl Acad Sci U S A. 2013;110:6937–42.
Pinsky ML, Montes HR Jr, Palumbi SR. Using isolation by distance and effective density to estimate dispersal scales in anemonefish. Evolution. 2010;64:2688–700.
Pinsky ML, Saenz-Agudelo P, Salles OC, Almany GR, Bode M, Berumen ML, Andréfouët S, Thorrold SR, Jones GP, Planes S. Marine dispersal scales are congruent over evolutionary and ecological time. Curr Biol. 2017;27:149–54.
Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945–59.
Puebla O, Bermingham E, McMillan WO. On the spatial scale of dispersal in coral reef fishes. Mol Ecol. 2012;21:5675–88.
Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. A practical guide to environmental association analysis in landscape genomics. Mol Ecol. 2015;24:4348–70.
Riegl BM, Purkis SJ, Al-Cibahy AS, Abdel-Moati MA, Hoegh-Guldberg O. Present limits to heat-adaptability in corals and population-level responses to climate extremes. PLoS One. 2011;6:e24802.
Riginos C, Crandall ED, Liggins L, Bongaerts P, Treml EA. Navigating the currents of seascape genomics: how spatial analyses can augment population genomic studies. Curr Zool. 2016;62:581–601.
Rousset F. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics. 1997;145:1219–28.
Rozenfeld AF, Arnaud-Haond S, Hernández-García E, Eguíluz VM, Serrão EA, Duarte CM. Network analysis identifies weak and strong links in a metapopulation system. Proc Natl Acad Sci U S A. 2008;105:18824–9.
Ryu T, Veilleux HD, Donelson JM, Munday PL, Ravasi T. The epigenetic landscape of transgenerational acclimation to ocean warming. Nat Clim Chang. 2018;8:504–9.
Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S. Estimating connectivity in marine populations: an empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. Mol Ecol. 2009;18:1675–776.
Sala E, Giakoumi S. No-take marine reserves are the most effective protected areas in the ocean. ICES J Mar Sci. 2018;75:1166–8.
Sala E, Lubchenco J, Grorud-Colvert K, Novelli C, Roberts C, Sumaila UR. Assessing real progress towards effective ocean protection. Mar Policy. 2018;91:11–3.
Sanford E, Kelly MW. Local adaptation in marine invertebrates. Annu Rev Mar Sci. 2011;3:509–35.
Savolainen O, Lascoux M, Merilä J. Ecological genomics of local adaptation. Nat Rev Genet. 2013;14:807–20.
Schiavina M, Marino IAM, Zane L, Melià P. Matching oceanography and genetics at the basin scale. Seascape connectivity of the Mediterranean shore crab in the Adriatic Sea. Mol Ecol. 2014;23:5496–507.
Schunter C, Carreras-Carbonell J, Macpherson E, Tintoré J, Vidal-Vijande E, Pascual A, Guidetti P, Pascual M. Matching genetics with oceanography: directional gene flow in a Mediterranean fish species. Mol Ecol. 2011;20:5167–81.
Selkoe KA, D'Aloia CC, Crandall ED, Iacchei M, Liggins L, Puritz JB, von der Heyden S, Toonen RJ. A decade of seascape genetics: contributions to basic and applied marine connectivity. Mar Ecol Prog Ser. 2016;554:1–19.
Sgrò CM, Lowe AJ, Hoffmann AA. Building evolutionary resilience for conserving biodiversity under climate change. Evol Appl. 2011;4:326–37.

Shafer ABA, Wolf JBW, Alves PC, Bergström L, Bruford MW, Brännström I, Colling G, Dalén L, De Meester L, Ekblom R, Fawcett KD, Fior S, Hajibabaei M, Hill JA, Hoezel AR, Höglund J, Jensen EL, Krause J, Kristensen TN, Krützen M, McKay JK, Norman AJ, Ogden R, Österling EM, Ouborg NJ, Piccolo J, Popović D, Primmer CR, Reed FA, Roumet M, Salmona J, Schenekar T, Schwartz MK, Segelbacher G, Senn H, Thaulow J, Valtonen M, Veale A, Vergeer P, Vijay N, Vilà C, Weissensteiner M, Wennerström L, Wheat CW, Zieliński P. Genomics and the challenging translation into conservation practice. Trends Ecol Evol. 2015;30:78–87.

Siegle MR, Taylor EB, Miller KM, Withler RE, Yamanaka KL. Subtle population genetic structure in yelloweye rockfish (*Sebastes ruberrimus*) is consistent with a major oceanographic division in British Columbia, Canada. PLoS One. 2013;8:e71083.

Stanley RRE, DiBacco C, Lowen B, Beiko RG, Jeffery NW, Van Wyngaarden M, Bentzen P, Brickman D, Benestan L, Bernatchez L, Johnson C, Snelgrove PVR, Wang Z, Wringe BF, Bradbury IR. A climate-associated multispecies cline in the northwest Atlantic. Sci Adv. 2018;4:eaaq0929.

Stuart-Smith RD, Edgar GJ, Barrett NS, Kininmonth SJ, Bates AE. Thermal biases and vulnerability to warming in the world's marine fauna. Nature. 2015;528:88–92.

Sunday JM, Popović I, Palen WJ, Foreman MGG, Hart MW. Ocean circulation model predicts high genetic structure observed in a long-lived pelagic developer. Mol Ecol. 2014;23:5036–47.

Taylor PD, Fahrig L, Henein K, Merriam G. Connectivity is a vital element of landscape structure. Oikos. 1993;68:571–3.

Thomas L, Bell JJ. Testing the consistency of connectivity patterns for a widely dispersing marine species. Heredity. 2013;111:345–54.

Thomas L, Kennington WJ, Stat M, Wilkinson SP, Kool JT, Kendrick GA. Isolation by resistance across a complex coral reef seascape. Proc Biol Sci. 2015;82:20151217.

Thorrold SR, Zacherl DD, Levin LA. Population connectivity and larval dispersal: using geochemical signatures in calcified structures. Oceanography. 2007;20:80–9.

Tischendorf L, Fahrig L. How should we measure landscape connectivity? Landsc Ecol. 2000;15:633–41.

Truelove NK, Kough AS, Behringer DC, Paris CB, Box SJ, Preziosi RF, Butler MJ IV. Biophysical connectivity explains population genetic structure in a highly dispersive marine species. Coral Reefs. 2017;36:233–44.

Van Wyngaarden M, Snelgrove PVR, DiBacco C, Hamilton LC, Rodríguez-Ezpeleta N, Jeffrey NW, Stanley RRE, Bradbury IR. Identifying patterns of dispersal, connectivity and selection in the sea scallop, *Placopecten magellanicus*, using RADseq-derived SNPs. Evol Appl. 2017;10:102–17.

Veilleux HD, Ryu T, Donelson JM, van Herwerden L, Seridi L, Ghosheh Y, Berumen ML, Leggat W, Ravasi T, Munday PL. Molecular processes of transgenerational acclimation to a warming ocean. Nat Clim Chang. 2015;5:1074–8.

Volkmann L, Martyn I, Moulton V, Spillner A, Mooers AO. Prioritizing populations for conservation using phylogenetic networks. PLoS One. 2014;9:e88945.

von der Heyden S. Making evolutionary history count: biodiversity planning for coral reef fishes and the conservation of evolutionary processes. Coral Reefs. 2017;36:183–94.

Wagner HH, Fortin M-J. A conceptual framework for the spatial analysis of landscape genetic data. Conserv Genet. 2013;14:253–61.

Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA. Genetic rescue to the rescue. Trends Ecol Evol. 2015;30:42–9.

Whitlock MC, McCauley DE. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. Heredity. 1999;82:117–25.

Wright S. Isolation by distance. Genetics. 1943;28:114–38.

Xuereb A, Benestan L, Normandeau É, Daigle RM, Curtis JMR, Bernatchez L, Fortin M-J. Asymmetric oceanographic processes mediate connectivity and population genetic structure, as revealed by RADseq, in a highly dispersive marine invertebrate (*Parastichopus californicus*). Mol Ecol. 2018;27:2347–64.

Index

A
Acartia tonsa (Copepoda), 82
Acclimation, 18–19, 22, 345, 346, 433
Acropora hyacinthus, 312, 435
Adaptation, 178, 199
  barnacles, 20
  clinal (*see* Clinal adaptation)
  DNA sequence variation, 20
  genomic basis, 77, 88–89
  gradients in sea, 306–310
  hypoxic zones, 20
  local, 433–434
  in marine environment, 22
  marine snail, 21
  MPA networks, 435–439
  parallel (*see* Parallel evolution)
  post-colonisation, 372–374
  pre-colonisation, 371–372
  SNPs, 20
  stickleback, 21
Adaptive radiation
  in stickleback, 250
Allozymes, 15, 83, 105, 109, 111, 117, 141, 278, 279, 283, 285, 288
Amphipods, 66, 69, 87
Amplicon-based sequencing, 12, 49
Ampli-seq, 76
Anthozoa, 106, 107, 117, 118
  asexual propagation, 122
  genetic patterns, 123
  genetic structure, 123–125
Asexual and sexual reproductive systems, 124
Assisted gene flow (AGF), 434, 439
Asymmetry, seascape genomics, 195–197
Atlantic herring, 15, 224, 232, 237, 408, 409
Aurelia aurita, 120, 121
Autocorrelation, 111, 180, 183, 184, 192–193, 195, 198, 225, 230, 231
Autonomous reef monitoring structures (ARMS), 45, 46

B
Bacteria, 8–11, 44–46, 48–50
Barcoding, 12–13, 18
Barnacles
  adaptive variation, 20
  cost-effective pool-seq approach, 141
  genetic variation
    DAPC approach, 153, 155
    mtDNA and nuDNA, 150–151
    neutral variation, 152
    PCA, 153–154
    quality filtering, 152, 153
  historical biogeography, 147
  phylogeography, 156
  population genomics challenges, 158–159
  technology bias *vs.* biological signal, 155–158
Basic Local Alignment Search Tool, 145
Biodiversity
  conservation and protection, 425
  eDNA, 12–14
  metagenomics, 9–11
  in ocean, 304–305
  zooplankton, 62–63
Biofilms, 45–46
Biogeography, 6, 11, 15, 63, 139–162

Marjorie F. Oleksiak and Om P. Rajora (eds.), *Population Genomics: Marine Organisms*, Population Genomics [Om P. Rajora (Editor-in-Chief)], https://doi.org/10.1007/978-3-030-37936-0, © Springer Nature Switzerland AG 2020

Bioindicators, 51, 335
community-level, 42
coral diseases, 42
direct reef monitoring methods, 43
measurements, 42
microbiomes (*see* Microbiomes)
microorganisms, 43–48
monitoring, 41
monotonicity, 41
practicality, 41
reef monitoring, 42, 43
relevant, 41
specificity, 41
variability, 41
water quality, 42
Bioinformatic tools, 160
Biological invasions
ecological and evolutionary consequences, 24
hybridisation, 369–371
post-colonisation adaptation, 372–374
pre-colonisation adaptation, 371, 372
propagule pressure, 365–369
Biomonitoring, 339
eDNA, 12
"Biophysical larval dispersal and fitness model," 186
Biophysical models, 179, 183–186, 195–197, 206, 231, 431, 438
Blooms, 105, 118, 122, 330, 332, 340

C

Calanus finmarchicus (Copepoda), 79–80
Calcarea, 108
Capture-seq, 8, 76
Catostylus mosaicus, 120
Centropages typicus (Copepoda), 76, 80–81
Chaetognaths, 67, 69
Chaotic genetic patchiness, 176–177, 190, 203–204
Chimerism, 113–114
Chip-seq, 8–9
Chromatin, 8, 326, 328, 331–333, 337, 373
Climate change, 5, 10, 17, 25, 39, 63, 87, 201, 206, 327, 330, 337, 345, 401, 410–411
genetic variation, 434–435
local adaptation in sea, 433–434
trans-generational effects, 433
Clinal adaptation
candidate loci, 234
clinal variation, 223
EAA, 224–225, 229–231
endogenous and background selection, 226–227
gene flow and polygenic traits, 236
genome scans, 234
local adaptation (*see* Local adaptation)
in marine environment, 22
neutral clines, 225–226
outlier analysis, 224–225, 228–229
QTL, 235
soft sweeps, 238
spatially varying selection, 223
species-wide scales, 223
transient, role, 237
Clines
candidate loci, 232, 233
endogenous and background selection, 226–227
IBD, 225–226
LDH allozymes, 226
neutral, 226
non-equilibrium processes, 226
outlier analysis, 229
See also Clinal adaptation
Cliona vermifera, 111, 112
Clonality, 113–114
Anthozoans, 123–125
Cnidaria, 16, 64, 66, 106–108, 114, 116
Anthozoa, 122–125
genetic markers, 117
Medusozoa, 118–122
Cnidarians, 106, 109, 117–125, 316, 317, 333, 334
Coastal zones, 4
Coding sequences (CDS), 8, 145, 146
Collinearity, 180, 185, 193, 231, 234
Connectivity, 104–106, 109, 115, 117–119, 121, 123, 124, 127
definition, 426
demographic, 427
functional, 427
genetic, 427
MPA networks, 435–439
population graphs, 431–432
structural, 426–427
Conservation, 24–28, 39, 53, 400, 401, 404, 409, 410
Conservation genomics, 105–107, 425–426
Constrained ordination, 230–231, 237
Contact zones, 280, 281, 288, 292–293, 295–297
Convergent evolution, 235, 257–258

Copepods, 63, 66, 67, 69, 74, 76, 79, 87, 88, 174
Coral-algae symbiosis, 311
Coral reefs
  bioindicators (*see* Bioindicators)
  conservation, 39, 53
  direct environmental measurements, 40
  ecological speciation (*see* Ecological speciation)
  management, 39
  microbiomes (*see* Microbiomes)
  monitoring programs, 40
  rainforest ecosystems, 39
  staggering rate, 52
  stressors and assessing, 39–40
Corals
  adaptation, 306–310
  biodiversity, ocean, 304, 305
  ecological speciation, 306
  genomics, 316, 317
  reproductive isolation
    spawning timing, 313–314
    sperm-egg recognition systems, 314–316
  speciation, ocean, 305
Crab and wave ecotype, 280–282, 286, 287, 292
Crambe crambe, 110, 114
Ctenophora, 16, 66, 84–85, 106, 107, 115–117
Ctenophores, 69, 115–118, 125–127
Cubozoa, 118, 121
Cultivation-independent methods, 49
Culture-based methods, 48
Cytochrome c oxidase I (COI), 111, 112, 116, 119, 120, 125

D
Demographic connectivity, 427, 429
Demospongiae, 108
De novo genome assembly, 140, 143, 145, 159, 160
Direct experimental tests, 286
Discriminant analysis of principal components (DAPC) approach, 153, 155
Dispersal, 104, 105, 108, 111, 113–115, 117, 119–124, 126, 127
DNA barcoding, 12, 63
DNA methylation
  description, 328
  genome distribution, 329
  histones, 331–333
  histone variants, 331–333
  marine environments, 329, 330
  PTM, 331, 332
  regulatory role, 329
DNA sequence variation, 20, 24, 28
DNAse-seq, 8–9

E
Ecklonia radiata, 10–11
Ecological genomics, 141, 142
Ecological segregation, marine invertebrates, 307–309
Ecological speciation, 255–256
  depth, coral reefs
    Eunicea spp., 310, 311
    Favia fragum, 311
    Orbicella spp., 310, 311
    Seriatopora hystrix, 311
    Symbiodinium, 311–313
  habitat segregation, 306
  natural selection, 306
Ecological stresses, 140
Ecosystem management, 40, 43, 52, 53
Ecotypes
  crab-wave ecotype setting, 280
  inter-ecotype matings, 287
  L. saxatilis, 279, 294
  parallel formation, 287–288
  reference transcriptome, 283
  snail system, 282
Ectodysplasin (Eda), 260, 262, 264–268
Effective populations sizes, 140
Elasmobranchs, 13
Endozoicomonas, 46
Environmental association analysis (EAA)
  allele frequency variation, 225
  constrained, 230–231
  as gene-environment association, 229–230
  genetic variants, 224–225
  relatedness and kinship approaches, 230
Environmental change, 177, 181, 182, 206
Environmental DNA (eDNA), 12–14, 374, 375, 382
  barcoding, 12–13
  biomonitoring, 12
  biotic and abiotic factors, 14
  characterization, 14
  Lithobates catesbeianus, 12
  management and policy decisions, 12
  mass-balance model, 14
  metabarcoding approach, 13
  multicellular organisms, 12
  shedding rate, 14

Environmental DNA (eDNA) (*cont.*)
shotgun sequencing approach, 13
16S rRNA genes, 12
water samples, 12
Environmental gradients
EAA/outlier analysis, 224
gene flow, 232–233
IBD, 234
local adaptation, 223
polygenic models, 235
transient, 237
Environmental stress, 43, 45, 49, 89, 161, 294, 335, 338, 339, 347, 433
Environmental stressors, 47, 82, 339, 340, 346, 348, 381, 435, 439
Epigenetics, 22–23
assessment
disease, 339, 340
stress exposure, 340
chromatin, 326
coral reef restoration, 346–348
definition, 326
eukaryotic cells, 326, 327
evolution, 337, 338
history, 326
inheritance modifications, 335, 336
mechanisms
DNA methylation (*see* DNA methylation)
regulatory networks, 334, 335
RNA-mediated regulation, gene expression, 333, 334
population parameters
age estimation, 341, 342
ecological applications, 344, 345
identification, subpopulations, 343
reprogramming, 336, 337
restoration and management, 345, 356
Epigenomics, 22–23
environmental, 325–348
Eukrohnia hamata, 73
Euphausia superba (Euphausiacea), 73, 83–84
Eurytemora affinis, 64
"Everyone Wins" principle, 269
Evolutionary significant units (ESUs), 434
Exons, 145, 146

F

Fisheries-induced evolution, 413, 414
Fisheries management, 25–26
and conservation, 399–416
Fishery forensics
assignment tests, 412
DNA tests, 412
FishPopTrace project, 412
Fishery genomics management
cheap, fast and reliable markers, 415
climate change, 410–411
description, 401
dispersal inferences, 413
evolution, 413–414
forensics, 411–412
issues, outcomes and applications, 415
local climatic adaptations, 415
management and conservation, 401
MPS, 401
representation, 416
SNP, 401
stock structure, 402–410
taxonomic group representation, 401
Fishery stock structure
challenges, 402
chromosomal rearrangements, 408–409
definitions, 402
finer-scale population structure, 404–407
frequency distribution study, 404
genetic connectivity, outlier detection, 409–410
overexploitation and disappearance, 403
populations *vs.* management units, 403
FishPopTrace project, 412
Florida Reef Tract (FRT), 15
FORAM Index, 46
Foraminifera, 46
Free-swimming larvae, 108
Freshwater-adapted genotype, 267, 268
F_{ST} values, 111
Functional connectivity, 427

G

Gasterosteus aculeatus, 21, 250, 251, 331
See also Threespine stickleback fish
Gene-environment association, 229–230
Gene flow, 104, 105, 111, 112, 115, 116, 119–121, 123–127
Genetic breaks, 111, 112, 115, 116
Genetic connectivity, 427, 429
Genetic diversity, 14–15, 151, 161, 278, 368, 369, 373, 400, 402, 411, 432, 439
chimerism, 113–114
clonality, 113–114
and demographic patterns, 127
distribution, 115, 119

hybridization, 113–114
and inbreeding, 112–113
intrapopulation, 109
spatial distribution, 104
and structure, 11, 73–75
Genetic-environment association analyses (GEAs), 175, 191, 193, 194, 200
Genetic markers, 278, 285, 288
Cnidaria, 117
sponges, 109
Genetic structure
Anthozoa, 123–125
cryptic, 15
definition, 25
fine-scale, 25
large-scale, 283–285
populations, 104
sponge species, 110
Genome assembly, 140, 142, 144, 146, 155, 159, 160
Genome scan, 259, 262–264
Genome size, 76, 79, 80, 87, 142–144, 283
in zooplankton, 64, 66–67
Genome-wide association studies (GWAS), 230, 239, 259–260, 293, 316
Genome-wide scans for selection, 223–225
Genomic markers, 77
seascape genetics and genomics, 187–188
Genomic resources, 19–21, 87
S. balanoides, 142–146
snail system, 283
zooplankton
genome size, 64, 66–67
mitochondrial genomes, 67–69
published, 64, 65
transcriptomics, 69–72
Genomics approaches
capture-seq, 8
chip-seq, 8–9
description, 6
DNAse-seq, 8–9
GBS, 7–8
Rad-seq, 7–8
RNA-seq, 8
whole-genome sequencing, 6–7
Genomic study, 405–406
Genotype-environment association (GEA), 193, 259, 262, 264, 438
Genotyping-by-sequencing (GBS), 7–8, 62, 76, 79, 161, 187
Global carbon cycle, 10
Global Coral Microbiome Project, 11
Great Barrier Reef, 40, 195, 311
Gut microbiota, 10, 44

H
Habitat-specific ecotypes, 279
Hardy-Weinberg equilibrium (HWE), 125, 189, 190
Hexactinellida, 108
High-throughput sequencing (HTS), 50, 75–76, 133, 159, 162
Homoscleromorpha, 108
Host genetic sequences, 51
Hybridisation
genotypes/phenotypes, 369
heterosis, 369
Orconectes propinquus, 371
Orconectes rusticus, 371
Spartina spp., 369
Tigriopus californicus, 370
Hybridization, 8, 24, 113–114, 126, 227, 288, 295, 313, 315, 316
Hybrid zones, 280, 287, 293, 295
Hydrozoa, 118, 121
Hypoxic zones, 20

I
i5K Pilot Project, 64
Illegal, unreported and unregulated (IUU) fishing, 25, 411
Immigration, 181–183
Integrative approach, MAPs
at-site processes, 435
between-site processes, 435
GEA, 439
link (edge) level analyses, 435, 437–438
local adaptation, 436, 438
node level analyses, 435, 438
representation, 436
Internal transcribed spacer 2 (ITS2) region, 49
Invasive species, 6, 12, 24–25, 105, 110, 115, 118, 337, 345, 378
Ircinia fasciculata, 114
Ircinia variabilis, 114
Isolation-by-distance (IBD) hypothesis, 184, 191, 196, 225–226, 228–230, 232–234, 429–430

K
Kelp's health, 11

L
Lactose dehydrogenase (LDH), 226
Landscape genomics, 173, 179, 192, 193
Last Glacial Maxima (LGM), 142, 147, 158
Linkage disequilibrium (LD), 268

Lithobates catesbeianus, 12
Littorina saxatilis, 21
contact zone snails, 292
ecotypes, 279
gene copy number variation, 295
gene flow, 292
habitat choice, 293
intertidal snail, 279
maximum parsimony network, 284
partial reproductive isolation, 295
plasticity, 281
reference genome, 291
reference transcriptome, 283
speciation continuum, 296
Swedish rocky shore, 280
Local adaptation, 69, 77, 78, 80–84, 223, 436
clinal adaptation, 223
crab and wave ecotypes, *L. saxatilis*, 279, 280
EAA methods, 230
gene flow and polygenic trait, 232, 233, 236
and genetic differentiation, 222
Littorina system, 288
of marine organisms, 62, 63
in sea, 433–434
snail system, 282
and speciation, 278
species-wide scales, 223
Local retention, 181–183
Long-reads, 140–141

M

Mammals, 10, 315, 328, 329, 334–336, 339, 341, 342, 345
Management units (MUs), 403–406, 414, 434
Mantel tests, 192
Marine connectivity
measuring and integrating, 428–430
MPA networks, 435–439
Marine conservation
genetic methods, 425
genetic variation, 434–435
genomic data, 425
integrative approach (*see* Integrative approach, MAPs)
molecular technology, 425
neutral and adaptive markers, 425
SNPs, 425
Marine ecosystems, 4, 5, 12, 13, 27–28, 41, 140, 327, 364, 400
Marine invasion genomics
biological invasions (*see* Biological invasions)
methods
environmental DNA, 374–375
gene expression, 381
population genetics, 376–380
QTL, 375–376
Marine invertebrates, 104–106, 116, 123, 158, 223, 307–309, 334, 337, 346, 347
Marine protected areas (MPAs), 6, 24, 26
definition, 424
networks (*see* MPA networks)
Marine snail, 21
contact zone snails, 292
crab and wave ecotypes, *L. saxatilis*, 279, 280
ecotypes, 279
fitness, 293
next-generation sequencing technology, 283
reciprocal transplants, 286
snail system, 282
toxin diversity, *Conus* snail, 294
Marine spatial planning, 425, 426
Mass-balance model, 14
Mastigias papua 121
Mediterranean, 17, 84, 112, 114, 115, 195, 198, 200, 312, 372, 378, 379
Medusozoa, 16, 106, 107
blooms, 118, 122
budding/metamorphosis/lacking, 118
Cubozoa, 121
food webs, 118
Hydrozoa, 121
Scyphozoa, 119–121
short-living larval stage, 118, 119
Meganyctiphanes norvegica (Euphausiacea), 84
Metabarcoding, 13, 63, 78, 375, 382
Metagenetics, 78
Metagenomics, 48, 50–52, 78
databases, 10
global carbon cycle, 10
Global Coral Microbiome Project, 11
marine cyanobacteria, 10
marine microbe and virus populations, 9
photosystem genes, 9
physical, chemical and biological measurements, 9
plasmid families, 10
Sargasso Sea, 9
whole-genome microbial sequencing, 11
zooplankton, 11

Metatranscriptomics, 48, 50–52
Microarrays, 18, 340
Microbial bioindicators, 43, 44, 53
Microbiomes
advantages and disadvantages, 48, 49
amplicon-based sequencing, 49
coral, 39–53
cultivation-independent methods, 49
cultivation techniques, 48
culture-based methods, 48
environmental pollution, 41
high-throughput tag sequencing techniques, 50
ITS2 region, 49
metagenomics, 50
metatranscriptomics, 50–51
and microorganisms, 43–45
natural cycles, 42
OTU, 50
ribosomal DNA tag sequencing, 49
16S and 18S, 49, 50
Microorganisms, 41–42, 51
ARMS, 45
biofilms, 45–46
in diverse ecosystems, 43
environmental stress, 43, 45
FORAM Index, 46
foraminifera, 46
microbe-focused monitoring techniques, 45
and microbiomes, 43–45
monotonicity, 47
reef health, 45, 48
rivet hypothesis, 46–47
stressors, 46
variability, 47
Microsatellites, 74, 109, 110, 113, 115–117, 122, 259, 260
Migration, 285, 289
Mitochondrial DNA, 67, 74, 79, 109, 115–117, 197, 255, 279, 340, 376
Mitochondrial genomes, 67–69, 82, 85, 105
S. balanoides, 141, 144, 145
Mitogenomes, 67–69
Mnemiopsis leidyi, 115–116
Molecular approaches, 63
Molecular markers, 14, 15, 76, 174, 279, 376
Molecular technologies, 425
Moran's eigenvector maps, 230, 231
MPA networks
climate change, 433–435
configuration, 426
connectivity, 426–427
definitions, 426
design, 430–433
frequency distribution, 426, 428
measuring and integrating marine connectivity, 428–430
Multilocus genotypes (MLGs), 114, 124

N
Natural laboratories, 140
Natural selection, 5, 16–18, 22, 23, 177, 178
NCBI, 64, 145
Nemopilema nomurai, 120
Next-generation sequencing (NGS), 172, 187, 188
Noncoding RNAs (ncRNA), 333, 334
Non-indigenous species (NIS), *see* Marine invasion genomics
Non-model barnacle species, 6
Non-neutral markers, 77
Northern acorn barnacle, 20, 141
See also Barnacles
Nuclear genetic fragments, 109
Nucleosomes, 331, 339
Nuisance fish, 21

O
Oceanography, 173, 176, 197, 203
Ocean processes, 73
Octocorals, 310, 312, 313
Operational taxonomic units (OTUs), 50, 78
Ostracods, 66
Outlier analysis, 224, 225, 228–230, 232, 233, 236–238
Outlier test, 191, 192

P
Panmixia, 118–121
Paraleucilla magna, 113
Parallel evolution
cichlid, salmonid fishes and *Anolis* lizards, 257
convergent, 257
phenotypic variation, 259
primary population genomics approaches, 259
QTL mapping, 261
standing genetic variation, 265
in threespine stickleback, 250, 252–256
variation, 258–259
Parallel formation
crab and wave ecotypes, 287–288

Parallel phenotypic evolution, 257–259
PCR-build approach, 67
PCR inhibitors, 51
Pelagia noctiluca, 73, 119
Pelagic biogeography, 63
Phylogenetic biodiversity, 63
Phylogeographic analysis, 75
Phylogeography, 109, 115
Pituitary homeobox transcription factor I (PitxI), 266
Plankton networks, 10
Pleurobrachia bachei (Ctenophora), 84–85
Pocillopora damicornis, 124
Polymorphic loci, 5, 8, 189, 193
Polymorphism, 187, 188
Pooled sequencing, 141, 149, 153, 156–157, 161
Population adaptive index (PAI), 438
Population biology, 4
Population genetics, 67, 78–83, 85
  Bayesian computation (ABC) method, 380
  characterization, 104
  distribution, 104
  diversity and structure, 73–75
  DNA sequencing technology, 376
  genomic approaches, 377
  genotype-environment interactions, 376
  management and conservation strategies, 105–107
  Microcosmus squamiger, 376, 377
  to population genomics, 75–77
  Pterois volitans, 378–380
  SNPs data, 146
  standing genetic variation, 133
  structure, 104, 105
Population genomics
  acclimation, 18–19
  adaptation, 20–22
  approaches, 6–9
  biodiversity, 9–14
  candidate loci for adaptation, 232, 235
  characteristics, 4–5
  coastal zones, 4
  environmental variation, 5
  epigenomics, 22–23
  evolution, 62, 63, 67
  fisheries management, 25–26
  genetic diversity, 14–15
  genetic markers, 62
  genome-wide scans for selection, 223–225
  identification, 5–6
  invasive species, 24–25
  locus-specific effects, 62
  marine ecosystems, 4, 27–28
  MPAs, 26
  natural selection, 5, 16–18, 22, 23
  neutral markers, 5
  outlier analyses to clinal adaptation, 229
  polymorphic loci, 5
  in population biology, 4
  to population genetics, 75–77
  seascape genetics and genomics
    adaptive evolution, 177–178
    biological characteristics, marine species, 174, 175
    chaotic genetic patchiness, 176–177
    local environment, 177
    ocean currents and environment, 176
    traits, 175
  seascape genomics, 17
  speciation, 23
  structure, 15–16
  sustainable management, 24–26
  zooplankton (*see* Zooplankton)
  See also Fishery genomics
Population graphs, 190, 191, 430–432
Porifera, 16, 106, 107
  Calcarea, 108
  chimerism, 113–114
  clonality, 113–114
  demospongiae, 108
  free-swimming larvae, 108
  genetic diversity, 112–113
  genetic markers, 109
  hexactinellida, 108
  homoscleromorpha, 108
  hybridization, 113–114
  inbreeding, 112–113
  population differentiation, 110–112
  sponges, 108–109
Posttranslationally modified (PTM), 331, 332, 336
Principal component analysis (PCA), 148, 152–155, 180, 190
Proteomics approaches, 19

Q

Quantitative trait loci (QTL) mapping, 375, 376
  genetic markers, 259
  of stickleback
    lateral plate phenotype, 260
    phenotypic variation, 260
    selection and parallel evolution, 262
    SNP markers, 260

R
Rainforest ecosystems, 39
Read error correction, 143
Reduced representation libraries (GBS/ddRAD), 161
Redundancy analysis (RDA), 193, 194
Reef health, 11, 40, 42, 45, 46, 48, 51
Reference genome, 283
  L. saxatilis, 291, 293, 296
Restriction fragment length polymorphism (RFLP), 76
Restriction site-associated DNA sequencing (RAD-seq), 7–8, 76–77, 260, 262, 264, 269
Ribosomal DNA tag sequencing, 49
Ribosomal internal transcribed spacer (ITS), 109, 112, 115–117
Rivet hypothesis, 46–47
RNA-seq, 8, 18–19, 76

S
Salpa thompsoni (Tunicata, Thaliacea), 85–86
Sargasso Sea, 9
Sbal2 de novo assembly, 143–144
Scopalina lophyropoda, 114
Scyphozoa, 119–121
Seascape genetics
  adaptive evolution, 177–178
  biological characteristics, marine species, 174, 175
  chaotic genetic patchiness, 176–177
  vs. genomics, 174
  local environment, 177
  ocean currents and environment, 176
  traits, 175
Seascape genomics, 17
  adaptive divergence in ocean, 199–200
  asymmetry, 195–197
  autocorrelation, 180
  biophysical modelling, 185–186
  causes, adaptive divergence, 200–202
  chaotic genetic patchiness, 203–204
  description, 172
  environmental change, 181–183
  factors, 179
  features and processes, 183–184
  fishing, 206
  focal organism's biology, 179
  genetic maps, 202–203
  vs. genetics, 174
  genomics methods, 187–188
  history, 172–173
  IBD hypothesis, 184
  inferences, 192–195
  isolation-by-environment, 184
  landscape genetics/genomics, 172
  marine metapopulation dynamics, 197–198
  multispecies comparisons, 204–205
  neutral and adaptive, 172
  number and rate, 173
  patterns and process, 189–191
  population genomics, 172
  population-level sampling, 188–189
  replicate sampling, 205
  response variables, 189
  sequencing technologies, 178
  "stratified-random" sampling design, 181–183
  temperature and salinity, 201
SeaSketch projects, 440
Semibalanus balanoides, 20
  biological signal, 155
  genetic differentiation, 142
  genetic variation, 150
  genomic resources
    exons and CDS, 145, 146
    mitochondrial genome and gene-rich, 144
    Sbal1, 142
    Sbal2 de novo, 143–144
    USCO genes, 142
  historical biogeography, mtDNA markers, 147–150
  natural populations, 158–159
  in the North Atlantic, 147
  pool-seq experiments, 141
  Sbal2, 141
  structure-informative markers, 155–156
Sequencing
  amplicon-based, 49
  genetic, 48
  high-throughput tag, 50
  host genetic, 51
  metatranscriptomic, 51
  16S and 18S, 48, 50
Short Read Archive (SRA), 64
Short reads, 140–141
Shotgun sequencing, 13, 67
Single-cell genomics, 52
Single-nucleotide polymorphisms (SNPs), 15–16, 20, 25, 75–77, 106, 187–190, 196, 197, 260–262, 425
Small-scale heterogeneity, 74
Small-sized organisms, 88
Snail system, 279, 282, 283

Spadella cephaloptera (Chaetognatha), 85
Spatial autocorrelation, 192–193, 195
Spatially varying selection, 223, 225–227, 233–234, 238
Spawning timing, 313–314
Speciation, 23
  in coral (*see* Coral reefs)
  genome rearrangements, 294
  and local adaptation, 278, 288
  reproductive isolation, 295
  "speciation continuum," 296
Sperm-egg recognition proteins, 314–316
Sponges
  cnidarians, 117
  microsatellites, 116
  molecular ecology, 125
  population genetics, 126
  Porifera, 108–114
16S rRNA gene, 12, 48–50
18S rRNA gene, 48–50
Standing genetic variation, 289–290
Stickleback, 20, 21, 223, 234, 237, 249–270, 289, 331, 344, 348
Stratified-random sampling design, 181–183
Stress signatures, 51
Structural connectivity, 426–427
Structure-informative markers, 155–156
Sustainable management, 24–26

T
The Genetics of Colonizing Species, 364
Threespine stickleback fish
  ecological speciation, 255–256
  Eda, gene of major effect, 264–265
  Everyone Wins principle, 269
  evolution and divergence, 251
  evolutionary biology, 250, 256
  freshwater populations, 251, 255
  genetic mapping of traits, 259
  genome, 257
  genome scans for selection, 262–264
  genomics and experimental tools, 250
  mapping (*see* Quantitative trait loci (QTL) mapping)
  marine/freshwater and lake/stream population pairs, 255
  as model system for adaptive evolution, 255
  parallel evolution, 250, 252–255
  phenotypic changes, 257
  phenotypic diversity, 250
  phenotypic variation and popular appreciation, 250, 251
  PitzI, independent mutations, 266
  polygenic adaptation, 266–267
  rapid evolution, 267
Thunnus maccoyii, 6
Tigriopus californicus (Copepoda), 73, 81–82, 370
Transcriptomics, 18–19, 384
  zooplankton, 69–72
Transporter hypothesis, 267

U
Universal single-copy orthologue (USCO) genes, 142, 144

W
Whole-genome analysis, 62
Whole-genome microbial sequencing, 11
Whole-genome sequencing, 6–7, 87, 262, 269, 270
Wright's *F*-statistics, 74

Z
Zooplankton, 11, 17, 118, 119
  Acartia tonsa (Copepoda), 82
  applications
    genomic basis of adaptation, 77
    metabarcoding, 78
    metagenetics, 78
    population genetics, 73–77
  biodiversity, 62–63
  biogeography, 63
  Calanus finmarchicus (Copepoda), 79–80
  Centropages typicus (Copepoda), 80–81
  Euphausia superba (Euphausiacea), 83–84
  genomic resources (*see* Genomic resources, zooplankton)
  global ocean, 87–88
  life histories, 63
  Meganyctiphanes norvegica (Euphausiacea), 84
  Pleurobrachia bachei (Ctenophora), 84–85
  Salpa thompsoni (Tunicata, Thaliacea), 85–86
  small-sized organisms, 88
  Spadella cephaloptera (Chaetognatha), 85
  species identification, 88
  Tigriopus californicus (Copepoda), 81–82