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The role of intraspecific variation in the ecological and evolutionary success of diatoms in changing environments

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Intraspecific variation in diatoms has been shown to play a key role in species' responses to several important environmental factors such as light, salinity, temperature and nutrients. Furthermore, modelling efforts indicate that this variation within species extends bloom periods, and likely provides sufficient variability in competitive interactions between species under hydrographically variable conditions. The intraspecific variation most likely corresponds to optimal fitness in temporary microhabitats and may help to explain the paradox of the plankton. Here, we examine the implications of intraspecific variation for the ecology and success of diatoms in general and emphasize the potential implications for our understanding of carbon metabolism in these important organisms. Additionally, data from palaeoecological studies have the potential for evaluating genetic variation through past climate changes, going thousands of years back in time. We suggest pathways for future research including the adoption of multiple strains of individual species into studies of diatom carbon metabolism, to refine our understanding of the variation within and between species, and the inclusion of experimental evolution as a tool for understanding potential evolutionary responses of diatom carbon metabolism to climate change.

This article is part of the themed issue 'The peculiar carbon metabolism in diatoms'.

1. Background

Diatoms arose approximately 200 Ma and have since then undergone extensive adaptive radiation. They have colonized freshwater, marine and terrestrial habitats with different shapes, sizes and specialized physiological characteristics, and are responsible for 40% of marine primary production [1,2]. Diatoms are particularly useful in evaluating the effects of climate change due to their high abundances and importance in global biogeochemical cycling, and because they leave a rich archive of microfossils in the sediment. Presently, the rewarding and exciting combination of palaeoecological studies of fossil data in combination with modern-day ecological theories is leading us towards a greater understanding of evolutionary processes and climate change effects on marine ecosystems in the past, present and future [3].

It is estimated that as many as 100 000 diatom species exists globally [4]. Recent findings based on DNA barcoding sequencing and light microscopy data provided from the Tara Oceans global circumnavigation indicate that very few diatom sequences (operational taxonomic units) are cosmopolitan, but that there is a worldwide distribution of several highly abundant diatom genera which have diversified locally to their specific environmental conditions [5]. This property of the diatoms, to exploit and prosper in a wide range of habitats and ecological niches, may be the explanation for the large differentiation observed at the species and intraspecific taxonomic levels, and also the cause of their high success in carbon metabolism and resulting importance for global biogeochemical cycling.

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The study of intraspecific variation requires a definition of species boundaries, which preferably is pluralistic. Ideally, for correct species identification, and estimation of intraspecific diversity, several individuals at all stages of the life cycle should be examined [6]. In diatoms, the valve, girdle, protoplast, reproduction mode, genome size, DNA sequences of primarily the rcbL and the rRNA gene (small subunit, large subunit, internal transcribed spacer), and the secondary structure of the internal transcribed spacer region have been used (e.g. [7–12]) to delimitate species boundaries. In each of the publications on diatom intraspecific variation cited in this literature review, the approaches and techniques applied for correct identification vary largely depending on the species studied. However, the respective scientists have all used a pluralistic mode to confirm that a representative set of clones is indeed the same species and that the generated data are intraspecific.

We posit that the high level of diatom intraspecific variation is of great importance as it allows individual species to survive in a highly variable environment. Here, we describe the genetic and phenotypic diversity that has been observed within species and what the implications of that diversity are for diatom success and carbon metabolism under climate change conditions. So far, the richness of diatom subspecies genetic diversity has been estimated mostly by using neutral molecular markers, although some exceptions exist. The diversity appears to be structured, among populations and among individual clones, and clearly indicates that populations and clones within a species are not all equally related. Moreover, this rich intraspecific genetic diversity allows for the possibility of local adaptation and for differentiation in important physiological characteristics that produces local populations that are exceptionally fit and competitive in their respective local habitat.

2. Genetic diversity

(a) Variation among individuals

Variation among individuals from within a single species influences species' evolution and ecology as well as the functioning of ecosystems. From an evolutionary perspective, the extent of genetic variation among individuals can set a species' adaptive potential and influence its persistence over time [13]. For these reasons, the amount of genetic variability within species has been a focus of population genetics and evolutionary biology for nearly 100 years [14–17]. From an ecological perspective, recent work in both terrestrial and marine systems has shown that intraspecific genetic diversity can modulate interspecific interactions and thus influence levels of primary productivity [18] and functional responses to changing environments [19].

All recent studies of intraspecific genetic variation in aquatic phytoplankton, including diatoms, have identified high levels of diversity. Using sensitive DNA fingerprinting techniques, these studies identified high levels of diversity regardless of species investigated or habitat sampled (table 1 and figure 1). Results obtained using DNA fingerprinting are supported by the whole-genome sequences of *Thalassiosira pseudonana*, *Phaeodactylum tricornutum* and *Thalassiosira oceanica* which all displayed extensive sequence variation between homologous chromosomes, suggesting high levels of diversity in field populations [37–39]. Together, these studies suggest that high diversity is a hallmark of diatoms that likely has influenced their ecological success and evolutionary longevity. The diversity observed in diatoms has a practical impact on diatom studies. It indicates that assessing the physiology or genetics of one individual is not sufficient to describe the capabilities of the entire species.

Genetic diversity in diatoms can be described in two different ways. The first is clonal diversity, or the number of unique clonal lineages in a sample [40]. Clonal diversity in a population is an important measure of standing genetic variation because it is readily available to lineage sorting [41] and partially determines the potential magnitude and rate of response to selection. Because diatoms divide predominantly asexually, there is the potential for populations to be comprised of a single or very few clonal lineages derived from vegetative growth, leading to a clonal diversity close to zero. In natural populations of diatoms, clonal diversities ranging from 92 to 100% have been observed revealing instead that populations are comprised of many clonal lineages (table 1 and figure 1). Clonal diversity in diatoms has been measured with high sensitivity due to development and application of DNA fingerprinting markers called microsatellites [42]. Microsatellites are neutral markers that are scattered throughout the genome and consist of di-, tri- or tetranucleotides that are tandemly repeated tens to hundreds of times. The number of repeat units at a given microsatellite locus can vary dramatically between individuals. This variation in length is hypothesized to result from a process known as strand slippage, which occurs when the DNA-synthesizing machinery essentially 'slips' during replication of the repetitive regions [43]. Thus, the length of a repeat array (allele size) can act as a part of a DNA fingerprint. As more loci are analysed, the DNA fingerprint for an individual becomes increasingly precise. For example, a three marker panel in the diatom *Ditylum brightwellii* had sufficient variability to potentially identify in the order of 6×10^6 different genotypes [22]. For most diatom studies, sample sizes are usually 30 individuals per population, making it impossible to compare census size with clonal population size. One example exists where a spring bloom of the diatom *D. brightwellii* was sampled repeatedly and over 600 individuals were genotyped. Census sizes reached greater than 10 000 cells l^{-1} and it was estimated that the blooming population was comprised of at least 2400 different genotypes [22]. High levels of clonal diversity suggest that these rapidly dividing organisms may be able to respond quickly to a changing environment through selection acting on standing genetic variation.

A second metric of genetic variation in diatoms is gene diversity or expected heterozygosity. This is the probability that, at a single locus, any two alleles chosen at random from the population will be different from each other. The average gene diversity over many loci is used as an estimate of the genetic variation in the population. Gene diversities of diatoms examined thus far range from 39 to 88% (table 1). This level of standing diversity in diatom populations suggests sufficient variation for diatoms to evolve in response to their environment.

Given the variation observed in neutrally evolving microsatellite markers and the extent of genome-wide nucleotide polymorphism measured, it is likely that variation exists among individuals in terms of genes involved in carbon metabolism in diatoms. If variation exists among those genes, then they also may be responsive to selection. Comparative genome sequencing of seven *T. pseudonana* strains revealed variation across the genome, including 7% of the genome that was identified as being under positive selection [44]. Among

Table 1. Gene and clonal diversities of different diatom species collected from predominantly coastal regions around the world.

	habitat	gene diversity (H_E)	clonal diversity	references
Ditylum brightwellii	NE Pacific, coastal, fjord	0.88	0.96	[20]
Ditylum brightwellii	NE Pacific, coastal, fjord	0.79	0.99	[21]
Ditylum brightwellii	NE Pacific, coastal, fjord	0.70	0.87	[22]
Ditylum brightwellii	NE Pacific, coastal, fjord	0.71	0.94	[23]
Pseudo-nitzschia pungens	North Sea, coastal	0.73	0.98	[24]
Pseudo-nitzschia pungens	North Sea, coastal	0.69	0.95	[25]
Pseudo-nitzschia pungens	global, coastal	0.53–0.83	n.a.	[26]
Pseudo-nitzschia multiseries	global, coastal	0.39–0.70	0.92	[27]
Pseudo-nitzschia multistriata	Mediterranean Sea, coastal	0.46–0.61	0.75	[28]
Skeletonema marinoi	North Sea, fjord	0.56–0.71	0.99	[29]
Skeletonema marinoi	North Sea, fjord	n.a.	1.00	[30]
Skeletonema marinoi	Baltic Sea, Inland Sea	0.52–0.74	0.99	[31]
Skeletonema marinoi	Baltic Sea, North Sea	0.62–0.77	1.00	[32]
Skeletonema marinoi	Baltic Sea, North Sea	0.68–0.74	0.99	[33]
Sellaphora capitata	Freshwater lakes	n.a.	0.89	[34]
Thalassiosira gravida	N. Atlantic, open ocean	0.88	0.97	[35]
Thalassiosira rotula	global, coastal	0.55–0.83	0.99	[36]

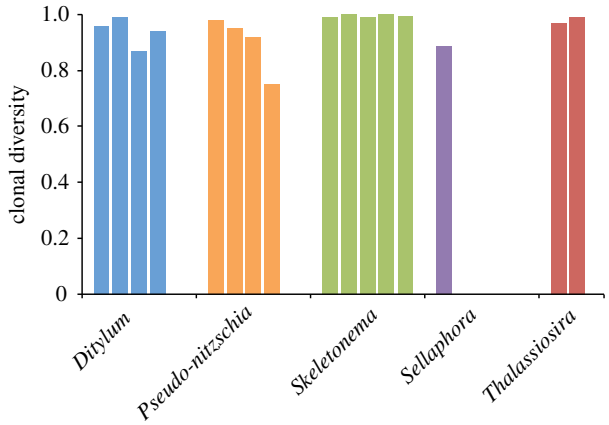

Figure 1. Measures of clonal diversity in five diatom genera. Each bar corresponds to a study that has reported clonal diversity using microsatellite markers (table 1). (Online version in colour.)

the genes and gene ontology (GO) terms that could be identified were those involved in sexual reproduction, cell wall formation, transcriptional regulation as well as a large portion of unknown or hypothetical proteins (59% of the positively selected genes). GO terms that were over-represented in the positively selected portion of the genome included biosynthetic and metabolic regulatory proteins. This finding opens the possibility that genes involved in carbon metabolism are under positive selection and that genetically different individuals may have metabolic capabilities that respond differently to environmental conditions.

(b) Variation among populations

Responses to natural selection at the individual level are coupled to evolutionary time scales by populations or groups of interbreeding individuals. Populations persist longer than individuals and thus act as repositories of genetic adaptation and variation for a species [45]. The adaptive potential of a species depends on how it is partitioned into distinct populations and in turn, relies heavily on the extent of diversity contained within those populations [46]. Distinct populations, the diversity they contain and the geographical ranges they occupy have the potential to exert a significant impact on diatom ecology and evolution.

Surprisingly, marine diatoms are often subdivided into genetically distinct populations despite their planktonic lifestyle of drifting passively with tides and currents, which should confer an enormous potential for long-range and persistent dispersal along with high levels of gene flow between populations. Genetic divergence has been related to increasing geographical distance, as in the diatom *Pseudo-nitzschia pungens* [26]. There are, however, examples of genetic divergence among populations in close proximity to each other. For example, within a single fjord ecosystem, four genetically distinct populations of the centric diatom *D. brightwellii* were identified that possessed unique physiological characteristics and formed blooms under distinct environmental conditions [21,23]. Similar genetic subdivision has been observed in the Skagerrak (NE Atlantic) in the diatom *Skeletonema marinoi* [29,30].

The extent of genetic differentiation between populations is particularly intriguing. In most pelagic marine organisms, F_{ST}, a measure of genetic divergence that ranges from 0 to 1, is rarely larger than approximately 0.01. For example, genetically distinct populations of Atlantic cod sampled along a 300 km stretch of coastline displayed F_{ST} values of only 0.0023 [47]. F_{ST} for *D. brightwellii* populations from two adjacent fjords in the northeast Pacific was as high as 0.245, indicating that these populations were unusually diverged. Similarly, *S. marinoi* populations from across a North Sea–Baltic Sea salinity gradient had F_{ST} values as high as 0.2 [48]. In this region, populations appear to maintain integrity via a combination of physical barriers and differential adaptation to environmental conditions [31,49]. The gene

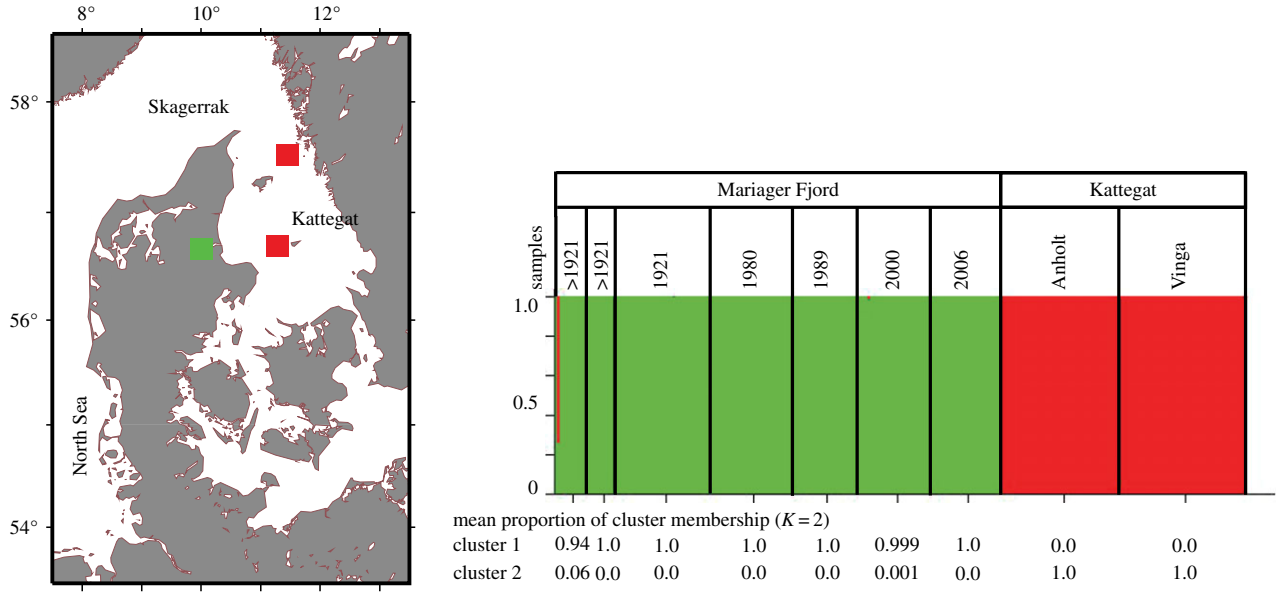

Figure 2. Map of Denmark with Mariager Fjord (green square) and Kattegat sites Anholt and Vinga (red squares) indicated. Population structure of *S. marinoi* based on eight microsatellite loci. The samples from the Mariager Fjord are groups of strains established from resting stages germinated from discrete layers of a sediment core. The approximate year when resting stages accumulated are as indicated. Samples Anholt and Vinga are groups of strains established from recent sediment collected in the Kattegat. Genetic clustering was estimated by STRUCTURE. Assignment of 245 individuals to $K = 2$ genetically distinguishable groups. Each individual is represented by a vertical bar coloured according to the assigned group (red or green), and the average proportions of membership of each sample to the two clusters are shown below. Figure redrawn from Härnström *et al.* [30].

flow that does exist among populations appears to be driven by dispersal via water currents [33].

Importantly, distinct populations can persist over long time periods. One *D. brightwellii* population was sampled within Puget Sound repeatedly during both bloom and non-bloom periods over the course of 7 years [12]. In the Gulf of Naples, the Mediterranean, two sympatric populations of *Pseudo-nitzschia multistriata* have been recorded over 4 years. The two populations produce viable hybrids that imply that gene flow occurs [28]. Each year during the growth season, there is a clear dominance of one of the populations which could be due either to environmental factors favouring one population over the other or intrinsic factors coupled to the obligate sexual life cycle of *P. multistriata* [50]. The most extreme example of population persistence to date is from a fjord ecosystem where sediment samples revealed the persistence of a single population of the diatom *S. marinoi* for over 100 years [30]. Interestingly, samples collected from the Kattegat outside the fjord revealed a second genetically distinct population, indicating that populations may be highly adapted to local conditions (figure 2). In the open ocean, populations appear to coexist, at least for short periods of time, and may result from the mixture of source populations from coasts, open ocean and resting spores from depth [35].

High divergence among persistent populations means low levels of gene flow between them and the opportunity for selection to act in different ways among unique populations [45]. Populations may thus evolve unique adaptations within their carbon metabolism that accommodate the specific environments which they experience.

3. Phenotypic diversity

Phenotypic variability is important and is commonly observed among phytoplankton species, including inter- and intraspecific responses to external changes. The phenotypic variability, i.e. plasticity, within a species, a genetic population or a clone will serve to buffer the immediate effects of swift environmental changes. Phenotypic diversity will moreover reflect the standing genetic variation for a species or a population in a particular area, and constitute the variability which selection can act upon and thereby influence species' and populations' long-term adaptation to a changing environment [13].

For the further discussion of phenotypic variability and the investigation of the operational scales, i.e. species, population and clonal variability in a changing environment, the concepts of population versus clonal phenotypic characteristics are delineated according to the following: (i) the clonal niche is substantially narrower than its population's niche and reflects preference or performance variation due to intrinsic traits, (ii) populations are composed of relatively generalized and relatively specialized clones, and (iii) within-clone variation may be greater than between-clone variation. Therefore, clonal niche realization will take fundamental and realized niche into account [51,52]. Using these criteria, we ask if there is substantial evidence for temporal, spatial and cross-habitat phenotypic differentiation in genetically diverse diatom populations.

(a) High levels of phenotypic variation among genetically diverse individuals

Phenotypic variability from closely related diatom species might give us insight into the possible ranges of intraspecific phenotypic variation. Clonal isolates of *Skeletonema* spp. from one single geographical location, but from different seasons (summer and winter), were found to have unique ratios of the photosynthetic pigments chlorophyll *a*, chlorophyll *c* and fucoxanthin [53,54]. When the temporally separated clones were transferred from high to low light, they adjusted

their respective pigment ratio uniquely and displayed distinct individual patterns of photoadaptation. Additionally, the clones displayed distinct photosynthetic unit (PSU) sizes, and the photosynthesis–irradiance (P–I) curves indicated differences in the functional organization of the PSU and the energy transfer from the light harvesting complex to the reaction centres [54].

Available literature data on diatom intraspecific variability of phenotypic characteristics are based on external manipulation and the responses from a limited set of clones kept in laboratory cultures. High-throughput phenotypic screening intended for massive replicated clonal phenomics is currently lacking. However, the existing records highlight unambiguously that whenever a multi-clonal approach is tested, individual clones will produce phenotypically variable responses.

Fundamental abiotic factors for diatom propagation, such as ranges of temperatures, have been demonstrated to yield unique phenotypic response curves in genetically distinct strains of *Asterionella formosa* [55]. Likewise, *D. brightwellii* clones from each of two populations displayed a variable response estimated as growth rates when exposed to different light intensities [21]. Different *S. marinoi* clones isolated from the same geographical location handled variation in salinity differently and displayed diverse responses in terms of growth rates, carbon uptake, particular organic nutrients and biogenic silica content. The responses of the individual clonal lineages ranged from unaffected to significant decline when exposed to suboptimal salinities [32].

Hence, at a single geographical site, the documented presence of intrapopulation genetic diversity is mimicked in phenotypic diversity for important functional traits, which probably accounts for the diatom species or population persistence at a particular site. Adapted clones will succeed each other and maintain the presence of the population (see §4), both throughout the short-term hydrographic dynamics and throughout seasonal changes.

(b) Population-level phenotypic adaptation to distinct environments

Intraspecific phenotypic comparisons of populations isolated from distinct habitats do support that populations at a particular site will adapt to their respective environmental conditions. Genetically differentiated populations of *S. marinoi* originating from the neritic habitat but from different latitudes displayed distinct population phenotypic characteristics [56]. The effect of temperature (Δ4°C) and pCO_2 (ambient versus 750 ppm) changes was tested on two populations, from the coastal Adriatic Sea (Mediterranean) and Skagerrak (NE Atlantic). Estimated growth rates at control conditions (20°C and ambient pCO_2) differed by a factor of 3 among eight Adriatic Sea clones, while the span was only half that among the clones isolated from higher latitudes. The large individual variation between strains cancelled out population experimental effects in the Adriatic Sea population, whereas the population from the Skagerrak had a homogeneous between-clone response to the manipulated conditions. The Skagerrak population is exposed to a wider seasonal temperature range with more extreme endpoints, and it is argued that high phenotypic plasticity and temperature tolerance on the clonal level is an advantage. On the other hand, in a seasonally more homogeneous location, the clonal isolates do not posses this inherit mechanism of plasticity as it will come at a fitness cost [56]. Studies from sites separated on much smaller spatial scales without apparent large hydrographic differences also support significant differentiated population-level phenotypic characteristics, which indicates that diatom populations phenotypically adapt to their local habitat. For example, genetically distinct *D. brightwellii* clones from two estuaries separated by less than 40 km displayed different population-wide phenotypic response to low and high light intensity [21].

(c) Drivers to maintain intrapopulation phenotypic diversity

Multi-variate experimental stress tests have shown that no single diatom strain displays the fastest growth under all stressful conditions. Laboratory populations of *S. marinoi* mixed to display different levels of genetic diversity (monoclonal, populations composed of five or 20 clones) were tested for salinity stress using primary production as the response variable. It was found that the response during the stress condition (3 PSU) deviated the least from the native conditions (5 PSU) in the high diversity population [32]. Another four clonal isolates of the same diatom species displayed distinct phenotypic responses in terms of growth rate and maximum biomass accumulation under salinity and temperature stress. Under control conditions (20°C and 26 PSU), a positive biodiversity effect was recorded; however, this was not the case in the stress conditions (27°C and 7 PSU, respectively) [57]. Hence, these manipulated experiments show that clones possess particular phenotypic characteristics and that they may dominate under a specific set of conditions, but as environmental conditions change, the clonal dominance will change. The suite of differentiated phenotypic properties intrinsic to the constituent clones would likely be of benefit for the population. The response of a diatom population to stress and how effectively it can handle short-term disturbances may in fact depend on the level of genetic and phenotypic diversity. A constant but varying external selection pressure in a highly dynamic environment would serve to maintain phenotypic diversity within the population.

So far, only abiotic factors have been discussed as drivers of diatom species or population phenotypic variability. However, studies of *A. formosa* indicate that viruses, bacteria or parasites could constitute drivers of clonal specialization. For instance, certain clones can survive infections by one particular virus but not by another and are metabolically active only when the former virus type is present but not the latter [58]. Further, *Chaetoceros debilis* infected by CdebDNAV virus revealed that the diatom population was composed of highly diverse host clones that differed widely in the virus sensitivity spectra [59]. Viruses, bacteria or parasites, e.g. the diatom microbiome, are probably important, and sometimes perhaps responsible, for the phenotypic characteristics observed. Currently, it is easier to document hydrographic spatial and temporal dynamics than micrometre-scale cellular and molecular interactions. Therefore, abiotic signals correlating with specific population phenotypic characteristics might be indirect drivers only. The entangling of causative abiotic and biotic factors as external drivers of phenotypic response will most certainly benefit from future community metagenomic analyses. In particular, if it can be combined with

methods that can monitor physical and chemical properties of the diatom microhabitat. The aim here has been to highlight the existence of intrapopulation phenotypic variability without explicit investigation of the causative drivers.

It is also plausible that life cycle traits of diatom species could influence the phenotypic variability of a particular population. Many diatom taxa form physiological dormant resting spores as a response to adverse conditions in the water column and sink to the bottom [60]. There they can remain alive for decades [61] and if resuspended under favourable conditions, they may germinate and contribute to the planktonic population. Clones with a phenotype not suitable under certain environmental conditions may stay dormant during non-favourable periods and subsequently provide an inoculum when the environment is suitable for that particular phenotype. Spore-forming diatom populations may therefore display higher genetic and phenotypic diversity and even different population structure in the same study area compared with non-spore-forming diatoms. Another perspective that is poorly understood, and not discussed here is the longevity of a clone and its phenotypic characteristics *in vivo*. Cell lines of a single clone will invariably accumulate mutations over time, and subsequently, they will differentiate to the extent that they display dissimilar phenotypic characteristics that will mediate different fitness and selection pressure in a changing environment.

4. Succession of species, populations and clones along continuous gradients

Diatom species succession over temporal and spatial environmental gradients is well documented across aquatic habitats [62]. Given the genetic and phenotypic diversity observed among populations and clones, do similar successional patterns exist on subspecies level (figure 3*a–c*)? Sampling methodology and the current poor resolution of high-throughput molecular markers applicable *in vivo* (as discussed above) are obstacles to correctly grasp the dynamic processes and test the hypothesis of, for example, clonal succession along a particular gradient. The literature available is based on data gathered from phenotypic laboratory experiments, genetic data from field isolates, statistical or mathematical modelling and correlation-based analyses, but it does indicate that succession on subspecies levels indeed takes place. Genetically differentiated populations manifest fitness maxima along environmental gradients (figure 3*b*) as indicated by two differentiated populations of *D. brightwellii*, seasonally separated in neighbouring estuaries. The population genotypes were correlated to *in vivo* concentrations of inorganic nutrients, temperature and solar irradiance, which suggests that environmental selection regulates the bloom dynamics of the genetically and temporally separated populations [23]. Adaptation along a salinity gradient ranging from 5 to 30 PSU provides more evidence that populations will succeed each other along environmental gradients. Salinity reaction norms were determined for clones of genetically differentiated populations of *S. marinoi* originating from different salinity regimes of the Baltic Sea (NE Atlantic), and each population displayed maximum growth rate at their local salinity [48]. As a consequence, genetic distance among the populations was significantly correlated with the Baltic Sea south-to-north salinity gradient [31]. However,

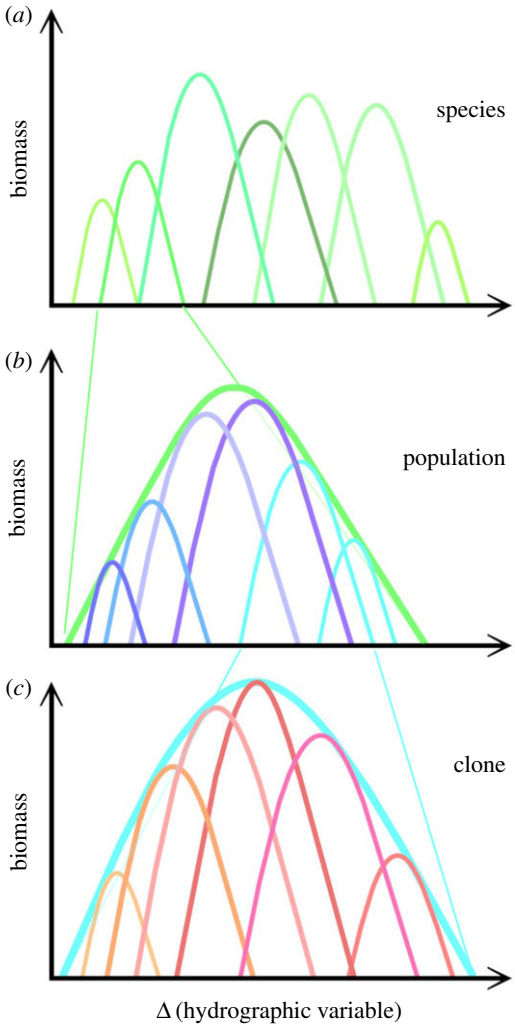

Figure 3. Hypothetical succession of (*a*) species, (*b*) populations and (*c*) clones along a hydrographic gradient. Diatom species succeed each other along environmental gradients, e.g. [62], because they are adapted to particular environmental conditions. A diatom species will consist of several populations, where each of the populations has its optimal fitness at specific hydrographic settings. The populations will succeed each other as the environment changes. Each population consists of several unique clones, which occupy a niche substantially more narrow than its population's niche. As the environment changes, successive clones will dominate the water column according to their performance variation at specific environmental conditions.

the succession of genetically structured populations along the salinity gradient (figure 3*b*) displayed an additional temporal succession pattern at any particular geographical location (figure 3*c*). In all populations, clones isolated during the initial phase of a diatom spring bloom were significantly separated from clones isolated during the later phase of the bloom. The initial and depleted bloom conditions, in this case silica concentration, selected for different clones during the bloom progression. This suggests that genetic differentiation may emerge over short temporal scales and that a bloom consist of clones with different growth optima. As the bloom progresses, the hydrographic conditions change and induce a shift to clones with optimized physiological adaptation to the new set of conditions. Such shifts are supported by the large genetic standing stock and the presence of phenotypic diversity observed on the subspecies level. A specific diatom bloom might consist of several short-lived groups of clones. Each

such group is adapted to a particular hydrographic condition, and will occupy that distinct temporal-hydrological niche and thus maintain the bloom over longer periods for the population. Succession of clones along a temperature gradient (figure 3*c*) has been demonstrated using experimental data on temperature-dependent reaction norms for clones of *A. formosa* isolated from a lake. The experimental data were modelled together with temperature monitoring data over 5 years. The model resulted in a succession pattern of two to several genotypes becoming numerically dominant depending on the temperature, and the fitness ranking order of the clones changed along the temperature gradient [55].

5. Adaptation to new environments: climate change

(a) Historical responses to changes in climate: the palaeo perspective

Potentially, we can increase our understanding of how diatoms will respond to future climate changes, and if diversity is important for their persistence, by analysing analogue scenarios from the past. The high temporal resolution of diatom microfossil records accumulated in aquatic sediments over long periods makes it possible to explore how species have responded to abrupt climate changes. Of interest are records when a species has persisted over several geological events of a specific hydrological characteristic, e.g. warming, which will indicate the species ability to adapt to the new environment. The ongoing climate warming is expected to enhance vertical thermal stratification in the oceans, which will reduce mixing and therefore the supply of nutrients into the upper mixed layer [63]. As diatoms rely on inputs of nutrients to succeed and outcompete other autotrophic plankton, such as coccolithophores and flagellates, reduced mixing is expected to limit their competitive abilities [64].

Microfossils accumulated in the sediment during the Holocene warming period and contrasted to periods of Heinrich events, which are millennial scale climate transitions linked to massive discharge of icebergs from the North Hemisphere into the North Atlantic with global effects, provide successive events of value for inferring diatom species adaptation to warming. Sediment cores from the Atlantic present two drops in sea surface temperatures (SST) linked to Heinrich events (16 000 and 13 000 BP), and an increase in SST during the Holocene warming period (10 000 BP). During the two cool periods, the diatom abundance and species richness increased, whereas the abundance and richness decreased during the Holocene [3,65]. The probability of sampling a given species during the climatic perturbations increased with its population density, and hence, the dominant species were more likely to live through the perturbation, whereas the rare taxa were subject to local extinction [3]. The documented hydrographic changes caused by Heinrich events in the eastern Pacific were indicative of enhanced subtropical influences and an oligotrophic upper mixed layer. Sediment cores displayed a drop in diatom abundance and species richness during three separate Heinrich events (84 000, 46 000, 16 000 BP). The abundance of *Thalassionema nitzschioides*, *Thalassiosira oestrupi* and *Cyclotella littoralis* increased in proportional abundance during all three nutrient-depleted periods and were identified as resistant to local extinction across the environmental change events [3,66].

The effects of Holocene warming and SST rise, and contrasting SST drop manifested as an increase in ice cover have been used to explore the response of the diatom community in Antarctica. From this study, diatom species evolvability was assessed by using the changes of proportional abundances of two local diatom species with similar ecology, *Thalassiosira antarctica* and *Porosia glacialis* [67]. The two taxa are important primary producers in Antarctic coastal waters, and ecological information from field surveys and laboratory experiments was used to formulate the hypothesis on interspecific adaptation to high and low SST. Subsequently, modern data were used to consolidate the observed relationship between the two taxa in the sediment cores together with other proxies which indicate SST changes [68]. The species relative abundance ratios were used to evaluate the two species successive fitness at their adapted niches over sequential periods of cooling and warming. A higher abundance ratio of *P. glacialis* accompanied changes to lower SST, whereas a lower ratio with higher proportional abundance of *T. antarctica* was indicative of warming periods.

Diatom species evolvability throughout warming and cooling periods has also been assessed by using morphological characteristics of the diatom frustule. Southern ocean high-nutrient–low-chlorophyll areas have low and fairly constant primary production in spite of high-nutrient concentrations because growth is limited by iron deficiency [69]. During glacial periods, land desertification and wind strength results in more atmospheric dust deposition into the open ocean, which enhances diatom net primary production. *Fragilariopsis kergulensis* is a common diatom species in the area, and the cells' surface-to-volume area has been recorded throughout sediment cores [70]. During the cool glacial period, the frustule attained maximum sizes, whereas during intermittent warm periods, with low iron concentrations, the cell size was smaller and surface-to-volume ratio increased. The larger ratio decreases the cell boundary layer, which will facilitate nutrient uptake [70]. Adaptive changes over the millennia, or altering the dominance of populations with small and large surface-to-volume ratios, will thus increase the competitive advantage of *F. kergulensis*.

The sediment record from the past is valuable, but the interpretation of a diatom species' ability to adapt to new environmental conditions, such as changes in SST, can be difficult. For instance, the interpretation of higher species flux to the sediment data can be due to longer duration of the growth season, which may not be perceived from the sediment record. Or it may, as inferred in the above studies, be because of improved conditions, such as deepening of the mixed layer that favours higher vegetative diatom production [67]. Another shortcoming with the diatom microfossil record is that routinely only light and scanning electron microscopes are used for species identification. Hence, the diatoms from the past are morphologically defined species with no way to discern genetic populations or clones. Therefore, we cannot know if a recurrent species in the sedimentary record is the result of temporal succession of several populations, or if it is one population that has adapted to the new conditions.

(b) Future responses to changes in climate: predicting evolutionary responses

Natural selection acts to shift the average phenotype of a population over many generations in response to a changed environment. In diatoms, the response to selection can occur in two different ways: de novo mutation and lineage sorting.

Classic experimental evolution approaches in microbes rely on de novo mutation arising from single lineages [71]. The experimental evolution approach has been applied infrequently in diatoms and the focus has been on the response to elevated CO_2. In the diatom *T. pseudonana*, long-term exposure to elevated CO_2 led to no significant changes in fitness [72]. Reduced transcription of one putative carbonic anhydrase was observed, although the transcription of three others and the small subunit of Rubisco did not change. Similarly, no significant changes in fitness were observed in the freshwater diatoms *Navicula pelliculosa* and *Nitzschia palea* after long-term exposure to high CO_2 [73]. These studies, which focused on de novo mutation in single cell lines, suggest that the physiology of carbon utilization may be conserved in a high CO_2 world, both in freshwater and marine diatoms.

In the field, lineage sorting [41] may be a more likely population-level response to elevated CO_2 than de novo mutation, given the phenotypic variation that has been observed among clonal lineages. One approach to examining the effects of lineage sorting is to conduct community-wide or mesocosm experiments. These experiments use all existing genetic variation in a population by subjecting the entire community (including competitors and predators) to a shift in CO_2 concentration. Following the experimental period, individual isolates are removed from the community and fitness effects are measured. This approach was used to show that growth rates of *S. marinoi* cells isolated from high CO_2 mesocosms were about 1.3 times faster than cells isolated from ambient CO_2 mesocosms [74]. The maximum number of mitotic divisions in the mesocosm experiment was about 100, making it unlikely that de novo mutations were responsible for the observed evolutionary changes and instead that lineage sorting took place in the population.

Although the underlying shifts in carbon metabolism in the mesocosm experiment are unknown, short-term experiments at high CO_2 exposure may provide some insight. When exposed to high CO_2, the diatom *T. pseudonana* reduced transcription of photosynthesis and respiration genes [75]. The downregulation included carbon concentrating mechanism and photorespiration genes that shared a cAMP-responsive *cis*-regulatory sequence, suggesting that these genes were co-regulated in response to CO_2 concentrations. By contrast, there was an upregulation of cAMP metabolism genes, which could potentially lead to altered signalling in cAMP-responsive pathways [75]. Interestingly, there is evidence that diatoms sense changes in external CO_2 concentrations, in part, through cAMP signalling [76,77]. The shifts in CO_2 sensing, signalling and metabolism observed by Hennon *et al*. [75] in response to short-term shifts in CO_2 concentrations combined with observations of lineage sorting in mesocosm experiments [74] suggest that standing variation in natural populations of diatoms exists in genes and gene clusters related to carbon metabolism. It also suggests that in natural populations, certain aspects of carbon metabolism may be responsive to the selection pressures imposed by climate change.

6. Conclusion

The literature on genetic and phenotypic variation produced during the last two decades clearly reveals that diatoms possess vast intraspecific diversity, and that phenotypic traits of species cannot be captured by studying the response of one clone only. We, therefore, stress the importance of including multiple clones in future experimental studies of diatom physiology including carbon metabolism. Inclusion of multiple clones will refine and extend our understanding of the diversity and variation within and between species.

Currently, the genomes of five diatom species have been published [37–39,78,79], and several others are expected to be released to public databases and published in the near future. In combination with new and emerging technologies such as high-resolution field samplers, *in situ* experimental robots, single-cell sequencing and phenotyping, high-throughput RNA, DNA and protein profiling, high-throughput phenotyping and bioinformatic techniques to merge and analyse large datasets, we will rapidly advance the understanding of the life of diatoms. Future studies on experimental evolution and resurrection ecology will increase our understanding of diatom carbon metabolism in a changing world. Exploiting diatom life cycle traits, such as their rapid vegetative growth, makes it possible to experimentally investigate the potential evolutionary effects of climate change on both genotype and phenotype and will provide powerful and important information for predicting diatoms' response to a changing environment.

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References

1. Armbrust E. 2009 The life of diatoms in the world's oceans. *Nature* **459**, 185–192. (doi:10.1038/nature08057)
2. Falkowski F, Barber R, Smetacek V. 1998 Biogeochemical controls and feedbacks on ocean primary production. *Science* **281**, 200–206. (doi:10.1126/science.281.5374.200)
3. Cermeño P, Marañon E, Romero O. 2013 Response of marine diatom communities to Late Quaternary abrupt climate changes. *J. Plankton Res.* **35**, 12–21. (doi:10.1093/plankt/fbs073)
4. Mann D, Vanormelingen P. 2013 An inordinate fondness? The number distribution and origins of diatom species. *J. Eucaryot. Microbiol.* **60**, 414–420. (doi:10.1111/jeu.12047)
5. Malviya S *et al.et al.* 2016 Insights into global diatom distribution and diversity in the world's ocean. *Proc. Natl Acad. Sci. USA* **113**, E1516–E1526. (doi:10.1073/pnas.1509523113)
6. Mann D. 1999 The species concept in diatoms. *Phycologia* **38**, 437–495. (doi:10.2216/i0031-8884-38-6-437.1)
7. Sarno D, Kooistra WHCF, Medlin LK, Percopo I, Zingone A. 2005 Diversity in the genus *Skeletonema* (Bacillariophyceae). II. An assessment of the taxonomy of *S. costatum*-like species with the description of four new species. *J. Phycol.* **41**, 151–176. (doi:10.1111/j.1529-8817.2005.04067.x)
8. Moniz M, Kaczmarska I. 2009 Barcoding diatoms: is there a good marker? *Mol. Ecol. Resour.* **9**, 65–74. (doi:10.1111/j.1755-0998.2009.02633.x)
9. Coleman A. 2009 Is there a molecular key to the level of 'biological species' in eukaryotes? A DNA

guide. *Mol. Phylogenet. Evol.* **50**, 197–203. (doi:10.1016/j.ympev.2008.10.008)

10. Amato A, Kooistra W, Levialdighiron J, Mann D, Proschold T, Montresor M. 2007 Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist* **158**, 193–207. (doi:10.1016/j.protis.2006.10.001)
11. Koester J, Swalwell J, Dassow PV, Armbrust E. 2010 Genome size differentiates co-occurring populations of the planktonic diatom *Ditylum brightwellii* (Bacillariophyta). *BMC Evol. Biol.* **10**, 1. (doi:10.1186/1471-2148-10-1)
12. Rynearson TA, Lin EO, Armbrust EV. 2009 Metapopulation structure in the planktonic diatom *Ditylum brightwellii* (Bacillariophyceae). *Protist* **160**, 111–121. (doi:10.1016/j.protis.2008.10.003)
13. Barrett RDH, Schluter D. 2008 Adaptation from standing genetic variation. *Trends Ecol. Evol.* **23**, 38–44. (doi:10.1016/j.tree.2007.09.008)
14. Chetverikov S. 1926 On certain aspects of the evolutionary process from the standpoint of modern genetics (Translated from Russian, 1961). *Proc. Am. Phil. Soc.* **105**, 167–195.
15. Fisher R. 1930 *The genetical theory of natural selection*. Oxford, UK: Clarendon Press.
16. Haldane JBS. 1932 *The causes of evolution*. London, UK: Harper.
17. Wright S. 1931 Evolution in Mendelian populations. *Genetics* **16**, 97–159.
18. Schöb C, Kerle S, Karley AJ, Morcillo L, Pakeman RJ, Newton AC, Brooker RW. 2015 Intraspecific genetic diversity and composition modify species-level diversity–productivity relationships. *New Phytol.* **205**, 720–730. (doi:10.1111/nph.13043)
19. Parkinson JE, Banaszak AT, Altman NS, LaJeunesse TC, Baums IB. 2015 Intraspecific diversity among partners drives functional variation in coral symbioses. *Sci. Rep.* **5**, 15667. (doi:10.1038/srep15667)
20. Rynearson TA, Armburst EV. 2000 DNA fingerprinting reveals extensive genetic diversity in a field population of the centric diatom *Ditylum brightwelli*. *Limnol. Oceanogr.* **45**, 1329–1340. (doi:10.4319/lo.2000.45.6.1329)
21. Rynearson TA, Armburst EV. 2004 Genetic differentiation among populations of the planktonic marine diatom *Ditylum brightwellii* (Bacillariophyceae). *J. Phycol.* **40**, 34–43. (doi:10.1046/j.1529-8817.2004.03089.x)
22. Rynearson TA, Armbrust EV. 2005 Maintenance of clonal diversity during a spring bloom of the centric diatom *Ditylum brightwelli*. *Mol. Ecol.* **14**, 1631–1640. (doi:10.1111/j.1365-294X.2005.02526.x)
23. Rynearson TA, Newton JA, Armbrust EV. 2006 Spring bloom development, genetic variation, and population succession in the planktonic diatom *Ditylum brightwelli*. *Limnol. Oceanogr.* **51**, 1249–1261. (doi:10.4319/lo.2006.51.3.1249)
24. Evans KM, Kühn SF, Hayes PK. 2005 High levels of genetic diversity and low levels of genetic differentiation in North Sea *Pseudo-nitzschia pungens* (Bacillariophyceae) populations. *J. Phycol.* **41**, 506–514. (doi:10.1111/j.1529-8817.2005.00084.x)
25. Casteleyn G, Evans KM, Backeljau T, D'hondt S, Chepurnov VA, Sabbe K, Vyverman W. 2009 Lack of population genetic structuring in the marine planktonic diatom *Pseudo-nitzschia pungens* (Bacillariophyceae) in a heterogeneous area in the Southern Bight of the North Sea. *Mar. Biol.* **156**, 1149–1158. (doi:10.1007/s00227-009-1157-6)
26. Casteleyn G, Leliaert F, Backeljau T, Debeer A-E, Kotaki Y, Rhodes L, Lundholm N, Sabbe K, Vyverman W. 2010 Limits to gene flow in a cosmopolitan marine planktonic diatom. *Proc. Natl Acad. Sci. USA* **107**, 12 952–12 957. (doi:10.1073/pnas.1001380107)
27. Evans KM, Hayes PK. 2004 Microsatellite markers for the cosmopolitan marine diatom *Pseudo-nitzschia pungens*. *Mol. Ecol. Notes* **4**, 125–126. (doi:10.1111/j.1471-8286.2004.00591.x)
28. Tesson SVM, Montresor M, Procaccini G, Kooistra WHCF. 2014 Temporal changes in population structure of a marine planktonic diatom. *PLoS ONE* **9**, e114984. (doi:10.1371/journal.pone.0114984)
29. Godhe A, Härnström K. 2010 Linking the planktonic and benthic habitat: genetic structure of the marine diatom *Skeletonema marinoi*. *Mol. Ecol.* **19**, 4478–4490. (doi:10.1111/j.1365-294X.2010.04841.x)
30. Härnström K, Ellegaard M, Andersen T, Godhe A. 2011 Hundred years of genetic structure in a sediment revived diatom population. *Proc. Natl Acad. Sci. USA* **108**, 4252–4257. (doi:10.1073/pnas.1013528108)
31. Godhe A *et al.et al.* 2016 Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom. *J. Biogeogr.* **43**, 1130–1142. (doi:10.1111/jbi.12722)
32. Sjöqvist C, Kremp A. 2016 Genetic diversity affects ecological performance and stress response of marine diatom populations. *ISME J.* **10**, 2755–2766. (doi:10.1038/ismej.2016.44)
33. Godhe A, Egardt J, Kleinhans D, Sundqvist L, Hordoir R, Jonsson PR. 2013 Seascape analysis reveals regional gene flow patterns among populations of a marine planktonic diatom. *Proc. R. Soc. B* **280**, 20131599. (doi:10.1098/rspb.2013.1599)
34. Evans KM, Chepurnov VA, Sluiman HJ, Thomas SJ, Spears BM, Mann DG. 2009 Highly differentiated populations of the freshwater diatom *Sellaphora capitata* suggest limited dispersal and opportunities for allopatric speciation. *Protist* **160**, 386–396. (doi:10.1016/j.protis.2009.02.001)
35. Chen G, Rynearson T. 2016 Genetically distinct populations of a diatom co-exist during the North Atlantic spring bloom. *Limnol. Oceanogr.* **61**, 2165–2179. (doi:10.1002/lno.10361)
36. Whittaker KA, Rynearson TA. 2017 Evidence for environmental and ecological selection in a microbe with no geographic limits to gene flow. *Proc. Natl Acad. Sci. USA* **114**, 2651–2656. (doi:10.1073/pnas.1612346114)
37. Armbrust E *et al.et al.* 2004 The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* **306**, 79–86. (doi:10.1126/science.1101156)
38. Bowler C *et al.et al.* 2008 The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* **456**, 239–244. (doi:10.1038/nature07410)
39. Lommer M *et al.et al.* 2012 Genome and low-iron response of an oceanic diatom adapted to chronic iron limitation. *Genome Biol.* **13**, R66. (doi:10.1186/gb-2012-13-7-r66)
40. Ellstrand NC, Roose ML. 1987 Patterns of genotypic diversity in clonal plant species. *Am. J. Bot.* **74**, 123–131. (doi:10.2307/2444338)
41. Becks L, Ellner S, Jones L, Hairston N. 2010 Reduction of adaptive genetic diversity radically alters eco-evolutionary community dynamics. *Ecol. Lett.* **13**, 989–997. (doi:10.1111/j.1461-0248.2010.01490.x)
42. Ellegren H. 2004 Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.* **5**, 435–443. (doi:10.1038/nrg1348)
43. Schlötterer C, Tautz D. 1992 Slippage synthesis of simple sequence DNA. *Nucleic Acids Res.* **20**, 211–215. (doi:10.1093/nar/20.2.211)
44. Koester JA, Swanson WJ, Armbrust EV. 2013 Positive selection within a diatom species acts on putative protein interactions and transcriptional regulation. *Mol. Biol. Evol.* **30**, 422–434. (doi:10.1093/molbev/mss242)
45. Hartl D, Clark A. 2007 *Principles of population genetics*. Sunderland, MA: Sinauer Associates.
46. Reed DH, Frankham R. 2003 Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**, 230–237. (doi:10.1046/j.1523-1739.2003.01236.x)
47. Knutsen H, Jorde PE, André C, Stenseth NC. 2003 Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. *Mol. Ecol.* **12**, 385–394. (doi:10.1046/j.1365-294X.2003.01750.x)
48. Sjöqvist C, Godhe A, Jonsson PR, Sundqvist L, Kremp A. 2015 Local adaptation and oceanographic connectivity patterns explain genetic differentiation of a marine diatom across the North Sea-Baltic salinity gradient. *Mol. Ecol.* **24**, 2871–2885. (doi:10.1111/mec.13208)
49. Sildever S, Sefbom J, Lips I, Godhe A. 2016 Competitive advantage and higher fitness in native populations of genetically structured planktonic diatoms. *Environ. Microbiol.* **18**, 4403–4411. (doi:10.1111/1462-2920.13372)
50. D'Alelio D, d'Alcalà MR, Dubroca L, Sarn D, Zingone A, Montresor M. 2010 The time for sex: a biennial life cycle in a marine planktonic diatom. *Limnol. Oceanogr.* **55**, 106–114. (doi:10.4319/lo.2010.55.1.0106)
51. Menden-Deuer S, Rowlett J. 2014 Many ways to stay in the game: individual variability maintains high biodiversity in planktonic microorganisms. *J. R. Soc. Interface* **11**, 200140031. (doi:10.1098/rsif.2014.0031)
52. Bolnick D, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003 The ecology of individuals: incidence and implications of individual

specialization. *Am. Nat.* **161**, 1–28. (doi:10.1086/343878)

53. Canesi KL, Rynearson TA. 2016 Temporal variation of *Skeletonema* community composition from a long-term time series in Narragansett Bay identified using high-throughput DNA sequencing. *Mar. Ecol. Prog. Ser.* **556**, 1–16. (doi:10.3354/meps11843)
54. Gallagher J, Wood A, Alberte R. 1984 Ecotypic differentiation in the marine diatom *Skeletonema costatum:* influence of light intensity on the photosynthesis apparatus. *Mar. Biol.* **82**, 121–134. (doi:10.1007/BF00394096)
55. Gsell A, de Senerpont Domis LN, Przytulska-Bartosiewicz A, Mooij WM, van Donk E, Ibelings BW. 2012 Genotype-by-temperature interactions may help to maintain clonal diversity in *Asterionella formosa* (Bacillariophyceae). *J. Phycol.* **48**, 1197–1208. (doi:10.1111/j.1529-8817.2012.01205.x)
56. Kremp A, Godhe A, Egardt J, Dupont S, Suikkanen S, Casabianca S, Penna A. 2012 Intraspecific variability in the response of bloom forming marine microalgae to changing climatic conditions. *Ecol. Evol.* **2**, 1195–1207. (doi:10.1002/ece3.245)
57. Rogers F, Godhe A, Gamfeldt L. 2012 Genetic diversity and ecosystem functioning in the face of multiple stressors. *PLoS ONE* **7**, e45007. (doi:10.1371/journal.pone.0045007)
58. DeBruin A, Ibelings BW, Rijkeboer M, Brehm M, van Donk E. 2004 Genetic variation in *Asterionella formosa* (Bacillariophyceae): is it linked to frequent epidemics of host-specific parasitic fungi? *J. Phycol.* **40**, 823–830. (doi:10.1111/j.1529-8817.2004.04006.x)
59. Tomaru Y, Nagasaki K. 2011 Diatom viruses. In *The diatom world* (eds J Seckbach, JP Kociolek), pp. 211–225. Berlin, Germany: Springer.
60. McQuoid M, Hobson L. 1996 Diatom resting stages. *J. Phycol.* **32**, 889–902. (doi:10.1111/j.0022-3646.1996.00889.x)
61. McQuoid M, Godhe A, Nordberg K. 2002 Viability of phytoplankton resting stages in the sediments of a coastal Swedish fjord. *Eur. J. Phycol.* **37**, 191–201. (doi:10.1017/S0967026202003670)
62. Potapova MG, Charles DF. 2002 Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. *J. Biogeogr.* **29**, 167–187. (doi:10.1046/j.1365-2699.2002.00668.x)
63. Barnett TP, Pierce DW, AchutaRao KM, Gleckler PJ, Santer BD, Gregory JM, Washing WM. 2005 Penetration of human-induced warming into the world's oceans. *Science* **309**, 284–287. (doi:10.1126/science.1112418)
64. Litchman E, Klausmeier C, Yoshiyama K. 2009 Contrasting size evolution in marine and freshwater diatoms. *Proc. Natl Acad. Sci. USA* **106**, 2662–2670. (doi:10.1073/pnas.0810891106)
65. Romero OE, Kim J-H, Donner B. 2008 Submillennial-to-millennial variability of diatom production off Mauritania, NW Africa, during the last glacial cycle. *Paleoceanography* **23**, PA3218. (doi:10.1029/2008PA001601)
66. Romero OE, Leduc G, Vidal L, Fischer G. 2011 Millennial variability and long-term changes of the diatom production in the eastern equatorial Pacific during the last glacial cycle. *Paleoceanography* **26**, PA2212. (doi:10.1029/2010PA002099)
67. Pike J, Crosta X, Maddison EJ, Stickley CE, Denis D, Barbara L, Renssen H. 2009 Observations on the relationship between the Antarctic coastal diatoms *Thalassiosira antarctica* Comber and *Porosira glacialis* (Grunow) Jørgensen and sea ice concentrations during the late Quaternary. *Mar. Micropaleontol.* **73**, 14–25. (doi:10.1016/j.marmicro.2009.06.005)
68. Pike J, Swann GEA, Leng MJ, Snelling AM. 2013 Glacial discharge along the west Antarctic Peninsula during the Holocene. *Nat. Geosci.* **6**, 199–202. (doi:10.1038/NGEO1703)
69. Pitchford JW, Brindley J. 1999 Iron limitation, grazing pressure and oceanic high nutrient-low chlorophyll (HNLC) regions. *J. Plankton Res.* **21**, 525–547. (doi:10.1093/plankt/21.3.525)
70. Marchetti A, Cassar N. 2009 Diatom elemental and morphological changes in response to iron limitation: a brief review with potential paleoceanographic applications. *Geobiology* **7**, 419–431. (doi:10.1111/j.1472-4669.2009.00207.x)
71. Collins S. 2011 Competition limits adaptation and productivity in a photosynthetic alga at elevated CO_2. *Proc. R. Soc. B* **278**, 247–255. (doi:10.1098/rspb.2010.1173)
72. Crawfurd K, Raven JA, Wheeler GL, Baxter EJ, Joint I. 2011 The response of *Thalassiosira pseudonana* to long-term exposure to increased CO_2 and decreased pH. *PLoS ONE* **6**, e26695. (doi:10.1371/journal.pone.0026695)
73. Low-Décarie E, Jewell MD, Fussmann GF, Bell G. 2013 Long-term culture at elevated atmospheric CO_2 fails to evoke specific adaptation in seven freshwater phytoplankton species. *Proc. R. Soc. B* **280**, 20122598. (doi:10.1098/rspb.2012.2598)
74. Scheinin M, Riebesell U, Rynearson TA, Lohback KT, Collins S. 2015 Experimental evolution gone wild. *J. R. Soc. Interface* **12**, 20150056. (doi:10.1098/rsif.2015.0056)
75. Hennon GMM, Ashworth J, Groussman RD, Berthiaume C, Morales RL, Baliga NS, Orellana MV, Armbrust EV. 2015 Diatom acclimation to elevated CO_2 via cAMP signalling and coordinated gene expression. *Nat. Clim. Change* **5**, 761–765. (doi:10.1038/nclimate2683)
76. Rayko E, Maumus F, Maheswari U, Jabbari K, Bowler C. 2010 Transcription factor families inferred from genome sequences of photosynthetic stramenopiles. *New Phytol.* **188**, 52–62. (doi:10.1111/j.1469-8137.2010.03371.x)
77. Matsuda Y, Nakajima K, Tachibana M. 2011 Recent progresses on the genetic basis of the regulation of CO_2 acquisition systems in response to CO_2 concentration. *Photosynth. Res.* **109**, 193–203. (doi:10.1007/s11120-011-9623-7)
78. Basu S *et al.* 2017 Finding a partner in the ocean: molecular and evolutionary bases of the response to sexual cues in a planktonic diatom. *New Phytol.* **215**, 140–156. (doi:10.1111/nph.14557)
79. Mock T *et al.* 2017 Evolutionary genomics of the cold-adapted diatom *Fragilariopsis cylindrus*. *Nature* **541**, 536–540. (doi:10.1038/nature20803)