Journal of
Plankton Research

academic.oup.com/plankt

J. Plankton Res. (2017) 39(2): 165–179. *First published online January 20, 2017 doi:10.1093/plankt/fbw098*

HORIZONS

Genetic diversity and evolution in eukaryotic phytoplankton: revelations from population genetic studies

KARIN RENGEFORS[1]*, ANKE KREMP[2], THORSTEN B.H. REUSCH[3] AND A. MICHELLE WOOD[4]

[1]AQUATIC ECOLOGY UNIT, DEPARTMENT OF BIOLOGY, LUND UNIVERSITY, ECOLOGY BUILDING, 22362 LUND, SWEDEN, [2]MARINE RESEARCH CENTRE, FINNISH ENVIRONMENT INSTITUTE, ERIK PALMENIN AUKIO 1, 00560 HELSINKI, FINLAND, [3]GEOMAR HELMHOLTZ-CENTRE FOR OCEAN RESEARCH KIEL, EVOLUTIONARY ECOLOGY, DÜSTERNBROOKER WEG 20, 24105 KIEL, GERMANY AND [4]DEPARTMENT OF BIOLOGY AND INSTITUTE OF ECOLOGY AND EVOLUTION, UNIVERSITY OF OREGON, EUGENE, OR 97403, USA

*CORRESPONDING AUTHOR: Karin.Rengefors@biol.lu.se

Received July 14, 2016; editorial decision December 20, 2016; accepted January 2, 2017

Corresponding editor: Pia Moisander

All authors have contributed equally to the text, the corresponding author has in addition led the work and taken responsibility for the submission.

Eukaryotic phytoplankton exhibit an enormous species richness, displaying a range of phylogenetic, morphological and physiological diversity. Yet, until recently, very little was known about the diversity, genetic variation and evolutionary processes within species and populations. An approach to explore this diversity and to understand evolution of phytoplankton is to use population genetics as a conceptual framework and methodology. Here, we discuss the patterns, processes and questions that population genetic studies have revealed in eukaryotic phytoplankton. First, we describe the main biological processes generating genetic variation. We specifically discuss the importance of life-cycle complexity for genetic and phenotypic diversity and consider how such diversity can be maintained during blooms when rapid asexual proliferation dominates. Next, we explore how genetic diversity is partitioned over time and space, with a focus on the processes shaping this structure, in particular selection and genetic exchange. Our aim is also to show how population genetics can be used to make inferences about realized dispersal and sexual recombination, as these processes are so difficult to study directly. Finally, we highlight important open questions and suggest promising avenues for future studies that will be made possible by new sequencing technologies.

KEYWORDS: population genetics; genetic diversity; evolution; phytoplankton; genetic differentiation

available online at academic.oup.com/plankt

INTRODUCTION

Phytoplankton form the base of aquatic food webs, and are directly involved in nutrient cycling (uptake and release) and carbon sequestration (Field *et al.*, 1998; Falkowski *et al.*, 2004). Despite superficial similarity in function and ecology with land plants, the group encompasses a broader range of phylogenetic diversity, representing more pigment systems and other aspects of the evolution of oxygenic photosynthesis (Falkowski and Knoll, 2007; Graham *et al.*, 2009; Simon *et al.*, 2009). The diversity of eukaryotic phytoplankton, in particular, is enormous and continues to be discovered (De Vargas *et al.*, 2015). Yet, our knowledge of *intra*specific diversity, genetic variation and evolutionary processes in microeukaryotic phytoplankton is very limited. Because they present a broader range of life history strategies (Dickey, 1991; Graham *et al.*, 2009; Von Dassow and Montresor, 2011) and evolve quite differently from prokaryotic phytoplankton (cyanobacteria), we here focus only on the eukaryotic phytoplankton.

Phytoplankton are subject to fluctuating environments on many different temporal and spatial scales (Harris, 1987; Reynolds, 1990). In addition, contemporary populations are also faced with major shifts associated with increasing human impact on both freshwater and marine environments. To some degree, these changes are directional (e.g. increased lake water temperature and ocean acidification), and to other extents, the changes will result in greater fluctuations, which leads to an unpredictable environment (Boyd *et al.*, 2016). Regardless of the intensity or direction of selection, phytoplankton may evolve in response to this selection, and that involves changes in genotype frequency. Population genetics, the branch of evolutionary biology that involves the study of genetic variation within populations, thus provides the mathematical and conceptual tools needed to understand the potential for phytoplankton species to evolve in response to environmental change (Lynch and Walsh, 1998; Hartl and Sunderland, 2000; Crow, 2008; Nielsen and Slatkin, 2013). It also allows for analyses of spatial structuring in phytoplankton populations, and provides a way to understand interactions between a species' life cycle, ploidy levels and possible routes of evolution.

In the sections below, we will discuss some outstanding issues that can be addressed using population genetics as well as challenges posed by phytoplankton. One of the major challenges is that population genetic theory was developed for predominantly diploid, sexually reproducing species (i.e. terrestrial animals). In contrast, most phytoplankton undergo long periods of asexual non-gametic reproduction followed by intermittent sexual recombination. This implies that some of the underlying assumptions may be erroneous, or that patterns will be different from expected patterns of sexually reproducing species. From the ecologist's perspective, population genetics can help us to understand dispersal and adaptive potential, which has implications for understanding invasive species and harmful algal blooms. Population genetics also provides the basis to explore the underlying processes that lead to population differentiation and eventually speciation. It thus offers the methodology to explore and establish what species concept to use in phytoplankton.

ADAPTIVE EVOLUTION IN RESPONSE TO ENVIRONMENTAL CHANGE

Population genetic theory predicts that, because of their large population sizes and fast replication rates (= short generation times), phytoplankton are capable of an evolutionary response to selection in relatively short time frames (Lynch *et al.*, 1991). Experimental studies provide some support for this hypothesis. Provided sufficient genetic variation in phenotypic traits for selection to act on, a rapid evolutionary response can be predicted (Wood *et al.*, 1987, 2005; Edgar and Theriot, 2003). Moreover, several authors have suggested that the seemingly cosmopolitan distribution and maintenance of relatively high abundances of many species of phytoplankton result from changes in genotype composition within species (cf. Brand, 1988; Wood, 1988; Lohbeck *et al.*, 2012; Reusch and Boyd, 2013; Kashtan *et al.*, 2014) much as originally suggested for krill (Ayala and Valentine, 1979). This hypothesis has been tested most thoroughly in cyanobacteria where the existence of "ecotypes" that show non-random distribution with regard to habitat is well established (Moore *et al.*, 1998; Zwirglmaier *et al.*, 2008; Sohm *et al.*, 2016). In addition, physiological and genomic adaptations associated with at least some ecotypes of cyanobacteria have been documented, suggesting some role of natural selection and adaptive evolution in maintaining or creating the non-random distribution (Moore *et al.*, 1998; Bibby *et al.*, 2003; Rocap *et al.*, 2003; Stuart *et al.*, 2013). However, the study of eukaryotic phytoplankton species has not yielded as clear-cut results, even if the existence of high genetic diversity within populations of eukaryotic phytoplankton has been established both by early works using techniques such as allozymes and random amplified polymorphic DNA (RAPD) (reviewed by Medlin *et al.* 2000) and subsequent work mainly based on microsatellites

and amplified fragment length polymorphism (AFLP) markers (Evans *et al.*, 2005a; Iglesias-Rodríguez *et al.*, 2006; Rynearson *et al.*, 2006a; Logares *et al.*, 2009; Masseret *et al.*, 2009).

Because eukaryotic phytoplankton encompass such a diversity of complex life cycles, it has been hard to demonstrate and generalize the role of microevolution. Examples of adaptive evolution have thus far only addressed phytoplankton populations undergoing mitotic life-cycle phases (e.g. asexually reproducing populations) in the laboratory. Very likely, complex processes relating to sexual reproduction may play a more important role in maintaining diversity and shaping the population genetic structure of eukaryotic phytoplankton than in prokaryotic phytoplankton.

Irwin *et al.* (Irwin *et al.*, 2015) suggest that a number of eukaryotic and prokaryotic phytoplankton species are adapting to the directional changes associated with climate change. However, understanding the mechanisms by which this can happen and understanding the nature of limits on an evolutionary response to environmental change in phytoplankton require more detailed knowledge of the biology of this taxonomically diverse functional group. This is particularly true with respect to the ways that genetic variation is generated and maintained during periods of both sexual and asexual growth, the role of a variety of life history stages including periods of dormancy, and the true nature of dispersal. Here, we consider what is known about these key topics, and suggest directions for future research that would improve our ability to use the power of population genetics to predict and model phytoplankton response to a changing environment.

THE BIOLOGY BEHIND GENETIC VARIATION

Genetic variation within a species is a prerequisite for evolutionary adaptation of phytoplankton populations. Genetic variation needs to be expressed at the level of phenotypes in order to "be visible" to selection. Selection thus always affects genetic diversity indirectly; how exactly depends on the complex correlation of the genotype with the phenotype. As a surrogate, however, diversity at genetic marker loci can be informative to evaluate genetic diversity in a more general sense (Table I), which is often correlated with selectively relevant diversity. Genetic diversity is generated and selected upon by different processes acting at alternating stages of the life cycles of phytoplankton species (Fig. 1). Clonal (= asexual reproduction) may happen in the haploid or diploid phase of the life cycle and is often thought to be the predominant mode of growth. However, most phytoplankton have complex life cycles including occasional sexual reproduction events as well as phases of reduced metabolic activity. As we discuss here, mutation, sexual reproduction and dormancy have remained understudied aspects of life history, despite their importance in allowing us to understand population genetics and the evolutionary process in phytoplankton.

Mutation—generation of new genetic variation

Mutation is the fundamental mechanism generating genetic variation in both the asexual and sexual phase of all organisms. The mutational supply is probably seldom a rate-limiting factor for evolution in eukaryotic phytoplankton because the mutation target is larger. This is in part due to large census size of many species in nature, mostly short generation times, the dominance of asexual reproduction, and in part due to often large genome sizes. Genome sizes in eukaryotic phytoplankton vary widely, from as low as 12 Mb in *Ostrecoccus* to greater than 100 Gb in species of dinoflagellates (Parker *et al.*, 2008), although there is a bias toward smaller genomes sequenced, due to difficulties of sequencing and assembling large repetitive genomes. Nevertheless, phytoplankton that achieve population sizes on the order of a billion cells in 1 m^3 of water can theoretically generate multiple mutations at every locus every doubling. While the number of selectively relevant mutations per doubling is determined by whether or not they occur in a functionally relevant portion of the genome, this high capacity for generation of new genotypes makes estimation of mutation rates and understanding of the common mechanisms and types of mutations in phytoplankton particularly important. There is increasing awareness of the evolutionary potential of prolonged growth in culture (Wood *et al.*, 2005; Lakeman *et al.*, 2009), and experimental evolution studies are increasingly reporting evidence for genetic adaptation by phytoplankton (López-Rodas *et al.*, 2008; Huertas *et al.*, 2010; Lohbeck *et al.*, 2012; Schlüter *et al.*, 2016). Whole-genome sequencing of cells in these types of studies especially before and after periods of selection or prolonged maintenance in culture provides an exceptional opportunity to assess mutation type and frequency in asexually growing cells, as has been done successfully in yeast experimental evolution studies (Lang *et al.*, 2013).

Mutations can range from exchange of single nucleotides resulting in SNPs (= single-nucleotide polymorphisms, see Box 1) to insertions, deletions, inversions at multiple scales, whole chromosome duplications or losses, or even whole-genome duplications. How mutations play out depends critically upon the ploidy of the population.

Table I: Genetic markers used in phytoplankton population genetic studies

Markers	Description	Key examples
Allo-enzymes, briefly allozymes	Polymorphic molecular forms of enzymes encoded by different alleles at specific gene loci	Gallagher (1980): *Skeletonema costatum*
	Relatively coarse markers with low reproducibility	Medlin *et al.* (1996): *Emiliania huxleyi*
	Used in early works on genetic variation of phytoplankton	Bolch *et al.* (1999): *Gymnodinium catenatum*
RAPD	Randomly amplified genomic DNA fragments	Lewis *et al.* (1997): *Fragillaria capucina*
	Sensitive method due to large number of loci	Bolch *et al.* (1999): *Gymnodinium catenatum*
	Dominant markers, heterozygotes are masked, not suitable for diploids	
	Not reproducible, no longer in use	
AFLP	Selectively amplified polymorphic restriction fragments of enzyme digested genomic DNA	De Bruin *et al.* (2004): *Asterionella formosa*
	Reproducible and statistically powerful fingerprinting technique	John *et al.* (2004): *Alexandrium tamarense*
	High amount of genome-wide loci	Logares *et al.* (2009): several freshwater dinoflagellate species
	Markers consist largely of non-coding DNA	Lebret *et al.* (2012): *Gonyostomom semen*
	Dominant markers, heterozygotes are masked, limited application in diploids	
Microsatellites	Short sequence (2–7 bases) repeats of variable length (polymorphic) in genomic DNA, mostly non-coding	Rynearson and Armbrust (2004), *Ditylum brightwellii*
	Highly reproducible and informative	Iglesias-Rodríguez *et al.* (2006): *Emiliania huxleyi*
	Codominant markers, detecting heterozygotes and allowing calculation of allele frequencies	Nagai *et al.* (2004): *Alexandrium tamarense*
	Alleles are inherited in Mendelian fashion, suitable for mating studies	Demura *et al.* (2014): *Chattonella marina*
	Identification of individuals possible prior sequence information required locus specific development, few loci in studies due to high costs per locus	
SNP	Single base pair mutations across the genome, polymorphisms in SNP patterns	No studies from eukaryotic phytoplankton
	Genome-wide detection of large numbers of SNPs requires *de novo* sequencing of whole-genome or defined genome subsets (e.g. RAD-seq) by high throughput sequencing techniques	Kashtan *et al.* (2014): *Prochlorococcus*, prokaryotic phytoplankton
	Highly informative codominant markers, can be linked to phenotype and selection, or designed to be delectively neutral	
	SNP development requires extensive sequence information *via* multiple transcriptomes or genomes	
	Population-level genotyping depends upon number of markers , can be PCR-based microfluidic technology (10–100 s of markers) or RAD (100–10 000 s of markers)	

When haploid, any non-neutral mutation is immediately expressed and subject to selection. This is not the case in the diplontic phase (Zeyl and Bell, 1997), where deleterious mutations, thus the loss of function, are recessive and masked by intact copies of the homologous chromosome. Conversely, *de novo* adaptation can only occur if the mutations are at least partially dominant, i.e. confers a gain of function. Data on spontaneous (point) mutation in phytoplankton are rare and papers addressing mutation rates vary in their estimates. Some studies indicate low rates (Ness *et al.*, 2012), while others report high frequencies in phytoplankton clonal cultures (Tesson *et al.*, 2013), suggesting that they contribute significantly to genetic change in clonal lineages. As a novel tool, experimental evolution studies have provided evidence for genetic adaptation by showing increased fitness in haploid and diploid clonal phytoplankton populations after multi-generation exposure to climate stressors e.g. warming or ocean acidification (Collins and Bell, 2004; Huertas *et al.*, 2011; Flores-Moya *et al.*, 2012; Lohbeck *et al.*, 2012; Schlüter *et al.*, 2014). However, the genetic basis of such adaptive change is currently elusive, thus we have no idea which type of underlying mutations or even epi-mutations have caused such adaptive change.

Occasional sex in clonal populations—the best of two worlds?

While mutation is the primary source of novel or new genotypes, recombination of existing alleles during sexual

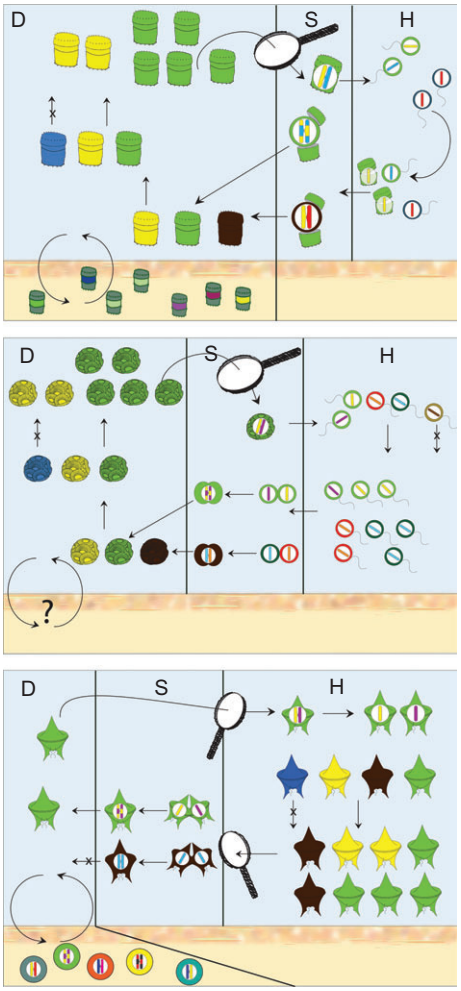

Fig. 1. The interplay between emergence of genetic diversity *via* sexual recombination (meiosis followed by syngamy S) alternating with different phases of asexual genotypic selection. In each panel, the left section corresponds to the diploid (D) phase, the right to the haploid (H) phase, both of which are connected *via* meiosis and syngamy (S). The three depicted typical life cycles of eukaryotic phytoplankton differ in their contribution of sexual and asexual propagation. Top panel: a typical diplont, such as a diatom; here only the diploid phase is propagating asexually. Mid panel: a haplo-diplont, such as a coccolithophore, here both ploidy stages undergo asexual reproduction and hence, genotypic selection. Bottom panel: a typical haplont such as a dinoflagellate, where asexual proliferation and selection occur in the haploid phase. Genotypic selection favors certain genotypes (different colors) and their specific allelic combinations in particular during bloom conditions. In all three life-cycle types, genetic diversity *via* recombination is generated during meiosis and syngamy (middle section in all three diagrams, chromosome sections in different colors symbolize recombination). In addition, genetic diversity is stored, and hence, N_e may be greatly expanded in diplonts and haplonts with resting stages, shown as additional chromosomal genotypes. The formation of resting genotypes may (Dinoflagellates) or may not (Diatoms) be linked to sexual recombination.

reproduction provides a substantial source of selectable phenotypic diversity, and nearly all eukaryotic species have some form of sexual reproduction. In both freshwater and marine phytoplankton, reproduction is commonly expressed as a mixture of alternating clonal and sexual reproduction. Figure 1 summarizes some life-cycle types of eukaryotic phytoplankton ranging from haplontic *via* haplo-diplontic to diplontic. The exact nature of such life cycles has both consequences on how population genetic processes play out, and makes the application of simple population genetic models questionable. Studies based on clonal cultures have documented sexual stages mostly for diatoms, raphidophytes and dinoflagellates, and revealed a variety of reproductive strategies and mating systems in these taxa (Chepurnov *et al.*, 2004; Blackburn and Parker, 2005; Figueroa and Rengefors, 2006). In addition, there are studies providing evidence for positive selection on sexual reproduction genes (Sorhannus, 2008). Although direct evidence of sex is limited in other classes of phytoplankton, their general potential for syngamy and meiosis has been suggested in e.g. chlorophytes and haptophytes, by the presence of respective genes in their transcriptomes and genomes (Derelle *et al.*, 2006; Speijer *et al.*, 2015). Using AFLP and microsatellite markers as well as phenotypic characters, crossing experiments have demonstrated how genetic variation can arise from sexual recombination in phytoplankton (Figueroa *et al.*, 2006; Tesson *et al.*, 2013; Godhe *et al.*, 2014).

Prolonged asexual growth results in the entire genome behaving like a single linkage group or "supergene", displaying mutations in a genealogical pattern, and resulting in strong linkage disequilibrium (LD) (see Box 1), the nonrandom association of particular alleles within the genome. It will also increase the load of deleterious mutations in clonal lineages, but how such genetic load is expressed and purged from the population depends on whether species are haplonts, diplonts or haplo-diplonts. Sexual recombination allows the purging of deleterious mutations from diploid populations (Kondrashov, 1988) while bringing combinations of favorable genes together, allowing their spread through the population. As a consequence, the population-level variance for fitness increases, and so does the rate of evolutionary adaptation, as demonstrated in the laboratory freshwater evolution model species *Chlamydomons rheinhardtii* (Kaltz and Bell, 2002).

Evolutionary theory predicts that a mixture of clonal (asexual) and sexual reproduction may be "the best of two worlds". Even infrequent sex in an asexually dividing organism may result in the same level of genetic diversity as found in obligate sexual species (Bengtsson, 2003). Unfortunately, there is only very limited data on the relative rates of sexual vs. asexual reproduction in any phytoplankton species in nature. The impact of sex on genetic diversity in facultatively sexual organisms is

Box 1. Glossary box

Allele frequency—the relative proportion of all alleles at a specified gene (or locus) represented by each allele individually.
Gene flow—exchange of genes among populations due to movement (dispersal) of individuals among subpopulations.
Genetic drift—the process by which chance alone determines a change in allele frequency. Typically happens only in very small populations or when populations go through bottlenecks.
Genotype frequency—the proportion of specific genotypes within a population.
Founder effect—happens when a few individuals establish a new population and the population goes through a bottleneck and is accompanied by random *genetic drift*. This tends to reduce genetic variation, affects distribution of alleles and leads to increased *linkage disequilibrium*.
Four-gamete test—a test for linkage disequilibrium (see below) and the amount of historical recombination. Assumes four haplotypes for each pair of biallelic loci, where the presence of all four haplotypes in a population indicates recombination or recurrent mutations.
F_{ST}—Fixation index, the most common measure of genetic differentiation between two populations. It allows an objective comparison of the effect of population structure in different organisms because it is expressed relative to the total genetic variance of one or many genetic loci.
Haplotype—combination of linked alleles. In asexually reproducing populations, some alleles may be lost as sex does not occur for many generations because haplotypes comprises specific allelic variants at a number of loci out-compete less fit combinations.
Hardy–Weinberg equilibrium (HWE)—the mathematical model describing the relation between allele frequencies and genotype frequencies based on a number of assumptions about an ideal population, such as random mating, large population size, diploid, as well as negligible migration, mutation and selection.
Heterozygous deficiency/excess—heterozygosity is the likelihood that there are different alleles at one genetic locus. In an ideal population, its fraction is determined by HWE. Deviations in the heterozygosity indicate inbreeding (deficiency) or heterozygote superiority or extensive clonality (excess).
Linkage disequilibrium (LD)—when alleles at different loci are not in random association. This can be caused by sampling effects in genetic drift, and by the genes interacting in fitness and selection, magnified by physical linkage (proximity on a chromosome). When genes are in linkage equilibrium they will not be associated, and any particular combination of alleles of these genes equal the product of the frequencies of those alleles. LD is one way to detect the predominance of asexual over sexual reproduction, as the multiplication of one certain genotype effectively creates one huge linkage block.
N_e—effective population size (see Box 2). The number of individuals in a theoretically ideal population, in principle the size of the breeding population, which is usually (much) lower than the census size. The ideal population would show the same level heterozygosity as the actual population in the field, but under assumed conditions of random mating and neutrality of alleles. The magnitude of N_e determines how much random genetic drift (small N_e) versus directional processes such as selection (large N_e) change the genetic composition of a population. When population sizes fluctuate strongly, as may be the case in eukaryotic phytoplankton, N_e is driven by the smallest population sizes.
Negative frequency-dependent selection (NFDS)—a mechanism that favors selection of a rare genotype, which increases in frequency. When it becomes more common, fitness decreases and the genotype is disfavored by selection again. NFDS can lead to equilibrium of many genotypes.
Pleiotropy—Situation in which a single gene has an effect on more than one phenotypic trait. Selection on one trait will have a correlated effect on the other.
Single-nucleotide polymorphism (SNP)—the smallest unit of polymorphism found at a particular nucleotide site. A locus is considered an SNP in a population if the nucleotide pair at that site differs among individuals. The SNP identifies two alleles for which there can be three genotypes in a diplont population, and four genotypes in a haplont.
Wahlund effect—apparent reduction in heterozygosity of a population when two or more genetically differentiated populations are pooled. The total expected heterozygosity according to HWE is then larger than what is measured, which may lead to erroneous inferences on inbreeding.

usually assessed by multiple genetic indices, such as heterozygote excess or deficiency, presence of repeated multilocus genotypes or LD, including four-gamete test (see Box 1) (Tibayrenc and Ayala, 1991; Halkett *et al.*, 2005). Studies that have calculated these indexes show that genetic signatures of both clonal and sexual reproduction can be found in phytoplankton populations. However, interpretation of the respective indices is not always straight forward, as they can be affected by null alleles, Wahlund effect (see Box 1) or inbreeding (Alpermann *et al.*, 2009; Godhe and Härnström, 2010; Sjöqvist *et al.*, 2015). Among the studies that have addressed reproduction patterns, several have reported weak or no LD (Tahvanainen *et al.*, 2012; Sassenhagen *et al.*, 2015; Van Den Wyngaert *et al.*, 2015), indicating that regular recombination events occur in phytoplankton populations, e.g. diatoms that display a biannual cycle (D'Alelio *et al.*, 2010). It has also been shown that sexuality indicators may be present in some and absent in other populations/subpopulations of the same species (Tahvanainen *et al.*, 2012; Van Den Wyngaert *et al.*, 2015). This suggests that the level of sexual reproduction (and consequently genetic diversity) is influenced by factors such as habitat conditions, life-cycle strategies or population history.

Dormancy—a survival strategy with consequences for genetic diversity?

As an additional complication in addition to the already complex life cycles, many phytoplankton species from seasonally variable environments produce dormant resting stages to survive periods of adverse conditions. These resting stages (or propagules) sink to lake or marine sediments and form propagule banks that, analogous to seed banks of angiosperms on land, seed new active planktonic populations when germinating (Fig. 1). In several classes, particularly haplonts such as dinoflagellates, chlorophytes and chrysophytes, these are a part of the sexual cycle representing the zygote stage (Fryxell, 1983). Sexual resting stage formation is generally considered to contribute to genetic diversity of phytoplankton populations (Von Dassow and Montresor, 2011) as the new annual growth phase will be inoculated entirely from sexually produced individuals. Regardless of their reproductive origin, dormant propagule banks should be expected to represent genetic reservoirs. As shown for zooplankton these may by far exceed the genetic diversity of the active planktonic population (Brendonck and De Meester, 2003). Dormant propagule banks can both slow down and enhance adaptive evolution, depending on whether the fraction of emerging genotypes is a random or non-random sample of the total gene pool (Hairston and De Stasio, 1988). So far, only one study has compared genetic diversity of a sediment propagule bank and the actively growing phytoplankton population and confirmed such an accumulation effect (Godhe and Härnström, 2010). However, the hypothesis is generally supported by high intraspecific phenotypic variation found in the propagule bank of various phytoplankton species (Ribeiro *et al.*, 2013; Kremp *et al.*, 2016). Since genetically diverse propagule banks regularly re-seed actively growing populations and thereby maintain diversity on longer time scales, species with dormant resting stages should be expected to be more diverse than species without such strategies. Successive recruitment of diverse genotypes has been considered as a factor explaining high genetic diversity in prolonged phytoplankton blooms (Lebret *et al.*, 2012). Recently, a seascape analysis approach linked spatiotemporal genetic structure of a diatom bloom to local propagule banks (Godhe *et al.*, 2016). We suggest that the role of the propagule bank in maintaining and providing genetic diversity, and its role in the persistence and evolvability of a species, should be a focus of further studies.

Genetic diversity of blooms—paradox of the plankton?

While the paradox of the plankton (Hutchinson, 1961) at the interspecific level is already difficult to explain, it is even more so for genotypes of the same species. How can blooms be diverse, given that the best genotype should prevail? Blooms are predicted to quickly become dominated by a few particularly well-adapted genotypes and show strong patterns of LD as a result of selective sweeps (De Meester, 1996). Nevertheless, most studies describing genetic diversity of blooming phytoplankton populations report high intraspecific variation (Rynearson and Armbrust, 2005; Alpermann *et al.*, 2010; Lebret *et al.*, 2012; Dia *et al.*, 2014; Krueger-Hadfield *et al.*, 2014), indicating that some sexual reproduction is occurring, mutation rates are high, or the direction of selection is rapidly fluctuating.

Several mechanisms have been suggested that are able to maintain diversity in asexually growing phytoplankton bloom populations. First, spatially and temporarily fluctuating selection has been proposed as an important process in heterogeneous environments (Bell, 1997; Rynearson and Armbrust, 2004; Gsell *et al.*, 2012; Godhe *et al.*, 2016). Second, NFDS, i.e. a relatively higher fitness of rare genotypes can cause oscillation and coexistence of multiple clonal lineages. As shown for experimental dinoflagellate populations, NFDS is intensified at high population densities (Minter *et al.*, 2015), suggesting that this mechanism may be particularly

effective during bloom conditions. Parasitism, which commonly occurs in phytoplankton blooms (Kagami *et al.*, 2007; Chambouvet *et al.*, 2008), has been considered an example of NFDS (the "kill-the-winner" hypothesis, Thingstad, 2000). Genotype-specific parasitism has been reported for some phytoplankton species (De Bruin *et al.*, 2004; Figueroa *et al.*, 2008) and shown to drive genetic diversification in blooms (Gsell *et al.*, 2013), clearly an area that deserves further research. Finally, other, less studied biotic interactions such as selective grazing and virus infection likely affect genetic diversity. Grazer presence, for example, enhanced clonal richness and evenness in experimental diatom populations (Sjöqvist *et al.*, 2014).

A role of viruses in genetic diversity of eukaryotic phytoplankton has not been systematically addressed so far, despite the well-established importance of virus infections in population dynamics (Suttle, 2007; Gustavsen *et al.*, 2014). Effects of viruses on genotype succession have been demonstrated in studies that include cyanobacteria (Muhling *et al.*, 2005) and prasinophytes (Baudoux *et al.*, 2015). Thus, phytoplankton–virus interactions probably influence the genetic diversity of phytoplankton populations and may be a source of new genes as well. This is an important area for further research from a population genetic standpoint.

PATTERNS AND PROCESSES GENERATING POPULATION GENETIC STRUCTURE

Processes generating population genetic structure

In order for populations to become genetically differentiated, one or more of the following processes are involved: natural selection, mutation or genetic drift, in combination with limited gene flow (i.e. realized dispersal). Dispersal barriers are never absolute and can be of physical, environmental or biological nature.

Population genetic structure is typically analyzed by determining genetic differentiation between sampling locations that are operationally defined "populations". One such widely used quantitative statistic is the fixation index, F_{ST}, a measure based on allele frequencies in populations (see Box 1). F_{ST} can range from 0 to 1, where values above 0.25 are considered to signify large genetic differentiation (Wright, 1978). Rynearson and Armbrust (Rynearson and Armbrust, 2004; Rynearson *et al.*, 2006b) were among the first to show that a marine diatom was made up of genetically differentiated populations in different but connected estuaries. However, later, it was shown that these are probably two cryptic species, where the second has formed after a recent whole-genome duplication event (Koester *et al.*, 2010). Nevertheless, similar observations of within-species differentiation were corroborated in other marine dinoflagellates and diatoms (Nagai *et al.*, 2007; Adams *et al.*, 2009; Godhe and Härnström, 2010). On the other hand, populations of a North Sea diatom (Evans *et al.*, 2005b) showed low levels of genetic differentiation in accordance with the idea of unlimited dispersal. In limnic phytoplankton, populations in different lakes showed moderate to high genetic differentiation (Logares *et al.*, 2009; Lebret *et al.*, 2013; Van Den Wyngaert *et al.*, 2015). These and other studies demonstrate that gene flow is usually, but not always, limited in phytoplankton, but were not able to address the underlying processes and mechanisms.

Physical forces that affect realized dispersal include oceanic currents, lack of or presence of hydrological connections among lakes, or simply geographic distance. The latter process leads to a pattern of genetic distance that increases with geographic distance between populations, also called isolation-by-distance (IBD) (Slatkin, 1987). The latter can be viewed as a null model in the absence of further complicating processes such as eddies, ocean fronts or environmental gradients. This pattern of genetic IBD is due to a decrease in the frequency of realized dispersal with geographic distance. In phytoplankton, there are only a limited number of studies to date addressing the IBD pattern. Nagai *et al.* (2007) showed a clear genetic IBD in populations of the marine dinoflagellate *Alexandrium tamarense* along the Japanese coast as did (Demura *et al.*, 2014) for the marine raphidophyte *Chattonella marina*. On the other hand, no IBD was found in a study of the marine diatom *Skeletonema marinoi* (Godhe *et al.*, 2013). Instead, local hydrologic connectivity was shown to structure the populations of that species (Godhe *et al.*, 2013; Sjöqvist *et al.*, 2015). In freshwater habitats, connectivity (streams) between lakes does not appear to enhance gene flow among populations of the raphidophyte *Gonyostomum semen*, and instead geographic distance appears to be more important at the regional level (Sassenhagen *et al.*, 2015).

Biological processes that may structure populations include local adaptation, founder/priority effects and reproductive isolation. These processes have been little explored in phytoplankton, as most studies to date have focused on determining how genetic variation is partitioned in phytoplankton populations, rather than how the differentiation came about. Local adaptation means that resident individuals have a higher fitness in their local environment than individuals from a different environment (Pigliucci, 2001). This process can happen

either by lineage selection on new arrivals (Wade, 2000), thereby preventing them from establishing, or by natural selection on standing genetic variation leading to selection of individuals best adapted to the local environment (Orsini *et al.*, 2013). These two processes will lead to different population genetic patterns, where the former will show correlation with environment (Nosil, 2008), and the latter likely an IBD/colonization pattern (Orsini *et al.*, 2013). Correlation of phenotypes with the environment has been observed in the Baltic diatom *S. marinoi*, in which both salinity and silica concentration appear to be involved (Sjöqvist *et al.*, 2015; Godhe *et al.*, 2016). Local adaptation has also been observed in some other species, to, for example, temperature or salinity (Boenigk *et al.*, 2007; Zhang *et al.*, 2014; Rengefors *et al.*, 2015), but has not been coupled to population genetic structure. Although local adaptation has been confirmed experimentally, there are still no studies investigating the genomic regions or loci in genes that may be under selection, and which would allow us to understand the underlying process. The lack of these types of data largely depends on the fact that most studies to date have utilized neutral markers such as microsatellites, or AFLP markers, which cannot easily be connected to genes. Here, population genomic approaches (e.g. RAD sequencing) clearly have a major role to play in elucidating how phenotypes may map onto the genomic space, and which genomic regions correspond to local adaptation.

Founder effects are a special case of a one-time strong genetic drift event. A small number of founding individuals, upon rapid propagation, determine the genetic structure of a population for a long time following the initial colonization. This effect involves the advantage of being first and quickly monopolizing resources, and thereby preventing new colonizers establishing easily. With time, and especially in cases where a resting propagule bank is established to replenish the population, local adaptation may enforce patterns of the initial founder effect. This combined process is referred to as the Monopolization Hypothesis (De Meester *et al.*, 2002) and was developed as an explanation of patterns observed in cladocerans, but may likely apply for phytoplankton with resting stages (e.g. Sassenhagen *et al.*, 2015; Sefbom *et al.*, 2015). The resulting population genetic patterns expected following founder effects is high genetic differentiation compared with nearby populations along with reduced genetic diversity. Founder effects have been used to explain patterns in marine (Godhe and Härnström, 2010) and limnic phytoplankton (Sassenhagen *et al.*, 2015). Priority effects have been demonstrated experimentally in cyanobacterial phytoplankton (Gremberghe *et al.*, 2009) and diatoms (Sefbom *et al.*, 2015).

Another key feature that may affect population genetic structure is population size, which is likely to differ profoundly between phytoplankton and higher organisms. Regardless of the effective population size (N_e, Box 2) discussed in the previous section, the census size of phytoplankton populations can have a dramatic effect on dispersal rates. Providing that all other factors remain identical, such as rate of dispersal, the actual number of dispersers will be higher in a larger population. However, studies on actual migration rates are few (except Godhe *et al.*, 2013) and population genetic studies on non bloom-forming or rare phytoplankton species are missing. The genetically effective population size (N_e) is a key parameter to determine the relative effects of selection (and hence adaptation) versus genetic drift (Box 1). Fluctuations in phytoplankton populations are commonplace and will have marked effects on N_e. If we hypothesize that census size and N_e are correlated, we would expect that species with widely fluctuating abundance have generally lower genetic diversity than those with constant intermediate levels of abundance. Currently, we do not know how rare species really become once they are so rare that they are missed in classical phytoplankton assessments, and we do not always know the correct spatial scale for describing a species as absent. For example, Utermöhl-counts have a lower detection limit of ~10 cells per liter which would still amount to millions of co-occurring individuals at a scale of kilometer. Likewise, we have no idea about the critical absolute densities of phytoplankton necessary to complete their sexual life cycle, or the spatial scale over which this needs to be assessed. Specifically, what is the spatial scale that determines the boundaries of a population which functions as a sexually recombining gene pool determining N_e (the genetic neighborhood in population genetics), and how much patchiness can be tolerated before assumptions of free recombination break down? These hypotheses regarding the structure of phytoplankton populations require urgent testing, which may become possible once population genetic data of more eukaryotic phytoplankton species are available. New theory and additional empirical observations are also needed to understand the significance of N_e in phytoplankton population genetics.

What is a species—how to make use of population genetics

For phytoplankton, it is often extremely difficult to establish whether or not morphologically similar, but genetically differentiated populations represent intraspecific variations or are separate species according to the

Box 2. **Why all the fuss about effective population size?**

All life-cycle phases combined eventually produce a population with a certain effective population size N_e (see Glossary Box for definition). N_e is a key concept to understand Darwinian adaptation, determining the relative importance of random versus selective processes, and hence, the rate of adaptation (Wright, 1969). The absolute number of genotypes in a population also determines the total mutational target size and, hence, whether or not there are waiting times for critical beneficial mutations to occur, notwithstanding any standing genetic variation. Given the complex life cycles of phytoplankton, it is currently impossible to estimate effective population sizes for most phytoplankton species (but see Watts *et al.*, 2013). To begin with, we do not know from which stage to which other sampled stage one would define 1 generation, which is prerequisite for example for temporal methods to estimate N_e (Do *et al.*, 2014). This is exacerbated by the enormous fluctuations that typical populations experience, from near absence in ecological census data to bloom situations with $>10^6$ cells per liter. Note that the effective population size is proportional to the harmonic mean between maximal/minimal time points in case of fluctuating abundances (Nei *et al.*, 1975). This implies that the overall N_e is driven by the smallest population sizes when species are rare and/or a few genotypes dominate, data that in turn are seldom available. This raises the interesting possibility that despite occasional mass occurrences, phytoplankton adaptation may be subject to random processes such as drift and losses of genetic diversity when population abundance is low although, as discussed below, the large census size of natural populations may mean that mutation overrides many effects of N_e.

biological species concept. When it comes to harmful algal bloom species, it may be particularly important to distinguish biological species e.g. (Brosnahan *et al.*, 2010), while in other ecological studies it is not necessarily relevant. In order to determine whether algal isolates belong to the same biological species, interbreeding experiments have been conducted to establish whether there is reproductive isolation. Examples of these are the experiments by Coleman and Amato *et al.* (Coleman, 2001; Amato *et al.*, 2007), who also showed an accompanying difference in the ITS rDNA region in isolates that were reproductively isolated. In other cases, differences in the ITS rDNA region did not prevent interbreeding (D'Alelio *et al.*, 2009). Population genetic analyses can be of help here, as it is possible to assess gene flow without having to perform crossing experiments or identifying molecular markers that are informative regarding hybridization. For instance, very high F_{ST} levels and lack of admixture would indicate that populations are no longer sharing genetic material.

Several recent studies suggest that previously recognized cosmopolitan species are actually composed of multiple populations or even multiple species. These can either replace each other temporally (but with overlap) as in the case of the marine diatom *Pseudo-nitzschia multistriata* (Tesson *et al.*, 2014) and *Skeletonema costatum* (Gallagher, 1982) or co-exist sympatrically as in the freshwater *Asterionella formosa* (Van Den Wyngaert *et al.*, 2015). In the latter case, the authors suggest that the populations are actually separate species. Moreover, Read *et al.* (2013) reveal a pan genome of the coccolithophore *Emiliania* spp, suggesting that what was previously considered a single species, is actually composed of multiple species. These patterns may have arisen either due to environmental adaptation followed by staggered blooms and eventually reproductive isolation, or by reproductive isolation by for example genome duplication followed by adaptation (Koester *et al.*, 2010), both leading to limited gene flow and differentiation. If reproductive barriers persist, leading to reproductive isolation, speciation may occur (Saez *et al.*, 2003).

FUTURE PERSPECTIVES

The genomics revolution is likely to also change our view of phytoplankton population genetic processes. New high throughput sequencing techniques offer the possibility to overcome the limitations imposed by using only a handful of microsatellite markers or many but non-informative AFLP loci. The new methods allow for population genomic analyses, which can be widely defined as the simultaneous study of many loci or regions of the genome to understand evolutionary processes and combines genomic technologies and concepts with population genetic objectives (Luikart *et al.*, 2003). A larger number of loci may be identified for example by sequencing transcriptomes or entire or partial (e.g. RAD-tag sequencing) genomes. For

example, whole-genome sequencing showed that oceanic subpopulations of the cosmopolitan coccolithophore *Emiliania huxleyi* lack genes essential for recombination (Von Dassow *et al.*, 2015), suggesting that these populations reproduce entirely as asexual diplonts. Novel analyses of population genomics data can also provide (semi)quantitative estimates of meiotic and mitotic recombination based on the size and structure of haplotype blocks (Tsai *et al.*, 2008; Magwene *et al.*, 2011). Since many phytoplankton species will remain uncultured, such indirect methods are an important and possibly, the only tool to assess the relative frequencies of sex and asexuality in natural populations. Phytoplankton genomics will also continue to provide insight into the delineation of populations and species at the genomic level (Read *et al.*, 2013; Biller *et al.*, 2015). We note that there is some resistance among phytoplankton biologists to apply (any) species concept to entities that have widely divergent gene content, morphology, and physiology (e.g. compare morphotypes of *Emiliania huxleyi* in Paasche (2001)). Genomic data may provide a more objective basis for species delineation (Annenkova *et al.*, unpublished paper). Another avenue is that based on single-cell genome amplification, which not only allows for sequencing of uncultured phytoplankton, but also circumvents the selection bias resulting from cultivation in the laboratory (Yoon *et al.*, 2011; Kashtan *et al.*, 2014). Finally, a field that warrants further work is establishing culturing protocols that incorporate all life-cycle transitions, with the goal to conduct more realistic adaptation experiments in response to abiotic and biotic factors.

ACKNOWLEDGEMENTS

We thank all the contributors of the session "*Population genetic and genomic advances in understanding the evolution of plankton*" at the Joint Aquatic Sciences Meeting in Portland, Oregon, 2014, for inspiring this Horizon paper. We thank Susanne Landis for preparing the illustration in Figure 1. In addition, we would like to thank Russ Lande, Bill Cresko and Patrick Phillips for useful discussions during the preparation of this paper as well as the valuable comments of two anonymous reviewers. We also acknowledge the NordForsk Researcher Network PRODIVERSA.

FUNDING

Nordic Council of Ministers (Nordforsk grant 44881 to K.R. and A.K.); Swedish Research Council (grant 621-2012-3726 to K.R.); Academy of Finland (grant 251564 to A.K.); Kiel Excellence Cluster "The Future Ocean" (to T.B.H.R.), National Oceanic and Atmospheric Administration (NOAA: grant NA07NOS4780195 to A. M.W). This is MERHAB contribution #201.

REFERENCES

Adams, N. G., Trainer, V. L., Rocap, G., Herwig, R. P. and Hauser, L. (2009) Genetic population structure of *Pseudo-Nitzschia pungens* (Bacillariophyceae) from the Pacific Northwest and the North Sea. *J. Phycol.*, **45**, 1037–1045.

Alpermann, T., Beszteri, B., John, U., Tillmann, U. and Cembella, A. D. (2009) Implications of life-history transitions on the population genetic structure of the toxigenic marine dinoflagellate *Alexandrium tamarense*. *Mol. Ecol.*, **18**, 2122–2133.

Alpermann, T., Tillmann, U., Beszteri, B., Cembella, A. D. and John, U. (2010) Phenotypic variation and genotypic diversity in a planktonic population of the toxigenic marine dinoflagellate *Alexandrium tamarense* (Dinophyceae). *J. Phycol.*, **46**, 18–32.

Amato, A., Kooistra, W. H. C. F., Levialdi Ghiron, J. H., Mann, D. G., Pröschold, T. and Montresor, M. (2007) Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist*, **158**, 193–207.

Annenkova, N. V., Ahrén, D. and Rengefors, K. (unpublished paper) The evolutionary relationships of recently diverged microbial species (Dinophyceae) resolved using phylogenomic analysis.

Ayala, F. J. and Valentine, J. W. (1979) Genetic variability in the pelagic environment: a paradox? *Ecology*, **60**, 24–29.

Baudoux, A. C., Lebredonchel, H., Dehmer, H., Latimier, M., Edern, R., Rigaut-Jalabert, F., Ge, P., Guillou, L. *et al.* (2015) Interplay between the genetic clades of Micromonas and their viruses in the Western English Channel. *Environ. Microbiol. Rep.*, **7**, 765–773.

Bell, G. A. C. (1997) Experimental evolution in *Chlamydomonas*. 1. Short-term selection in uniform and diverse environments. *Heredity*, **78**, 490–497.

Bengtsson, B. O. (2003) Genetic variation in organisms with sexual and asexual reproduction. *J. Evol. Biol.*, **16**, 189–199.

Bibby, T. S., Mary, I., Nield, J., Partensky, F. and Barber, J. (2003) Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem. *Nature*, **424**, 1051–1054.

Biller, S. J., Berube, P. M., Lindell, D. and Chisholm, S. W. (2015) *Prochlorococcus*: the structure and function of collective diversity. *Nat. Rev. Microbiol.*, **13**, 13–27.

Blackburn, S. I. and Parker, N. (2005) Microalgal life cycles: encystment and excystment. In: Anderson, R. A.*Algal Culturing Techniques*. Elsevier Academic Press, Burlington, pp. 269–286.

Bolch, C. J. S., Blackburn, S. I., Hallegraeff, G. M. and Vaillancourt, R. E. (1999) Genetic variation among strains of the toxic dinoflagellates *Gymnodinium catenatum* (Dinophyceae). *J. Phycol*, **35**, 356–367.

Boenigk, J., Jost, S., Stoeck, T. and Garstecki, T. (2007) Differential thermal adaptation of clonal strains of a protist morphospecies originating from different climatic zones. *Environ. Microbiol*, **9**, 593–602.

Boyd, P. W., Dillingham, P. W., Mcgraw, C. M., Armstrong, E. A., Cornwall, C. E., Feng, Y. Y., Hurd, C. L., Gault-Ringold, M. *et al.* (2016) Physiological responses of a Southern Ocean diatom to complex future ocean conditions. *Nat. Clim. Change*, **6**, 207-+.

Brand, L. E. (1988) Review of genetic variation in marine phytoplankton species and the ecological implications. *Biol. Oceanogr.*, **6**, 397–409.

Brendonck, L. and De Meester, L. (2003) Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. *Hydrobiologia*, **491**, 65–84.

Brosnahan, M. L., Kulis, D. M., Solow, A. R., Erdner, D. L., Percy, L., Lewis, J. and Anderson, D. M. (2010) Outbreeding lethality between toxic Group I and nontoxic Group III Alexandrium tamarense spp. isolates: Predominance of heterotypic encystment and implications for mating interactions and biogeography. *Deep Sea Res. Part II Top. Stud. Oceanogr.*, **57**, 175–189.

Chambouvet, A., Morin, P., Marie, D. and Guillou, L. (2008) Control of toxic marine dinoflagellate blooms by serial parasitic killers. *Science*, **322**, 1254–1257.

Chepurnov, V. A., Mann, D. G., Sabbe, K. and Vyverman, W. (2004) Experimental studies an sexual reproduction in diatoms. *Int. Rev. Cytol.*, **237**, 91–154.

Coleman, A. W. (2001) Biogeography and speciation in the *Pandorina/Volvulina* (Chlorophyta) superclade. *J. Phycol.*, **37**, 836–851.

Collins, S. and Bell, G. (2004) Phenotypic consequences of 1,000 generations of selection at elevated CO2 in a green alga. *Nature*, **431**, 566–569.

Crow, J. F. (2008) Mid-century controversies in population genetics. *Annu. Rev. Genet.*, **42**, 1–16.

D'Alelio, D., Amato, A., Kooistra, W., Procaccini, G., Casotti, R. and Montresor, M. (2009) Internal Transcribed Spacer Polymorphism in *Pseudo-nitzschia multistriata* (Bacillariophyceae) in the Gulf of Naples: Recent Divergence or Intraspecific Hybridization? *Protist*, **160**, 9–20.

D'Alelio, D., Dubroca, L., Sarno, D., Zingone, A., D'alcalá, M. R. and Montresor, M. (2010) The time for sex: a biennial life cycle in a marine planktonic diatom. *Limnol. Oceanogr.*, **55**, 106–114.

De Bruin, A., Ibelings, B. W., Rijkeboer, M., Brehm, M. and 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.

De Meester, L. (1996) Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. *Ecoscience*, **3**, 385–399.

De Meester, L., Gómez, A., Okamura, B. and Schwenk, K. (2002) The Monopolization Hypothesis and the dispersal-gene flow paradox in aquatic organisms. *Acta Oecol.*, **23**, 121–135.

De Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C. *et al.* (2015) Eukaryotic plankton diversity in the sunlit ocean. *Science*, **348**, 1261605.

Demura, M., Nakayama, T., Kasai, F. and Kawachi, M. (2014) Genetic structure of Japanese Chattonella marina (Raphidophyceae) populations revealed using microsatellite markers. *Phycol. Res.*, **62**, 102–108.

Derelle, E., Ferraz, C., Rombauts, S., Rouze, P., Worden, A. Z., Robbens, S., Partensky, F., Degroeve, S. *et al.* (2006) Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. *Proc. Natl. Acad. Sci. USA*, **103**, 11647–11652.

Dia, A., Guillou, L., Mauger, S., Bigeard, E., Marie, D., Valero, M. and Destombe, C. (2014) Spatiotemporal changes in the genetic diversity of harmful algal blooms caused by the toxic dinoflagellate *Alexandrium minutum*. *Mol. Ecol.*, **23**, 549–560.

Dickey, T. D. (1991) The emergence of concurrent high-resolution physical and biooptical measurements in the upper ocean and their applications. *Rev. Geophys.*, **29**, 383–413.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J. and Ovenden, J. R. (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour.*, **14**, 209–214.

Edgar, S. M. and Theriot, E. C. (2003) Heritability of mantle areolar characters in *Aulacoseira subarctica* (Bacillariophyta). *J. Phycol.*, **39**, 1057–1066.

Evans, K. M., Kuhn, S. F. and Hayes, P. K. (2005a) High levels of genetic diversity and low levels of genetic differentiation in North Sea *Pseudo-nitzschia pungens* (Bacillariophyceae) populations. *J. Phycol.*, **41**, 506–514.

Evans, K. M., Kühn, S. F. and Hayes, P. K. (2005b) High levels of genetic diversity and low levels of genetic differentiation in North Sea *Pseudo-nitzschia pungens* (Bacillariophyceae) populations. *J. Phycol.*, **41**, 506–514.

Falkowski, P. G., Katz, M. E., Knoll, A. H., Quigg, A., Raven, J. A., Schofield, O. and Taylor, F. J. R. (2004) The evolution of modern eukaryotic phytoplankton. *Science*, **305**, 354–360.

Falkowski, P. G. and Knoll, A. H. (2007) *Evolution of primary producers in the Sea*. Elsevier Science Publishing, San Diego, US.

Field, C. B., Behrenfeld, M. J., Randerson, J. T. and Falkowski, P. (1998) Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, **281**, 237–240.

Figueroa, R. I., Bravo, I., Garcés, E. and Ramilo, I. (2006) Nuclear features and effect of nutrients on *Gymnodinium catenatum* (Dinophyceae) sexual stages. *J. Phycol.*, **42**, 67–77.

Figueroa, R. I., Garces, E., Massana, R. and Camp, J. (2008) Description, host-specificity, and strain selectivity of the dinoflagellate parasite *Parvilucifera sinerae* sp nov (Perkinsozoa). *Protist.*, **159**, 563–578.

Figueroa, R. I. and Rengefors, K. (2006) Life cycle and sexuality of the freshwater raphidophyte *Gonyostomum semen* (Raphidophyceae). *J. Phycol.*, **42**, 859–871.

Flores-Moya, A., Rouco, M., Jesus Garcia-Sanchez, M., Garcia-Balboa, C., Gonzalez, R., Costas, E. and Lopez-Rodas, V. (2012) Effects of adaptation, chance, and history on the evolution of the toxic dinoflagellate *Alexandrium minutum* under selection of increased temperature and acidification. *Ecol Evol*, **2**, 1251–1259.

Fryxell, G. A. (1983) *Survival strategies of the algae*, Vol, Cambridge University Press, New York.

Gallagher, J. C. (1980) Population genetics of *Skeletonema costatum* (Bacillariophyceae) in Narragansett Bay. *J. Phycol.*, **16**, 464–474.

Gallagher, J. C. (1982) Physiological variation and electrophoretic banding patterns of genetically different seasonal populations of *Skeletonema costatum* (Bacillariophyceae). *J. Phycol.*, **18**, 148–62.

Godhe, A., Egardt, J., Kleinhans, D., Sundqvist, L., Hordoir, R. and Jonsson, P. R. (2013) Seascape analysis reveals regional gene flow patterns among populations of a marine planktonic diatom. *Proc. R. Soc. Lond. B Biol. Sci.*, **280**, 20131599.

Godhe, A. and Härnström, K. (2010) Linking the planktonic and benthic habitat: Genetic structure of the marine diatom *Skeletonema marinoi*. *Mol. Ecol.*, **19**, 4478–4490.

Godhe, A., Kremp, A. and Montresor, M. (2014) Genetic and microscopic evidence for sexual reproduction in the centric diatom *Skeletonema marinoi*. *Protist*, **165**, 401–416.

Godhe, A., Sjöqvist, C., Sildever, S., Sefbom, J., Hardardóttir, S., Bertos-Fortis, M., Bunse, C., Gross, S. *et al.* (2016) Physical barriers and environmental gradients cause spatial and temporal genetic

differentiation of an extensive algal bloom. *J. Biogeogr.*, **7**, doi:10.3389/fmicb.2016.00517.

Graham, L. E., Graham, J. M. and Wilcox, L. (2009) *Algae*, 2nd edn. Benjamin Cummings Pearson, San Francisco.

Gremberghe, I., Vanormelingen, P., Van Der Gucht, K., Souffreau, C., Vyverman, W. and De Meester, L. (2009) Priority effects in environmental populations of the cyanobacterium *Microcystis*. *Environ. Microbiol.*, **11**, 2564–2573.

Gsell, A. S., De Senerpont Domis, L. N., Przytulska-Bartosiewicz, A., Mooij, W. M., Van Donk, E. and Ibelings, B. W. (2012) Genotype-by-temperature interactions may help to maintain clonal diversity in *Asterionella formosa* (Bacillariophyceae). *J. Phycol.*, **48**, 1197–1208.

Gsell, A. S., Domis, L. N. D. S., Verhoeven, K. J. F., Van Donk, E. and Ibelings, B. W. (2013) Chytrid epidemics may increase genetic diversity of a diatom spring-bloom. *ISME. J.*, **7**, 2057–2059.

Gustavsen, J. A., Winget, D. M., Tian, X. and Suttle, C. A. (2014) High temporal and spatial diversity in marine RNA viruses implies that they have an important role in mortality and structuring plankton communities. *Front. Microbiol.*, **5**, 10.3389/fmicb.2014.00703.

Hairston, N. G. and De Stasio, B. T. (1988) Rate of evolution slowed by a dormant propagule pool. *Nature*, **336**, 239–242.

Halkett, F., Simon, J.-C. and Balloux, F. (2005) Tackling the population genetics of clonal and partially clonal organisms. *Trends Ecol. Evol.*, **22**, 194–201.

Harris, G. P. (1987) *Phytoplankton Ecology: Structure, Function, and Fluctuation.* Chapman and Hall Ltd, London, UK.

Hartl, D. L. and Sunderland, M. A. (2000) *A Primer of Population Genetics*. Sinauer Associates, Sunderland, USA.

Huertas, I. E., Rouco, M., Lopez-Rodas, V. and Costas, E. (2010) Estimating the capability of different phytoplankton groups to adapt to contamination: herbicides will affect phytoplankton species differently. *New Phytol.*, **188**, 478–487.

Huertas, I. E., Rouco, M., López-Rodas, V. and Costas, E. (2011) Warming will affect phytoplankton differently: evidence through a mechanistic approach. *Proc. R. Soc. Lond. B. Biol. Sci.*, **278**, 3534–43.

Hutchinson, G. E. (1961) The paradox of plankton. *Am. Nat.*, **XCV**, 137–145.

Iglesias-Rodríguez, M. D., Schofield, O. M., Batley, J., Medlin, L. and Hayes, P. K. (2006) Intraspecific genetic diversity in the marine coccolitophore *Emiliania huxleyi* (Prymnesiophyceae): The use of microsatellite analysis in marine phytoplankton population studies. *J. Phycol.*, **42**, 526–536.

Irwin, A. J., Finkel, Z. V., Müller-Karger, F. E. and Troccoli Ghinaglia, L. (2015) Phytoplankton adapt to changing ocean environments. *Proc. Natl. Acad. Sci. USA*, **112**, 5762–6.

John, U., Groben, R., Beszteri, B. and Medlin, L. (2004) Utility of amplified fragment length polymorphisms (AFLP) to analyse genetic structures within the *Alexandrium tamarense* species complex. *Protist*, **155**, 169–179.

Kagami, M., De Bruin, A., Ibelings, B. W. and Van Donk, E. (2007) Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. *Hydrobiologia.*, **578**, 113–129.

Kaltz, O. and Bell, G. (2002) The ecology and sgenetics of fitness in *Chlamydomonas*. XII. Repeated sexual episodes increase rates of adaptation to novel environments. *Evolution*, **56**, 1743–1753.

Kashtan, N., Roggensack, S. E., Rodrigue, S., Thompson, J. W., Biller, S. J., Coe, A., Ding, H., Marttinen, P. *et al.* (2014) Single-cell genomics reveals hundreds of coexisting subpopulations in wild Prochlorococcus. *Science*, **344**, 416–20.

Koester, J. A., Swalwell, J. E., Von Dassow, P. and Armbrust, E. V. (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.

Kondrashov, A. S. (1988) Deleterious mutations and the evolution of sexual reproduction. *Nature*, **336**, 435–440.

Kremp, A., Oja, J., Letortorec, A. H., Hakanen, P., Tahvanainen, P., Tuimala, J. and Suikkanen, S. (2016) Diverse seed banks favour adaptation of microalgal populations to future climate conditions. *Environ. Microbiol.*, **18**, 679–691.

Krueger-Hadfield, S. A., Balestreri, C., Schroeder, J., Highfield, A., Helaouet, P., Allum, J., Moate, R., Lohbeck, K. T. *et al.* (2014) Genotyping an *Emiliania huxleyi* (prymnesiophyceae) bloom event in the North Sea reveals evidence of asexual reproduction. *Biogeosciences*, **11**, 5215–5234.

Lakeman, M. B., Von Dassow, P. and Cattolico, R. A. (2009) The strain concept in phytoplankton ecology. *Harmful Algae*, **8**, 746–758.

Lang, G. I., Rice, D. P., Hickman, M. J., Sodergren, E., Weinstock, G. M., Botstein, D. and Desai, M. M. (2013) Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations. *Nature*, **500**, 571–574.

Lebret, K., Kritzberg, E., Figueroa, R. I. and Rengefors, K. (2012) Genetic diversity within and genetic differentiation between blooms of a microalgal species. *Environ. Microbiol.*, **14**, 2395–2404.

Lebret, K., Kritzberg, E. and Rengefors, K. (2013) Population genetic structure of a microalgal species under expansion. *PLoS One*, **8**, 1–9 e82510.

Lewis, R. J., Jensen, S. I., DeNicola, D. M., Miller, V. I., Hoagland, K. D. and Ernst, S. G. (1997) Genetic variation in the diatom *Fragilaria capucina* (Fragilariaceae) along a latitudinal gradient across North America. *Plant Syst. Evol.*, **204**, 99–108.

Logares, R., Boltovskoy, A., Bensch, S., Laybourn-Parry, J. and Rengefors, K. (2009) Genetic diversity patterns in five protist species occurring in lakes. *Protist*, **160**, 301–317.

Lohbeck, K. T., Riebesell, U. and Reusch, T. B. H. (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. *Nat. Geosci.*, **5**, 346–351.

López-Rodas, V. L., Marva, F., Rouco, M., Costas, E. and Flores-Moya, A. (2008) Adaptation of the chlorophycean *Disctyosphaerium chlorelloides* to stressful acidic, mine metal-rich waters as a result of pre-selective mutations. *Chemosphere*, **72**, 703–707.

Luikart, G., England, P. R., Tallmon, D., Jordan, S. and Taberlet, P. (2003) The power of population genomics: from genotyping to genome typing. *Nat. Rev. Genet.*, **4**, 981–994.

Lynch, M., Gabriel, W. and Wood, A. M. (1991) Adaptive and demographic responses of plankton populations to environmental change. *Limnol. Oceanogr.*, **36**, 1301–1312.

Lynch, M. and Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, USA.

Magwene, P. M., Kayıkçı, Ö., Granek, J. A., Reininga, J. M., Scholl, Z. and Murray, D. (2011) Outcrossing, mitotic recombination, and life-history trade-offs shape genome evolution in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA*, **108**, 1987–1992.

Masseret, E., Grzebyk, D., Nagai, S., Genovesi, B., Lasserre, B., Laabir, M., Collos, Y., Vaquer, A. *et al.* (2009) Unexpected genetic

diversity among and within populations of the toxic dinoflagellate *Alexandrium catenella* as revealed by nuclear microsatellite markers. *Appl. Environ. Microbiol.*, **75**, 2037–45.

Medlin, L. K., Barker, G. L. A., Campbell, L., Green, J. C., Hayes, P. K., Marie, D., Wrieden, S. and Vaulot, D. (1996) Genetic characterization of *Emiliana huxleyi* (Haptophyta). *J. Mar. Systems*, **9**, 13–31.

Medlin, L. K., Lange, M. and Nothig, E. M. (2000) Genetic diversity in the marine phytoplankton: a review and a consideration of Antarctic phytoplankton. *Antarct. Sci.*, **12**, 325–333.

Minter, E. J. A., Watts, P. C., Lowe, C. D. and Brockhurst, M. A. (2015) Negative frequency-dependent selection is intensified at higher population densities in protist populations. *Biol. Lett.*, **11**, 20150192.

Moore, L. R., Rocap, G. and Chisholm, S. W. (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature*, **393**, 464–7.

Muhling, M., Fuller, N. J., Millard, A., Somerfield, P. J., Marie, D., Wilson, W. H., Scanlan, D. J., Post, A. F. *et al.* (2005) Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton. *Environ. Microbiol.*, **7**, 499–508.

Nagai, S., Lian, C., Hamaguchi, M., Matsuyama, Y., Itakura, S. and Hogetsu, T. (2004) Development of microsatellite markers in the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae). *Mol. Ecol. Notes*, **4**, 83–85.

Nagai, S., Lian, C., Shimada, H., Kaga, S., Yamauchi, H., Nishikawa, T., Kim, C.-H. and Hogetsu, T. (2007) Microsatellite markers reveal population genetic structure of the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae) in Japanese coastal waters. *J. Phycol.*, **43**, 43–54.

Nei, M., Maruyama, T. and Chakraborty, R. (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Ness, R. W., Morgan, A. D., Colegrave, N. and Keightley, P. D. (2012) Estimate of the spontaneous mutation rate in *Chlamydomonas reinhardtii*. *Genetics*, **192**, 1447.

Nielsen, R. and Slatkin, M. (2013) *An Introduction into Population Genetics*. Sinauer, Sunderland, USA.

Nosil, P. (2008) Heterogeneous genomic differentiation between walking-stick ecotypes: 'isolation by adaptation' and multiple roles for divergent selection. *Evolution*, **62**, 316–336.

Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J. and De Meester, L. (2013) Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Mol. Ecol.*, **22**, 5983–5999.

Paasche, E. (2001) A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. *Phycologia*, **40**, 503–529.

Parker, M. S., Mock, T. and Armbrust, E. V. (2008) Genomic insights into marine microalgae. *Annu. Rev. Genet.*, **42**, 619–45.

Pigliucci, M. (2001) *)* Phenotypic Plasticity: Beyond Nature and Nurture. Johns Hopkins University Press, Baltimore, MD.

Read, B. A., Kegel, J., Klute, M. J., Kuo, A., Lefebvre, S. C., Maumus, F., Mayer, C., Miller, J. *et al.* (2013) Pan genome of the phytoplankton *Emiliania* underpins its global distribution. *Nature*, **499**, 209–213.

Rengefors, K., Logares, R., Laybourn-Parry, J. and Gast, R. J. (2015) Evidence of concurrent local adaptation and high phenotypic plasticity in a polar microeukaryote. *Environ. Microbiol.*, doi:10.1111/1462-2920.12571.

Reusch, T. B. H. and Boyd, C. M. (2013) Experimental evolution meets marine phytoplankton. *Evolution*, **67**, 1849–1859.

Reynolds, C. S. (1990) Temporal scales of variability in pelagic environments and the response of phytoplankton. *Freshwat. Biol.*, **23**, 25–53.

Ribeiro, S., Berge, T., Lundholm, N. and Ellegaard, M. (2013) Hundred years of environmental change and phytoplankton ecophysiological variability archived in coastal sediments. *PLoS One*, **8**, e61184.

Rocap, G., Larimer, F. W., Lamerdin, J., Malfatti, S., Chain, P., Ahlgren, N. A., Arellano, A., Coleman, M. *et al.* (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature*, **424**, 1042–7.

Rynearson, T. A. and Armbrust, E. V. (2004) Genetic differentiation among populations of the planktonic marine diatom *Ditylym brightwellii* (Bacillariophyceae). *J. Phycol.*, **40**, 34–43.

Rynearson, T. A. and Armbrust, E. V. (2005) Maintenance of clonal diversity during a spring bloom of the centric diatom *Ditylum brightwellii*. *Mol. Ecol.*, **14**, 1631–40.

Rynearson, T. A., Newton, J. A. and Armbrust, E. V. (2006a) Spring bloom development, genetic variation, and population succession in the planktonic diatom *Ditylum brightwellii*. *Limnol. Oceanogr.*, **51**, 1249–1261.

Rynearson, T. A., Newton, J. A. and Armbrust, E. V. (2006b) Spring bloom development, genetic variation, and population succession in the planktonic diatom *Ditylum brightwellii*. *Limnol. Oceanogr.*, **51**, 1249–1261.

Saez, A. G., Probert, I., Geisen, M., Quinn, P., Young, J. R. and Medlin, L. K. (2003) Pseudo-cryptic speciation in coccolithophores. *Proc. Natl. Acad. Sci. USA*, **100**, 7163–7168.

Sassenhagen, I., Sefbom, J., Säll, T., Godhe, A. and Rengefors, K. (2015) Freshwater protists do not go with the flow: Population structure in *Gonyostomum semen* independent of connectivity among lakes. *Environ. Microbiol.*, **17**, 5063–5072.

Schlüter, L., Lohbeck, K. T., Gutowska, M. A., Groeger, J. P., Riebesell, U. and Reusch, T. B. H. (2014) Adaptation of a globally important coccolithophore to ocean warming and acidification. *Nat. Clim. Change*, **4**, 1024–1030.

Schlüter, L., Lohbeck, K. T., Gröger, J. P., Riebesell, U. and Reusch, T. B. H. (2016) Long-term dynamics of adaptive evolution in a globally important phytoplankton species to ocean acidification. *Sci. Adv.*, **2**, e1501660.

Sefbom, J., Sassenhagen, I., Rengefors, K. and Godhe, A. (2015) Priority effects in a planktonic bloom-forming marine diatom. *Biol. Lett.*, **11**, 20150184.

Simon, N., Cras, A.-L., Foulon, E. and Lemee, R. (2009) Diversity and evolution of marine phytoplankton. *C. R. Biol.*, **332**, 159–170.

Sjöqvist, C., Kremp, A., Lindehoff, E., Bamstedt, U., Egardt, J., Gross, S., Jonsson, M., Larsson, H. *et al.* (2014) Effects of grazer presence on genetic structure of a phenotypically diverse diatom population. *Microb. Ecol.*, **67**, 83–95.

Sjöqvist, C., Godhe, A., Jonsson, P. R., Sundqvist, L. and Kremp, A. (2015) Local adaptation and oceanographic connectivity patterns explain genetic differentiation of a marine diatom across the North Sea-Baltic Sea salinity gradient. *Mol. Ecol.*, **24**, 2871–2885.

Slatkin, M. (1987) Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.

Sohm, J. A., Ahlgren, N. A., Thomson, Z. J., Williams, C., Moffett, J. W., Saito, M. A., Webb, E. A. and Rocap, G. (2016) Co-occurring *Synechococcus* ecotypes occupy four major oceanic regimes defined by temperature, macronutrients and iron. *ISME J.*, **10**, 333–45.

Sorhannus, U. (2008) The effect of positive selection on a sexual reproduction gene in *Thalassiosira weissflogii* (Bacillariophyta):results obtained from maximum-likelihood and parsimony-based methods. *Mol. Biol. Evol.*, **20**, 1326–1328.

Speijer, D., Lukes, J. and Elias, M. (2015) Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *Proc. Natl. Acad. Sci. USA*, **112**, 8827–8834.

Stuart, R. K., Brahamsha, B., Busby, K. and Palenik, B. (2013) Genomic island genes in a coastal marine *Synechococcus* strain confer enhanced tolerance to copper and oxidative stress. *ISME. J.*, **7**, 1139–49.

Suttle, C. A. (2007) Marine viruses – major players in the global ecosystem. *Nat. Rev. Microbiol.*, **5**, 801–812.

Tahvanainen, P., Alpermann, T. J., Figueroa, R. I., John, U., Hakanen, P., Nagai, S., Blomster, J. and Kremp, A. (2012) Patterns of post-glacial genetic differentiation in marginal populations of a marine microalga. *PLoS ONE*, **7**, e53602.

Tesson, S. V. M., Legrand, C., Van Oosterhout, C., Montresor, M., Kooistra, W. H. C. F. and Procaccini, G. (2013) Mendelian inheritance pattern and high mutation rates of microsatellite alleles in the diatom *Pseudo-nitzschia multistriata*. *Protist*, **164**, 89–100.

Tesson, S. V. M., Montresor, M., Procaccini, G. and Kooistra, W. (2014) Temporal changes in population structure of a marine planktonic diatom. *PLoS One*, **9**, e114984.

Thingstad, T. F. (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnol. Oceanogr.*, **45**, 1320–1328.

Tibayrenc, M. and Ayala, F. J. (1991) Towards a population-genetics of microorganisms - the clonal theory of parasitic protozoa. *Parasitol. Today*, **7**, 228–232.

Tsai, I. J., Bensasson, D., Burt, A. and Koufopanou, V. (2008) Population genomics of the wild yeast *Saccharomyces paradoxus*: Quantifying the life cycle. *Proc. Natl. Acad. Sci. USA*, **105**, 4957–4962.

Van Den Wyngaert, S., Möst, M., Freimann, R., Ibelings, B. W. and Spaak, P. (2015) Hidden diversity in the freshwater planktonic diatom *Asterionella formosa*. *Mol. Ecol.*, **24**, 2955–2972.

Von Dassow, P., John, U., Ogata, H., Probert, I., Bendif, E. M., Kegel, J. U., Audic, S., Wincker, P. *et al.* (2015) Life-cycle modification in open oceans accounts for genome variability in a cosmopolitan phytoplankton. *ISME J.*, **9**, 1365–1377.

Von Dassow, P. and Montresor, M. (2011) Unveiling the mysteries of phytoplankton life cycles: patterns and opportunities behind complexity. *J. Plankton Res.*, **33**, 3–12.

Wade, M. J. (2000) Epistasis as a genetic constraint within populations and an accelerant of adaptive divergence among them. In Wolf, J. B., Brodie, E. D. B. and Wade, M. J. (eds.), *Epistasis and the evolutionary process*. Oxford University Press, Oxford, pp. 213–231.

Watts, P. C., Lundholm, N., Ribeiro, S. and Ellegaard, M. (2013) A century-long genetic record reveals that protist effective population sizes are comparable to those of macroscopic species. *Biol. Lett.*, **9**, 20130849.

Wood, A. M. (1988) Molecular biology, single cell analysis, and quantitative genetics: new evolutionary approaches in phytoplankton ecology. In: Yentsch, C. M., Mague, F. and Horan, P. K. *Immunochemcial Approaches to Coastal Estuarine and Oceanographic Questions*. Springer-Verlag, 41–71.

Wood, A. M., Everroad, R. C. and Wingard, L. M. (2005) Measuring algal growth rates in microalgal cultures. In: Anderson, R. A. *Algal Culturing Techniques*. Elsevier Academic Press, Burlington, pp. 269–285.

Wood, A. M., Lande, R. and Fryxell, G. A. (1987) Quantitative genetic analysis of morphological variation in an Antarctic diatom grown at two light intensities. *J. Phycol.*, **23**, 42–54.

Wright, S. (1969) *Evolution and the Genetics of Populations, Vol. 2 The Theory of Gene Frequencies*. University of Chicago Press, Chicago.

Wright, S. (1978) *Evolution and the Genetics of Populations, Vol. 4 Variability Within and Among Natural Populations*. University of Chicago Press, Chicago.

Yoon, H. S., Price, D. C., Stepanauskas, R., Rajah, V. D., Sieracki, M. E., Wilson, W. H., Yang, E. C., Duffy, S. *et al.* (2011) Single-cell genomics reveals organismal interactions in uncultivated marine protists. *Science*, **332**, 714–717.

Zeyl, C. and Bell, G. (1997) The advantage of sex in evolving yeast populations. *Nature*, **388**, 465–468.

Zhang, Y., Klapper, R., Lohbeck, K. T., Bach, L. T., Schulz, K. G., Reusch, T. B. H. and Riebesell, U. (2014) Between- and within-population variations in thermal reaction norms of the coccolithophore *Emiliania huxleyi*. *Limnol. Oceanogr.*, **59**, 1570–1580.

Zwirglmaier, K., Jardillier, L., Ostrowski, M., Mazzard, S., Garczarek, L., Vaulot, D., Not, F. and Massana, R. (2008) Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. *Environ. Microbiol.*, **10**, 147–161.