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Opinion

Finding genes and pathways that underlie coral adaptation

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Mass coral bleaching is one of the clearest threats of climate change to the persistence of marine biodiversity. Despite the negative impacts of bleaching on coral health and survival, some corals may be able to rapidly adapt to warming ocean temperatures. Thus, a significant focus in coral research is identifying the genes and pathways underlying coral heat adaptation. Here, we review state-of-the-art methods that may enable the discovery of heat-adaptive loci in corals and identify four main knowledge gaps. To fill these gaps, we describe an experimental approach combining seascape genomics with CRISPR/Cas9 gene editing to discover and validate heat-adaptive loci. Finally, we discuss how information on adaptive genotypes could be used in coral reef conservation and management strategies.

Highlights

Marine heat waves are having unprecedented impacts on reef-building corals worldwide.

Corals within the same species have different sensitivity to heat stress, and part of this variation is due to genetic adaptation.

Finding coral genotypes that underpin heat adaptation would allow the identification of adapted populations so that reef managers could protect and promote the spread of these adapted populations.

Seascape genomics identifies candidate adaptive genotypes by associating variation in genotype frequencies across a reef system with historical thermal conditions.

CRISPR/Cas9-based gene editing could assess and validate the functional roles of putative adaptive genotypes, and validated genotypes could then be used in conservation.

The search for heat-adaptive alleles in corals

The worldwide decline of reef-building corals is a clear example of how climate change affects marine communities [1,2]. This decline is mainly attributed to anomalous heat waves disrupting the vital symbiosis between corals and the unicellular algae inside their tissue [3,4]. Important differences in thermal tolerance can be observed among corals from the same species [5–9], and recent research suggests that these differences are heritable [9–18]. The heritability of thermal tolerance is driven by the genotypes of the host and the algal symbiont, and the relative importance of each can change based on the species or life stage involved [12,18–21].

The heritability of thermal tolerance implies the existence of coral alleles that promote tolerance to increasing ocean temperatures. Therefore, a promising future direction for coral research is identifying heat-adaptive alleles, characterizing their functions, and using this genetic information to predict how coral may adapt to climate change. For example, heat-adaptive loci could be used as biomarkers to identify thermally adapted corals and rank reefs based on their adaptive potential (i.e., the frequency of thermally adapted corals) [22]. Reefs with high adaptive potential could be designated as protected areas, preserving wild populations of heat-adapted corals from local stressors (e.g., water eutrophication, increased sedimentation due to coastal development, and destructive fishing practices [23–26]). Moreover, corals with adaptive traits could be used in breeding and restoration programs [27,28], although this strategy might be feasible only for small reef areas [29].

Here, we review recent efforts to study coral heat adaptation and summarize our current understanding of the genetic architecture of coral heat tolerance. We then identify and describe four major knowledge gaps that need to be addressed to effectively characterize heat-adaptive alleles in corals. Finally, we discuss how a combination of genotype-by-environment association analysis (referred to as '**Seascape genomics**'; see Glossary) and CRISPR/Cas9 technology may constitute a powerful new approach to characterize natural adaptive genotypes for use in coral reef management and conservation.

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Recent coral heat-adaptation studies

The results of recent experiments to identify candidate alleles underpinning coral heat adaptation are reviewed in Table 1 and visually summarized in Figure 1. These experiments are generally a combination of three steps: (i) sampling of corals from reefs with differing thermal histories; (ii) measuring heat-related phenotypes in these sampled corals; and (iii) characterizing genetic variation that may underlie adaptive traits (Figure 2).

Sampling approaches

There are typically three main approaches for sampling: (i) corals are collected from a single reef (Figure 2A; 3/19 reviewed studies) [14,15,17]; (ii) corals are collected from a pair of reefs experiencing distinct thermal conditions (Figure 2B; 7/19 studies) [5,11,12,16,30–34]; or (iii) corals are collected from multiple reefs spanning a natural gradient of thermal conditions across a large spatial scale (Figure 2C; 9/19 studies) [9,10,20,21,35–39]. These three approaches result in substantial differences in sample size and geographical extent of the study area, from a few dozen colonies collected in neighboring locations (e.g., [31,34]) to hundreds of colonies distributed across hundreds of kilometers (e.g., [21,36,40]). On the one hand, sampling at one or two reefs allows researchers to cost-effectively apply demanding field techniques, such as phenotyping of heat responses [5,12,32] or sampling corals for breeding experiments [11,12,14–16,30]. The focus on a small spatial scale also reduces the risk of sampling corals from populations that are genetically isolated and that might not share adaptive alleles (e.g., cryptic species [41]). On the other hand, sampling across multiple sites allows researchers to characterize heat adaptation at spatial scales more relevant for coral reef management and conservation [42].

Phenotyping approaches

Several approaches have been developed to estimate heritable heat-related phenotypes in corals, and these methods can be used alone or in combination. The first approach is the *in situ* measurement of coral phenotypes in response to natural heat exposure (Figure 2D; 4/19 reviewed studies) [5,9,17,21,34]. Before these measurements, corals (entire colonies or fragments) are usually transplanted between reefs exposed to different thermal conditions. Heat-related phenotypes, such as visual bleaching severity [9,17], chlorophyll content [5,21], or photochemical efficiency [9], are measured directly in the field after an acclimation period. Researchers then compare how corals originating from different environments respond to heat stress in a common setting. This approach allows for the identification of heritable phenotypes (i.e., determined by the genetics of the coral holobiont) and those that are not (i.e., determined by the environment).

A second approach is *ex situ* thermal profiling, where corals from different reefs are either acclimated to a common environment or reciprocally transplanted, before being exposed to controlled heat stress conditions in an aquarium setting (Figure 2E; 4/19 studies) [10,12,17,32]. Similarly to the *in situ* experiments, a wide range of heat-related phenotypes can be measured [43], such as changes in symbiont abundance [10], photochemical efficiency [12,17], antioxidant capacity [17], or pigmentation [32]. Compared to *in situ* measurements, these controlled experiments allow for more reliable comparisons across studies (but see [44] for a detailed discussion of standardization in coral heat experiments).

The third approach is to breed corals from a single reef or from a pair of environmentally contrasting reefs (Figure 2F; 6/19 studies) [11,12,14–16,30]. Adult colonies are typically collected immediately before natural spawning so that gametes can be recovered and crossed in the laboratory. The resulting larvae display a range of heat-related phenotypes, and researchers can correlate parental genotype with this phenotypic variation (e.g., the survival rate of larvae under thermal stress) [11,12,14–16]. In addition, breeding experiments allow researchers to perform intrafamily

comparisons, which are an optimal setting to estimate the **narrow-sense heritability** of heat-related phenotypes [45].

Genetic approaches

In most of the past studies (15/19 studies), corals were genotyped using a reduced genomic representation technique, such as RNA sequencing (RNAseq) or restriction site-associated DNA sequencing (RADseq) [9–12,14–17,30,31,34–38]. These cost-effective methods produce high-quality genotypes across thousands of loci from every sampled colony [46]. Over the past 3 years, low-coverage whole-genome sequencing (lcWGS) has become increasingly popular (3/19 studies) [20,21,32]. Compared to RADseq and RNAseq, lcWGS produces genotypes at millions of variable sites across the genome, although this approach leads to less confidence in nucleotide calling across the genome. However, this uncertainty may be acceptable for some analyses, such as investigating broad patterns of genetic variation across a large number of individuals from a population [47].

Following genotyping, three main approaches are generally used to search for loci underpinning thermal tolerance. Genotype-by-phenotype association analysis (used in 10/19 studies) aims to identify candidate adaptive alleles by correlating allelic frequencies with variation in a given heat-related phenotype (Figure 2G) [9–11,14–17,21,30,33]. The strategies to identify such alleles usually depend on the sampling approach and phenotypes measured [48]. For example, **quantitative trait loci (QTL)** mapping typically uses animals generated from experimental breeding, which have a known familial history, enabling the random segregation of chromosomes and the identification of broad genomic regions associated with phenotypic variation [11,16]. In another strategy, **genome-wide association studies (GWAS)** typically require large numbers of wild individuals from populations with historic admixture, which enables the identification of single nucleotide polymorphisms (SNPs) or haplotypes that correlate with a phenotype of interest [10,21].

Other genetic approaches can detect candidate adaptive loci without directly correlating genotypes with variation in a phenotype (Figure 2H; 12/19 studies) [12,16,17,20,21,31,32,34–38,40]. In **population-genomic scans**, genome-wide allelic frequencies can be compared between populations displaying varying heat-associated phenotypes or living in habitats with distinct environmental conditions. These experiments can identify candidate adaptive alleles the frequencies of which deviate from the neutral patterns of allelic variation across populations. Typically, the fixation index (F) is used to calculate the degree of allelic frequency differentiation of each locus between coral populations from two reefs [31,32,34,36–38]. Loci with F_{st} values higher than the genome average are candidates for being under selection. Importantly, population-genomic scans can also be performed on corals sampled from many reefs without any *a priori* knowledge of phenotypes or environmental conditions [48]. One common statistic for this type of analysis is the pairwise nucleotide diversity (π), which is calculated by comparing nucleotide diversity across all possible pairs of corals sampled from many reefs. The π statistic summarizes genome-wide changes in genetic diversity in a population and can be used to identify genomic regions where genetic diversity is anomalously low (perhaps resulting from recent selection due to environmental stress [20]) or anomalously high (perhaps resulting from balancing selection in an heterogeneous environment [21]). When haplotype data are available, conceptually similar approaches [e.g., extended haplotype homozygosity (EHH)] can be used to identify genomic regions that may have arisen from recent selection [49].

In a related approach, seascape genomics correlates genomic variants within a population with historical environmental parameters that characterize the habitat of the population (Figure 2I) [17,35,37,40,50]. In seascape genomics studies, data sets derived from satellite imagery are

Glossary

Genome-wide association studies (GWAS): method to identify adaptive loci by correlating genetic and phenotypic variation in a large number of individuals from the same population.
Narrow-sense heritability: fraction of phenotypic variation that can be attributed to the additive effects of genes.
Population-genomic scan: method to identify adaptive loci by analyzing changes in patterns of genetic variation in a population along the genome.
Quantitative trait loci (QTL): method to identify adaptive loci by correlating genetic and phenotypic variation in individuals with a known family history (e.g., originated from directed breeding).
Seascape genomics: method to study local adaptation by correlating variation of genotype frequencies in a wild population with the environmental variation across the habitat of the population.

Table 1. Recent efforts to uncover heat-adaptive loci in corals[a]

Study	Sampling	Phenotyping	Genotype association	No. of reefs	No. of colonies	Spatial scale	Type of sequencing	Species	Region	No. of candidate loci	Molecular functions
Bay and Palumbi [34]	Pair of reefs	*In situ* measure	Genotype	2	23	<10 km	RNA	*Acropora hyacinthus*	American Samoa	114 SNPs	PF, UBI, APO
Dixon *et al.* [11]	Pair of reefs	Cross-breeding	Phenotype	2	4	~500 km	RNA	*Acropora millepora*	North GBR	2 regions	MT
Howells *et al.* [12]	Pair of reefs	*Ex situ* thermal profiling, cross-breeding	Genotype	2	18	~500 km	RAD-tag	*Platygyra daedalea*	Persian Gulf, Sea of Oman	Not investigated	Not investigated
Jin *et al.* [17] (follow-up Lundgren *et al.* [50])	Multiple reefs	*In situ* measure, *ex situ* thermal profiling	Phenotype, genotype, environment	1 (25)[b]	10	(>1000 km)[b]	RNA	*A. millepora*	GBR	2 SNPs	OXY, UBI
Thomas *et al.* [37]	Multiple reefs	–	Genotype, environment	6	70	>1000 km	RAD-tag	*Pocillopora damicornis*	Western Australia	159 SNPs	UBI, FLU, IMM
Devlin-Durante and Baums [36]	Multiple reefs	–	Genotype	18	96	>1000 km	RAD-tag	*Acropora palmata*	Caribbean	13 loci	CS, IMM, CM
Kirk *et al.* [15]	Single reef	Cross-breeding	Phenotype	1	19	–	RAD-tag	*P. daedalea*	Persian Gulf	131 SNPs	UBI, TR
van Oppen *et al.* [31] (follow-up by Marhoefer *et al.* [33])	Pair of reefs	–	Genotype [31], phenotype [33]	2	96	<10 km	RAD-tag	*P. damicornis*	Southern GBR	16 SNPs	IMM, MET, UBI, TR, CS
Dziedzic *et al.* [10]	Multiple reefs	*Ex situ* thermal profiling	Phenotype	7	43	10–50 km	RAD-tag	*Orbicella faveolata*	Panama	12 SNPs in 9 linkage groups	OXY, APO, PF
Quigley *et al.* [16]	Pair of reefs	Cross-breeding	Phenotype, genotype	2	6	~500 km	RAD-tag	*Acropora spathulata*	GBR	87 SNPs	IMM, CM, MET
Fuller *et al.* [21]	Multiple reefs	*In situ* measure	Phenotype, genotype	12	44	~300 km	Whole Genome	*A. millepora*	Central GBR	1 region (population genomic scan)	PF
Cooke *et al.* [20]	Multiple reefs	–	Genotype	5	150	~300 km	Whole Genome	*Acropora tenuis*	Central GBR	71 regions	CS
Selmoni *et al.* [40]	Multiple reefs	–	Genotype, environment	12	155	~500 km	Whole Genome	*Acropora digitifera*	Ryukyu Archipelago	18 SNPs	DNR, DNR, CS
Drury and Lirman [9]	Multiple reefs	*In situ* measure	Phenotype, genotype	10	10	50–100 km	RAD-tag	*Acropora cervicornis*	Florida Reef Tract	58 SNPs	IMM, CS, APO
Howells *et al.* [30]	Pair of reefs	Cross-breeding	Phenotype	2	87	~500 km	RAD-tag	*P. daedalea*	Persian Gulf	542 SNPs	PF, OXY, IMM
Selmoni *et al.* [35]	Multiple reefs	–	Genotype, environment	20	395	~500 km	RAD-tag	*Pocillopora acuta, P. damicornis, A. millepora*	New Caledonia	9–25 SNPs	APO, PF, DNR, CS
Elder *et al.* [14]	Single reef	Cross-breeding	Phenotype	1	6	–	RAD-tag	*P. daedalea*	Central GBR	34–336 SNPs	APO, MET, CM
Smith *et al.* [38]	Multiple reefs	–	Genotype	14	40	>1000 km	RAD-tag	*P. daedalea*	Persian Gulf	1 scaffold	CM, CC
Thomas *et al.* [32]	Pair of reefs	*Ex situ* thermal profiling	Genotype	2	85	<10 km	Whole genome	*A. tenuis*	Western Australia	2 regions	UBI, PF, MET, CM

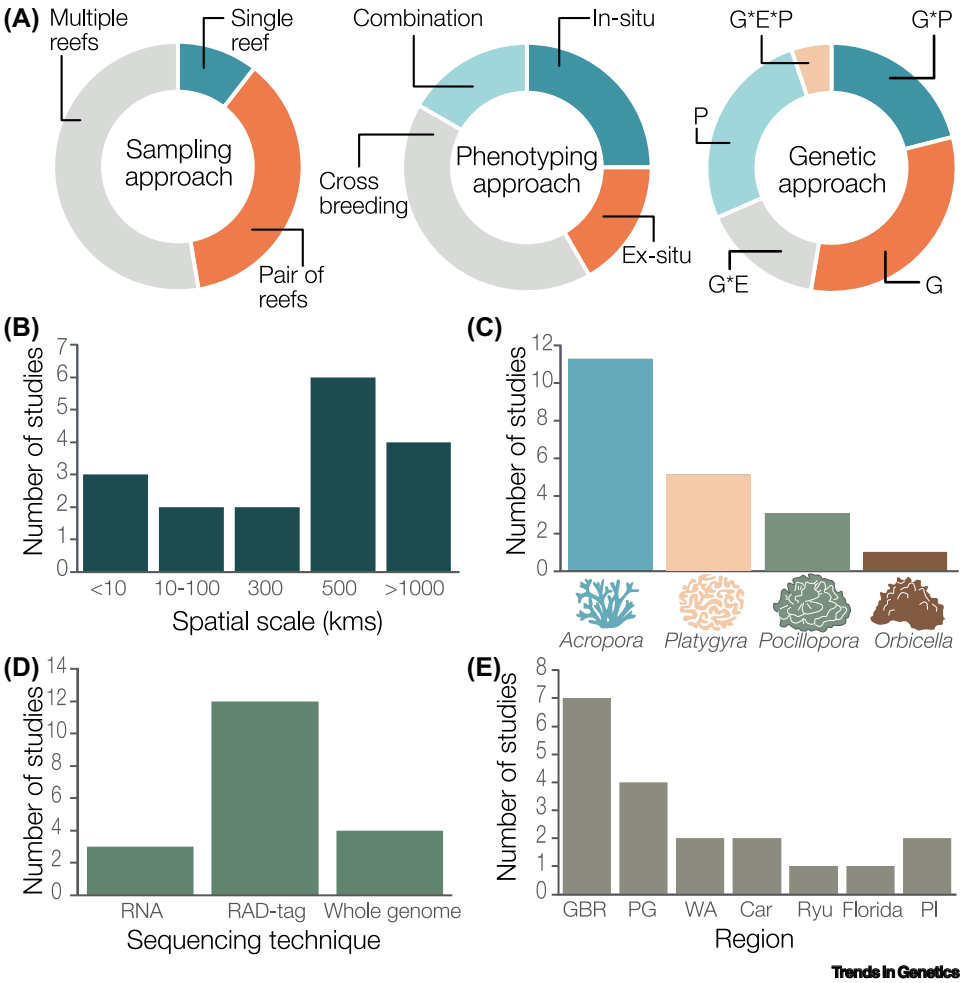

Figure 1. Experimental designs of recent coral heat-adaptation studies. A summary of the experimental designs from 19 studies on coral heat adaptation. (A) Summary of the number of reefs sampled (sampling approach), the methods used to phenotype the corals (phenotypic approach), and the methods used to identify loci associated with heat adaptation (genetic approach) in these studies. The sampling approach can be at single, pairs, or multiple reefs. The phenotypic approach can involve *in situ* field or *ex situ* laboratory measurements of heat-related phenotypes, cross-breeding of corals, or a combination of thereof. Candidate alleles can be found using population genomic scans (G), genotype-by-phenotype correlations (P), genotype-by-environment correlations (E), or a combination thereof. The spatial scale (B), number of genera surveyed (C), sequencing technology used (D), and geographic location (E) of these studies are also summarized. The details of the studies reviewed in this metanalysis are shown in Table 1. Abbreviations: Car, Caribbean; GBR, Great Barrier Reef; PG, Persian Gulf; PI, Pacific Islands; Ryu, Ryukyu Archipelago; WA, Western Australia.

used to characterize the environmental history of a reef system [51–53]. These environmental data typically cover large spatial scales (reefs spanning hundreds of kilometers) over several decades [35,37,40]. Typically, sampling locations are identified at multiple reefs that have been exposed to varying thermal and environmental histories. Without information on the

Notes to Table 1:
[a]Abbreviations: APO, apoptosis; CC, cell cycle regulator; CM, cell membrane structure and process; CS, cell signaling; DNR, DNA repair or processing; FIU, fluorescence; IMM, immune system; MET, metabolism; MT, mitochondrial; OXY, oxidative stress response; PF, protein folding; RAD, restriction site-associated DNA; SNP, single nucleotide polymorphism; TR, transcriptional regulator; UBI, ubiquitination.
[b]Sampling locations of preliminary seascape genetics experiment.

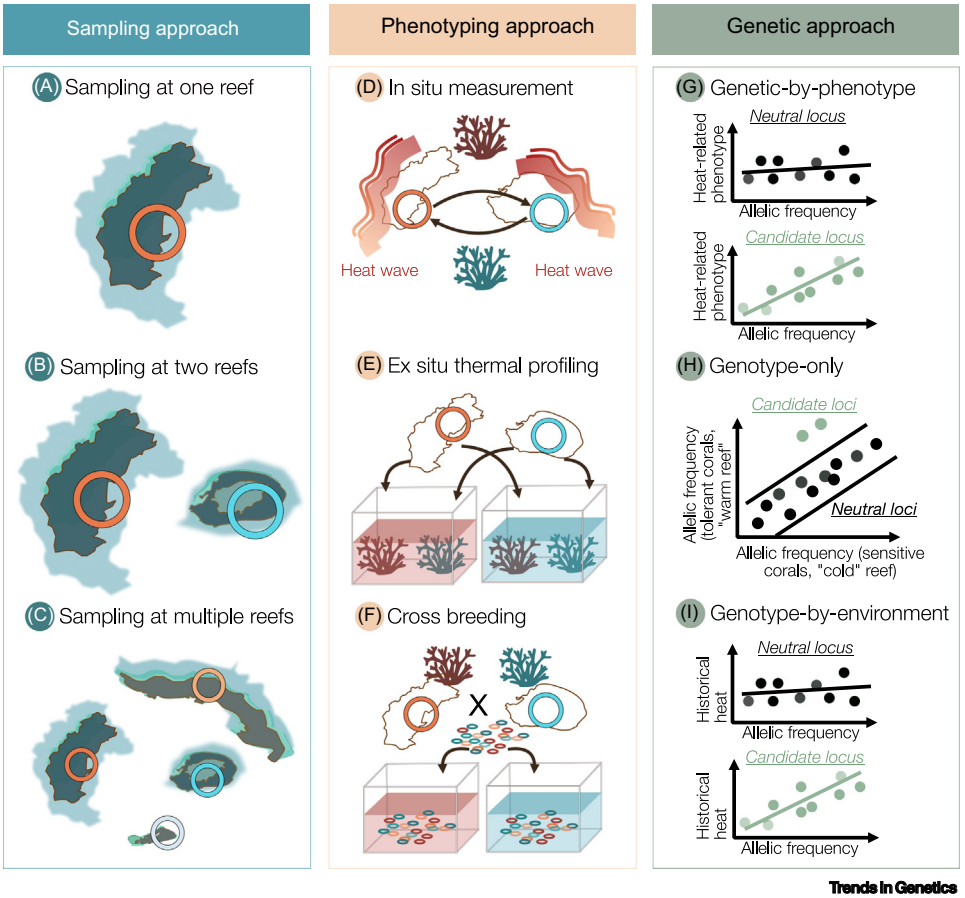

Figure 2. Current strategies to identify candidate loci underlying coral heat adaptation. Current methods to study coral heat adaptation are typically a combination of the steps shown here. (A–C) First, a sampling approach is used to collect corals (circles represent sampling locations from thermally contrasted reefs). (D–F) Second, sampled corals can be phenotyped in the presence or absence of heat stress. (G–I) Third, sampled corals are genotyped, and genetic variation is associated with the heat-related phenotypes and/or environmental variation at the sampling locations.

phenotypes of the corals, candidate adaptive alleles are identified as those with frequencies that correlate with historical environmental gradients. Coral seascape genomics experiments have primarily focused on heat-related environmental variation (e.g., the number or intensity of past heat waves) [35,37,40]. However, the approach has also been used to discover alleles associated with water clarity [50], pH [54], salinity [40], and chlorophyll concentration [35]. Although less common than genotype-by-phenotype and population-genomic scan studies, seascape genomics experiments (4/19 studies) have become more frequent over the past 5 years.

Current understanding and gaps in coral heat-adaptive genetics

Most of the previous studies (14/19) suggest that the genetic architecture of coral heat tolerance is polygenic, comprising dozens to hundreds of candidate genetic loci that are significantly associated with variation in heat-related phenotypes [9,10,14–16,21,30,33], historical heat gradients [35,37,40,50], or show significant variation in allele frequencies in heat-exposed populations [9,16,20,31,34,35,37,40]. For instance, Kirk and colleagues ran a genotype-by-phenotype analysis on *Platygyra daedalea* larvae from a single reef of the Persian Gulf and found 131 loci associated with variation in thermal tolerance [15]. Together, these 131 loci explained ~69% of the observed variation in thermal tolerance [15]. In a genotype-by-phenotype

study on *Acropora cervicornis* from ten sites along the Florida Reef Tract, researchers found 58 candidate adaptive SNPs that explained ~27% of the variation in bleaching tolerance in the field [9]. In another study focusing on the west coast of Australia, population-genomic scans and genotype-by-environment analyses across six thermally contrasted reefs uncovered 159 candidate loci for heat adaptation in *Pocillopora damicornis* [37]. These loci displayed a strong degree of differentiation (i.e., the F_{st} index) across thermally contrasted reefs compared to the neutral loci of the genome. However, there are also a few studies that report single genetic variants with remarkably strong association with variation in heat-related phenotypes [17], historical heat gradients [35], or outstanding variation in allele frequencies in heat-exposed populations [20,21]. For example, Fuller and colleagues conducted a population-genomic scan in *Acropora millepora* from 12 thermally contrasted reefs of the Australian Great Barrier Reef. This study identified one locus with high levels of nucleotide diversity (π) [21]. The high levels of nucleotide diversity at this locus, which contained the gene encoding Sacsin (see below), indicated the presence of two dominant haplotype blocks likely maintained by balancing selection. The researchers hypothesized that one haplotype might be positively selected in heat-exposed reefs and the other might be selected for in nonheat-exposed reefs. However, the precise environmental conditions driving this outstanding variation in allele frequency could not be identified, and this locus was not significantly associated with variation in bleaching in the field.

Once putative adaptive loci are uncovered, it is necessary to identify the causal gene(s) and understand how they impact thermal adaptation. One common strategy is to identify genes located near candidate adaptive genetic variants, because these genes may be controlling the trait of interest. For example, the candidate locus found by Fuller *et al.* in *A. millepora* included the gene encoding Sacsin, a putative co-chaperone of Heat Shock Protein 70, which refolds unfolded proteins resulting from heat stress [21]. The authors hypothesized that Sacsin may be involved in controlling heat tolerance, but this hypothesis is yet to be validated at the molecular level. When multiple candidate loci are discovered and the lists of proximal genes become longer, researchers often summarize the molecular functions of proximal genes with Gene Ontology (GO) enrichments [55,56]. This approach led to the discovery of molecular pathways that appeared as enriched across multiple studies, including those implicated in protein folding [21,30,32,34,35], oxidative stress response [10,17,30], immune system [16,31,36], and apoptosis [9,10,14,34,35] (see detailed review in Table 1). Some of these pathways (e.g., protein folding or oxidative stress response) underpin well-known cellular responses to heat stress [57]. However, the GO enrichment approach usually generates long and variegated lists of pathways (up to hundreds of GO terms per study), and it is often not possible to interpret these results and determine the functional role of each adaptive genotype. For example, the *A. cervicornis* study mentioned above found 58 candidate SNPs in proximity to genes that are associated with 23 GO terms describing a variety of biological processes, and some of these processes are difficult to relate to thermal adaptation without additional evidence (e.g., 'lipopolysaccharide core region metabolic process', 'negative regulation of multicellular organismal process', and 'regulation of sprouting angiogenesis').

Despite their shortcomings, these studies opened the way to the discovery of putative heat-adaptive loci in corals. Below, we describe four outstanding knowledge gaps and propose solutions that may enable the use of adaptive loci in reef conservation and management.

Gap 1: small geographical scale

Half of the reviewed studies focus on corals originating from a single reef or from two contrasted reefs (Figure 1) [11,12,14–16,30,34]. These sampling strategies allowed researchers to improve the understanding of important aspects of coral heat adaptation (such as the phenotypic variability in thermal responses and the heritability of heat tolerance), but these sampling approaches have

limitations when it comes to the discovery of candidate adaptive alleles. With only limited sampling at two reefs, it is difficult to determine whether the potentially adaptive alleles discovered represent the suite of alleles among the entire population. Other adaptive alleles in the same reef but at different depths [33], habitat types (e.g., reef flat vs. reef slope) [31], or at neighboring reefs may be missed. Indeed, candidate alleles found at one site might not impact thermal tolerance at other locations [58] because various environmental factors might interact with thermal stress (e.g., different types of temperature variation, turbidity levels, or nutrient availability [59]). When sampling only at a pair of reefs, it is not possible to distinguish the effects of heat stress from those of other environmental factors that vary between the two sampled reefs. These limitations make it difficult to determine how genetic variation contributes to coral heat tolerance across a reef or an entire reef system.

Gap 2: unbalanced representativity of taxa

Almost all the previous studies investigated coral adaptive genetics in a single species, with only one study focusing on multiple species [35]. Additionally, the representation of different coral taxa is highly unbalanced, with a single coral genus, *Acropora*, being used in more than half of the reviewed studies (Table 1). For other important reef-building corals, such as *Porites*, knowledge of the genetic architecture underpinning heat adaptation is limited. The prioritization of certain species is partly due to the fact that heat-sensitive taxa (e.g., *Acropora*) receive more attention than heat-tolerant taxa (e.g., *Porites* [60–62]) (but see research on *Platygyra* [14,15]). However, characterizing the genetic architecture in more heat-tolerant species may reveal mechanisms of heat tolerance that may be missed in heat-sensitive species. Taken together, the taxonomic bias makes it difficult to anticipate how reefs comprising diverse coral communities will respond to future warming, which limits the ability of researchers to design reef management strategies accordingly.

Gap 3: low genomic resolution

Most of the studies used reduced representation genotyping techniques (e.g., RNAseq or RADseq) that only genotype a small percentage (i.e., generally <10%) of the genome [63]. Given this coarse genomic resolution, these studies might have missed single loci with outstanding adaptive signals (i.e., remarkably strong association with phenotypic or environmental variation or with exceptional variation in allele frequencies across reefs). It is important to identify single loci with outstanding adaptive signals because they might underpin large effects in thermal tolerance. If identified, large-effect loci could be used as biomarkers in rapid and inexpensive genetic assays to predict the adaptive potentials of coral populations [27] or to breed thermally adapted animals for restoration purposes. While it may be possible to breed thermally tolerant corals if the trait requires the cumulative effect of many loci, identifying one or few loci with large effects on thermal tolerance will simplify breeding efforts [22,29].

Gap 4: lack of validation of adaptive genotypes

The search for heat-adaptive loci in wild populations is notoriously prone to false discoveries [48]. Neutral loci can have heterogeneous distributions across reefs due to demographic processes and genetic drift. These loci can be wrongly identified as contributing to a trait of interest when their frequencies across reefs happen to correlate with phenotypic variation (genotype-by-phenotype analyses), correlate with environmental gradients (genotype-by-environment analyses), or deviate from neutral patterns of allele frequencies (population-genomic scans). False positive loci can confuse downstream GO enrichment analyses with spurious molecular functions. Indeed, candidate adaptive alleles in corals are associated with a kaleidoscope of molecular pathways. Often, only a few of these pathways are thoroughly discussed and are biased toward those that have been previously associated with heat exposure or studied in detail by other researchers (e.g., based

on previous gene expression analyses [64]). The role of the remaining pathways in heat adaptation remains to be determined. Therefore, there is an urgent need to validate the function of alleles predicted to control heat tolerance and identify which candidate alleles are truly adaptive. Once identified, these validated alleles could be used effectively in reef management and conservation.

New technologies to fill gaps in our understanding of coral heat adaptation

Solutions to Gaps 1 and 2: explore the diversity of candidate alleles with seascape genomics

There is an urgent need to discover and describe the genetic basis of coral heat tolerance across larger spatial scales and in diverse coral taxa to support reef management strategies that include adaptation to climate change. Unfortunately, phenotyping (i.e., *in situ* measurement, *ex situ* thermal profiling, and directed breeding) is both time and resource consuming, making it difficult and expensive to apply to multiple coral species across a large reef system. Large-scale experiments using approaches that do not require phenotyping, such as population-genomic scans and seascape genomics, should be more feasible.

Seascape genomic studies are typically applied across large spatial scales and at multiple sites. This is because the sampling strategies in seascape genomics emphasize environmental variation and large geographic range, such that candidate alleles for heat adaptation can be found at reefs sharing similar thermal conditions but located hundreds of kilometers apart [35]. The large sample size of seascape genomics studies can also be used to conduct population-genomic scans, whereas the sampling strategies for population-genomic scans are often inadequate (i.e., low number of sampled reefs and spatial scale is too small) to correlate allele frequencies with environmental gradients. Moreover, large-scale seascape genomic sampling designs can test the association of genotypes against multiple environmental factors in a single experiment (e.g., temperature related [37], nutrient load, and water turbidity [35,40]). Seascape genomics also has the advantage of producing data to characterize neutral genetic variation across large spatial scales. This allows examination of structure and connectivity through migration across the population, which are key to understanding how sea current patterns may favor the spread of adaptive alleles in the wild [65].

Current implementations of seascape genomics have two main challenges. First, genotype-by-environment association studies are limited by the quality and resolution of the environmental data. Often the remote sensing data are too coarse (1–5-km resolution) to capture fine-scale environmental variation within a reef [66]. For example, there can be reef microhabitats where diel temperature variation exposes corals to extreme (e.g., tide pools) or infrequent (e.g., ocean-exposed reef slopes) heat [5,8,67,68]. These types of temperature variation could contribute to genetic adaptation [34], but are not captured with current satellite technologies. One possible solution is to complement remote sensing with *in situ* measurements (e.g., using temperature loggers), but this would be costly and require more labor. Alternatively, in future seascape genomic studies, researchers could record local reef characteristics (e.g., depth and reef habitat type) surrounding each sampled coral. These more fine-scale descriptors could be used to infer local environments that may help explain variation in genotype frequencies and can be easily extracted from the worldwide geomorphic map of the Allen Coral Atlas [69]. In the future, recent advances in photogrammetry may allow for the rapid and affordable modeling of reef 3D structure from underwater videos [70], paving the way for fine-scale genotype-by-environment association analyses that account for topography within a reef [71].

The second challenge of seascape genomics is that genotype-by-environment association studies can only identify correlative relationships between genes and environment, but causal relationships

cannot be easily determined [48]. Therefore, it is often unclear why certain loci are associated with an environmental variable and further investigation is needed to link these loci with particular traits. Comparisons across well-replicated seascape genomics studies, corroboration with other population genomics and phenotyping-based experiments, and validation using new gene-editing approaches can identify true adaptive alleles that can be used in conservation efforts.

Solutions to Gap 3: whole-genome resolution to identify adaptive loci

The diversity of loci potentially underpinning thermal adaptation should be studied across the entire genome. This can be done using lcWGS, because the costs of this genotyping method are now comparable with RADseq and RNAseq [47]. As more loci are genotyped, it should be possible to find more loci with outstanding adaptive signals (e.g., the locus containing the gene encoding Sacsin [21]) and to determine their effects on thermal tolerance [72]. In addition, lcWGS characterizes loci from genomic regions that were unexplored by reduced representation methods, such as noncoding regulatory regions that are overlooked by RNAseq genotyping. Genotyping these unexplored genomic regions might shed light on new molecular mechanisms underpinning thermal adaptation.

A main weakness of lcWGS is that the genotypes of particular colonies at individual sites can be unclear due to low-coverage sequencing. Colony-level genotyping may be necessary for coral populations that exhibit widespread genetic and phenotypic variation within reefs [73]. One solution is to genotype a subset of the individuals using high-coverage whole-genome sequencing and then use the resulting haplotype panels to impute individual genotypes of all the samples [47]. The first application of this approach to corals resulted in a significant correlation between real and imputed genotypes [21].

Solutions to Gap 4: elucidate the function of adaptive alleles using CRISPR/Cas9

Given that methods to uncover candidate heat-adaptive loci are prone to false discoveries, there is a need to validate such loci so they can be used effectively in conservation efforts. CRISPR/Cas9 genome editing has revolutionized genetic manipulation across model and non-model species to study specific genes, pathways, and variant functions [74]. CRISPR/Cas9 allows the introduction of genetic changes at precise genomic locations [74–76], and these modifications can knock out a gene or knock in new sequences [77,78]. CRISPR/Cas9 knockout technology was recently applied to corals [79–81], making it an attractive tool for understanding molecular pathways and validating the role of candidate heat-adaptive loci. In one of these first applications, newly fertilized *A. millepora* zygotes were successfully microinjected with CRISPR/Cas9 reagents, generating mutant larvae for a transcription factor implicated in heat stress (heat shock factor 1; *HSF1*). These mutant larvae showed reduced survival when exposed to heat stress [80]. This study showed that it is possible to functionally characterize genes in coral and that mutations in a single gene could result in a large fitness impact in corals. In a later study, CRISPR/Cas9 was used to determine the role of a duplicated bicarbonate transporter in skeleton formation in coral polyps [81], showing that coral phenotypes can be studied after the larval stage.

Using CRISPR/Cas9, it should be possible to test the requirement of candidate adaptive loci for controlling heat tolerance. This approach can simultaneously edit several targeted loci at once with either a single guide RNA (sgRNA) or by multiplexing multiple sgRNAs [79,80]. Therefore, targeting several adaptive loci in the same experiment may be possible to test their cumulative fitness effects. In addition to knockouts, it may be possible to knock in a target sequence to determine whether a candidate locus is sufficient to change heat tolerance. Although this technology is not currently available in reef-building corals, CRISPR/Cas9-based genomic knock-in has been successful in several cnidarians, including an anemone (*Nematostella vectensis)* [82–84], a hydroid

(*Hydractinia symbiolongicarpus)* [85], and a non-reef-building temperate coral (*Astrangia poculata*) [86]. Therefore, it should be possible to extend CRISPR/Cas9 methods to allow for knock-ins and allelic replacements in reef-building corals [77]. Once developed, researchers can directly insert or change candidate adaptive SNPs to dissect their impact on heat tolerance.

Despite the promises, the field of coral CRISPR/Cas9 is still in its infancy, with only three reported experiments [79–81]. Current methods involve microinjecting CRISPR/Cas9 reagents into 1-cell zygotes immediately after coral spawning. The techniques to prepare and microinject CRISPR/Cas9 reagents are routine in many genetics and developmental biology laboratories and have been used in diverse organisms [87]. However, the seasonality of wild spawning limits the number of researchers able to perform CRISPR/Cas9 experiments. An intriguing solution to this limitation is the emergence of techniques to spawn corals in the laboratory [88,89]. Coupling laboratory spawning methods with CRISPR/Cas9 technology may increase the number of research groups performing functional genetics in corals.

A new framework to find coral heat-adaptive loci for conservation

Combining seascape genomics with CRISPR/Cas9 gene editing is a promising approach to find and characterize adaptive alleles for coral management and conservation. In Figure 3, we summarize a possible framework to combine these methods. First, remote-sensing data are used to reconstruct the environmental history across a reef system of interest [51]. Based on this environmental characterization, multiple reefs maximizing environmental and thermal contrasts are visited for sampling [90,91]. Colony-level descriptors of the sampling location are recorded (e.g., depth or reef habitat type) and corals are sampled from these reefs. Sampled corals are genotyped using lcWGS, such that genetic variants can be discovered across the entire genome [20,21,32]. Next, a genotype-by-environment analysis is performed to uncover candidate alleles associated with heat stress while controlling for fine-scale habitat variation. The list of candidate alleles will then be prioritized for CRISPR/Cas9 validation experiments [79,80]. For example, single or multiple loci could be mutated using CRISPR/Cas9, and the consequence of such mutations on coral thermal tolerance can be assessed in both larvae and adult corals. Given that the function of heat-adaptive alleles may change across genetic backgrounds, CRISPR/Cas9-based validation should be performed with corals of the same population and species as the seascape genomics experiment. Therefore, it is important as a field to expand CRISPR/Cas9 methods to additional species which should be possible with current methods (reviewed in [87]).

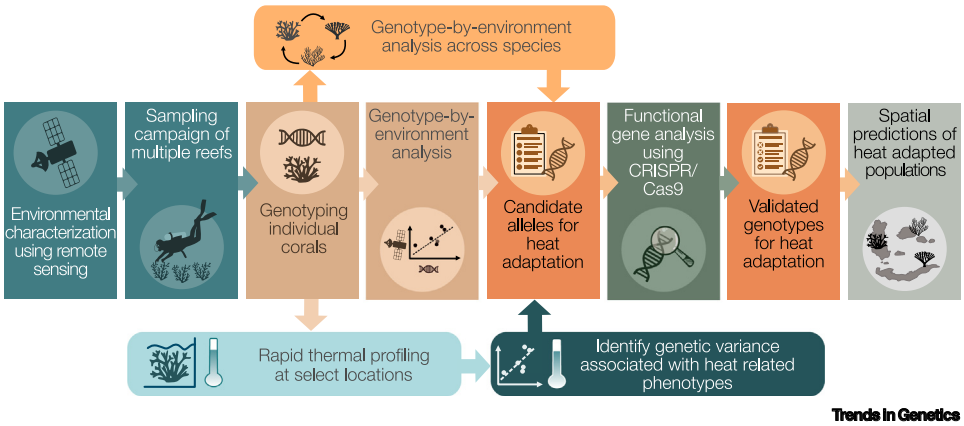

Figure 3. Framework to identify and test genotypes underlying heat adaptation in corals and predict their spatial distribution across the reefs.

The main limitation of the proposed framework is that it will be logistically impractical to systematically validate every candidate locus in every coral species with CRISPR/Cas9. For this reason, we suggest three independent approaches to screen the candidate loci found with seascape genomics. First, population-genomics scans (such as F_{st} or π analyses [20,21,31]) could be run on the same samples used for the seascape genomics analysis. Loci with frequencies that both correlate with thermal gradients and deviate from neutral patterns of genetic variation will be prominent candidates for downstream functional validation [92]. Second, parallel seascape genomics analyses or other complementary genomic scans should be run on the same or different coral species collected from the same sampling locations [35,50]. If the same variants are associated with adaptive signals in multiple experiments or in different species, the underlying loci are likely to be strong candidates for adaptation. Third, thermal profiling experiments could be performed on some of the corals collected for the seascape genomics analysis. To limit the logistic burden, thermal profiling could be performed at key sites immediately after sampling. For example, researchers could use portable tanks to measure changes in corals' photosynthetic efficiency of corals under rapid (18 h) heat stress assays [93]. Voolstra and colleagues showed that changes in photosynthetic efficiency measured with these rapid assays significantly correlated with changes in photosynthetic efficiency in traditional long-term (21 days) heat stress experiments. Alternatively, researchers could use portable incubators equipped with oxygen sensors to track changes in the metabolic rates of corals (e.g., respiration or photosynthesis) across 12 h of controlled heat ramps. This experiment measures the changes in metabolic rates across a range of temperatures (i.e., thermal performance curves) of individual colonies and identifies the temperature at which metabolism reaches the maximum rate (called the 'thermal optimum'). After genotyping, researchers could compare how changes in photosynthetic efficiency or thermal optimum correlate with colony genotype at a candidate adaptive locus. However, measurements of algal physiology (e.g., photosynthetic efficiency) may not be controlled by the host; thus, care should be made in interpreting these types of experiment to avoid false positive interactions. Candidate loci from seascape genomics that are also associated with a change in heat-related phenotypes can be considered strong candidates for heat adaptation [17]. Importantly, these complementary thermal-profiling experiments can also define the phenotypic effects (e.g., the increase in thermal threshold) of adaptive genotypes and can be designed to detect any potential trade-offs (e.g., reduced growth rates) associated with increasing tolerance to heat.

By intersecting the results across genomics and phenotyping experiments, it should be possible to prioritize key variants, genes, and pathways to functionally test with CRISPR/Cas9. For example, researchers could systematically knock out genes in a genomic region with an outstanding adaptive signal to determine which gene(s) impact heat tolerance. After the causal gene(s) have been identified, individual variants (e.g., SNPs) can be mutated to determine their impact on the function of the causal gene. However, the latter may be more time consuming due to the number of variants associated with each causal gene and the varying effect sizes of each. As more genome-wide analyses are performed in the search for adaptive alleles, researchers should conduct meta-analyses across experiments to identify alleles that are recurrently associated with adaptive signals. This approach might discover conserved adaptive genetic responses across coral species. If conserved adaptive alleles exist, they may be used as biomarkers to predict the future adaptive potential at a community level for individual reefs [94–99]. Finally, spatial predictions of the distribution of thermally adapted corals could be intersected with models of reef connectivity to predict the future spread of adaptive alleles across reefs [40,100]. This approach may identify reefs that act as a source of adaptive alleles for the neighboring reefs of the region [22,39,40,100,101], and marine protected areas could be established at such sources to protect them from local disturbances [102].

Concluding remarks

As climate change continues to impact coral reefs worldwide, improving our understanding of the genetic basis of coral heat adaptation is urgent (see Outstanding questions). Seascape genomics can be a foundational approach to identify candidate adaptive loci in wild populations because the search for adaptive alleles can cover larger spatial scales with multiple coral species and use whole-genome sequencing. The additional application of CRISPR/Cas9 gene editing can be used to validate the adaptive value of candidate alleles and elucidate the genetic basis and the molecular pathways underpinning these adaptive processes. Once validated adaptive genotypes are identified, the alleles can be used in reef management and conservation efforts.

Outstanding questions

How does the adaptive genetic diversity of corals distribute across large scales (e.g., entire reef systems or across latitudinal gradients)?

What is the genetic architecture of heat adaptation in corals (e.g., monogenic or polygenic)?

Are there molecular pathways that are preferential targets for heat adaptation across coral populations/species from different regions (e.g., protein-folding machinery, oxidative stress response, or immune system)?

How does the occurrence of adaptive genotypes in response to historical heat stress differ between taxa (e.g., species with branching vs. massive growth forms or species with a broadcast spawning vs. brooding reproductive strategies)?

Can knowledge of coral adaptive genetics lead to cost-effective strategies for reef conservation (e.g., protecting refuges of thermally adapted corals or assisted breeding of adapted corals for restoration)?

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Declaration of interests

The authors declare that they have no competing interests.

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