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Genetic diversity in the marine phytoplankton: a review and a consideration of Antarctic phytoplankton

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Abstract: Molecular analysis of phytoplankton population structure has lagged behind other groups and has usually been inferred from physiological data determined from relatively few clones. Nearly every physiological measurement has shown that no single clone of any phytoplankton species can be considered truly representative of that species. One important reason why studies of phytoplankton population structure are perhaps 20 or more years behind those of other organisms is because of the necessity to establish clonal cultures prior to genetic analysis and the inability to perform fine-scale sampling under most conditions. Isozyme analysis, performed for a few species, has revealed heterozygosity between populations. In addition, fingerprinting analyses, such as Random Amplified Polymorphic DNAs (RAPDs) or multi-locus probes, have shown that phytoplankton blooms are not mono-clonal, are highly diverse and isolates are related by geographic origin. In the Southern Ocean, only two studies have been made of the population structure of phytoplankton. The first, based on quantitative genetic analysis of morphometric features, suggests that there is sufficient genetic variation in populations of *Thalassiosira tumida* to allow speciation in terms of major shifts in morphology under conditions of continued directional selection. The second, using sequence data from the noncoding regions of the internal transcribed spacer region (ITS) in the ribosomal cistron as a molecular marker, shows that populations of *Phaeocystis antarctica* within continental water masses are homogenous with little evidence of population structure. Populations found within the Antarctic Circumpolar Current are genetically distinct from others, suggesting the currents also play an important role in determining population structure in phytoplankton populations.

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Introduction

Genetic diversity at the population level of a species plays an important role in the interactions of a species with the environment. These interactions will structure the ecosystem, so that spatial and temporal partitioning of genetic diversity will occur. Such structuring has seldom been measured in the marine planktonic community and studies of genetic diversity are virtually non-existent in pelagic ecosystems. Until recently, it was assumed that marine organisms with high dispersal capacities would be genetically homogeneous over their entire range, with no geographically isolated populations. Support for this has come mainly from phenotypic comparisons based initially on biogeographic studies of net phytoplankton and later on isozyme variation. Studies of phytoplankton diversity and population structure have lagged behind those of other organisms because of their small size, lack of morphological markers, and ability to bring into culture only a small part the known biodiversity. The lack of knowledge of their breeding systems makes genetic or demographic studies difficult. Logistical problems of collecting samples for long term seasonal studies in open ocean environments or for doing fine-scale sampling are two more reasons. The former is particularly true for polar waters like the Southern Ocean; strong seasonality and heavy ice cover prevent researchers from using appropriate sampling strategies and winter data are lacking for phytoplankton as well as other groups.

Protists in the sea

Protists and prokaryotes numerically dominate the world's oceans. In this microscopic community, the small photosynthetic organisms or marine phytoplankton contribute the bulk of primary production in oceanic and neritic waters and have long been considered to be high dispersal taxa with large population sizes. If we rely solely on net samples and bulk process measurements, such as chlorophyll *a* (chl *a*) and ^{14}C biomass estimates, we soon realise how very little we really know about the marine phytoplankton. Observations on net plankton have permitted large scale biogeographic comparisons to be made (Hasle 1976) and have permitted broad generalizations to be made regarding (1) the cell morphology best suited to a particular light regime, thus enabling predictions to be made about succession in different water masses (Reynolds 1997) and (2) the types of dinoflagellates and other flagellate species that can be found along a mixing stratification or turbulence regime (Smayda 2000). Such ordering of species allows for a prediction of species occurrences in time and space if the abiotic factors

constructing such arrays are known. Nevertheless, whole water samples analysed with flow cytometry, epifluorescence microscopy and HPLC have revealed other new insights into the marine phytoplankton, such as new size classes in the plankton (e.g. picoplankton < 3 μm), new picoplanktonic algal classes (Pelagophyceae and Bolidophyceae), and the domination of the picoeukaryotes and *Prochlorococcus/ Synechococcus* in open ocean oligotrophic ecosystems (Johnson & Sieburth 1979, 1982, Chisholm *et al.* 1988, Waterbury *et al.* 1979, Anderson *et al.* 1993, 1996, Guillou *et al.* 1999).

Phytoplankton population structure in the Antarctic

Phytoplankton studies in the Antarctic can be traced back to the middle of the last century when many expeditions were made focusing on taxonomic and biogeographic surveys. El-Sayed & Fryxell (1993) have traced the changes in the history of Antarctic phytoplankton research as emphasis has changed from cataloguing to measuring of bulk processes to remote sensing, permanent moorings and modelling. Early studies concentrated on the distribution of net plankton and showed that most larger planktonic diatoms were dominant in open waters and near the ice edge. Patchiness in open waters is well documented with relatively low levels of standing crop, comparable to that of the oligotrophic areas of the world oceans (Priddle *et al.* 1986). Large pulses of phytoplankton growth are regularly recorded along the dynamic ice edge, at frontal zones, and in neritic waters (Bathmann *et al.* 2000).

As in other oceans, studies of the nano- and, to a lesser extent, the pico-fractions of the Antarctic phytoplankton have increased considerably in the last two decades (see review in Knox 1994, Kang *et al.* 1993, 1997, Bidigare *et al.* 1996. These fractions can contribute up to 70% of the total chl *a* (Knox 1994) and appear to exist year-round in the plankton with their blooms tending to last longer than those of the net plankton (Kang *et al.* 1997, Kopczynska *et al.* 1998).

Seasonal variations in abundance are marked with many species exhibiting extreme morphological variability that can be indicative of environmental conditions, e.g. winter and summer forms of *Eucampia antarctica* (Castr.) Mang. (Fryxell & Praasad 1990). In fact, physical processes of horizontal circulation and vertical mixing play a dominant role in controlling the distribution and abundance of Antarctic phytoplankton (El-Sayed & Fryxell 1993, Kang & Fryxell 1993, Bathmann *et al.* 1997). Temporal studies of Antarctic phytoplankton are notably lacking because of the logistic problems involved in time series sampling in the Antarctic (Clarke & Leaky 1996).

Despite these new insights into phytoplankton biodiversity, knowledge of population structure or genetic diversity in the marine phytoplankton on a spatial and temporal scale is still very limited.

Genetic diversity in the plankton

Although as early as 1975, Doyle (1975) hypothesized that planktonic algae must consist of a multitude of competing genotypes, it has been assumed that these taxa may have little genetic structure over very large geographic areas. Marine planktonic organisms live in an ever-changing three-dimensional environment and it has been assumed that organisms with distributions determined by current systems must be highly dispersed with no trace of genetic structure. Recent evidence suggests that speciation and dispersal mechanisms in marine planktonic organisms may be very different from those on land (Palumbi 1992). Thus, generalizations about terrestrial plant diversity and population structure may not apply to marine ecosystems, making our knowledge of phytoplankton genetic diversity even less certain.

With the advent of nucleic acid methods, however, the older view on the lack of genetic structure in the marine phytoplankton has been seriously challenged. Genetic structure and physical, spatial partitioning within biogeographic regions is now known. The idea of a single globally distributed species is no longer tenable. Temporal genetic change can often be greater than spatial change or change between species, calling into question the idea of temporal stasis (Brand 1982, Gallagher 1980, 1982, Hedgecock 1994). This may very well apply to bloom populations. The rate of genetic change can and does occur on ecological time scales (Palumbi 1992). Reasons for this are unclear but such changes may play a role in determining how local adaptations and speciation can occur in apparently homogeneous populations. The concept of a 'super species' with the ability to exploit a wide spectrum of environmental conditions may lay the groundwork for temporal genetic change.

History of molecular studies in phytoplankton

An excellent introduction into the variety of molecular techniques available for use in studying biodiversity at all taxonomic levels can be found in Karp *et al.* (1998) and a review of biodiversity in the marine environment can be found in Ormond *et al.* (1998). Much of our limited knowledge about phytoplankton genetic diversity stems from the difficulty of finding polymorphic markers for ecological genetic studies. Most molecular genetic markers used in early studies (e.g. isozymes) evolve at such slow rates that closely related populations cannot be distinguished. This fact has undoubtedly influenced some of the early assumptions about the lack of genetic diversity in marine phytoplankton. The emergence of high resolution DNA fingerprinting techniques overcomes this problem and has thus opened up a previously intractable area for investigation.

DNA fingerprinting (Fp) rests on the fact that genomes of most eukaryotes contain tandemly arrayed, highly repetitive DNA sequences called VNTRs (variable number tandem repeats). The high variability at each locus and the large

number of loci, these multi-locus fingerprints (MLP's) make literally individual-specific but can provide, ironically, too much variation in the case of population studies. Banding patterns can quickly become so complex that they cannot be analysed in terms of allele frequencies (the data of population genetic measures). Random amplified polymorphic DNAs (RAPDs) are multi-locus fingerprints with less quantitative resolution. The main disadvantage of RAPDs is that they lack analytical power. First, RAPDs are dominant Mendelian markers. Second, they are obtained using PCR and the peculiarities of RAPD PCR kinetics combined with unique/unusual properties of genomic organization of the organisms of interest can cause problems when applying RAPDs at narrow population levels where the signal to noise ratio will/can be too high. For these reasons RAPDs must be coupled with other data measurements in an investigation of population level genetic diversity. AFLPs (Amplified Fragment Length Polymorphisms) are similar to RAPDs but rely on the restriction enzyme digestion of genomic DNA followed by the PCR amplification of these fragments using special oligonucleotide adapters to the DNA fragments to generate the multi-locus fingerprints (Vos *et al.* 1995).

The shortcomings of MLPs have prompted development of methods that offer the same high resolution but with electrophoretic patterns that are easier to interpret in natural population level studies and that lend themselves to standard population genetic methods of analysis. These are the so-called single-locus fingerprinting probes (SLPs). Among these are microsatellites, which are short repeated sequences of 1–5 nucleotides in length such as (TA)n (Fig. 1). Microsatellites can be selected that provide very high levels of heterozygosity so that the resolution obtainable with a given probe can be selected to match the population question. This is why microsatellites offer such enormous promise for natural population studies. Moreover, microsatellite fingerprinting can be converted into a PCR-based assay. Finding microsatellites involves the establishment of a genomic library for each target species, screening the library with oligonucleotide probes, sequencing the positive clones, and using these as probes.

Phytoplanktologists do not have the luxury of walking down to the shore and picking up 100 or so individuals for analysis. At present most genetic studies for phytoplankton must rely on clonal cultures for their analyses. These single-cell isolations, made from natural populations, can be difficult to perform at sea. If only 10–30% of clonal isolates from natural populations survive, then this may mean that the range of genetic diversity determined from a bank of clonal isolates may not be a true reflection of the genetic diversity in the original population and may not be adequate for the level of genetic diversity

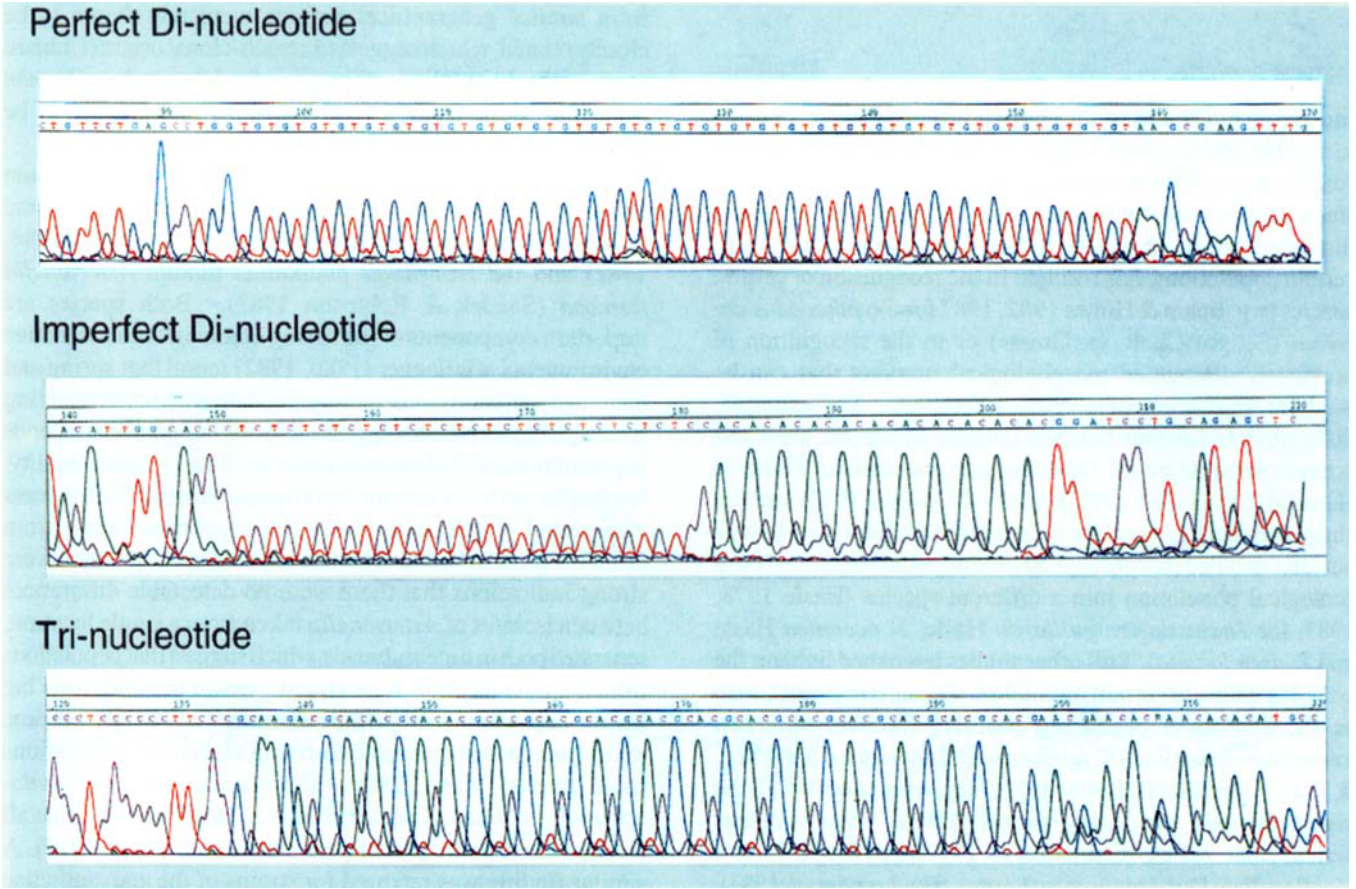

Fig. 1. An example of three different kinds of microsatellites found in *Emiliania huxleyi* (Rodriguez *et al.* unpublished data).

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being addressed (see Hillis *et al.* 1996, table 2.1 for a detailed description of how many samples need to be taken to estimate genetic diversity given certain confidence intervals). In many algal groups, life histories are incomplete or unknown, and if the algae undergo sexual reproduction during culturing, then this may also alter the type of genetic analysis performed or the interpretation of the data. In addition the ploidy level of the clonal isolates may be unknown, especially in the haptophytes, which have a haplo-diploid life cycle, and in many heterokonts, where the life cycle is completely unknown. Pre-established cruise tracks may mean that oceanic populations can only be sampled at depth along a transect rather than in a gridlike fashion as is usually done for hierarchical population studies. Even when research programmes, such as the Arabian Sea project, are able to provide fine-scale sampling in a grid like fashion, no genetic studies were included as part of the research program. Samples can be unknowingly taken from two water masses if current regimes in the study area are not well known, which may also bias sampling strategies. Despite these many problems, significant genetic diversity has been inferred to exist within and between phytoplankton populations primarily from physiological/biochemical measurements (Waterbury *et al.* 1986, Brand 1989, Wood & Leatham 1992, Partensky *et al.* 1993). These data have been used to speculate on hidden biodiversity and on temporal and spatial structuring of genetic diversity or gene flow.

Isozyme analysis

Most early molecular studies have relied on isozyme analysis to resolve species-level issues concerning microalgae with conflicting or little morphological resolution rather than to study genetic structure within bloom populations. In many instances, genetic diversity, detected by isozyme analysis in certain populations, has resulted in the recognition of cryptic species (e.g. Beam & Himes 1982, 1987 for *Crypthecodinium cohnii* (Seligo) Chatt. ex Grasse) or in the recognition of previously discounted morphological markers that can be used for separation of a species complex. For example, differences in isozyme banding patterns in neretic, shelf and oceanic populations of *Thalassiosira pseudonana* Hasle & Heimdal (Brand *et al.* 1981, Murphy & Guillard 1976) initially suggested that the species was composed of clinal populations but later detailed morphological investigations separated each ecological population into a different species (Hasle 1978, 1983, for *Thalassiosira guillardii* Hasle, *T. oceanica* Hasle and *T. pseudonana*). Still other studies have shed light on the complex nature of certain microalgal species complexes, such as the PSP-toxin producing dinoflagellate *Alexandrium tamarense* (Leb.) Bal./*A. fundyense* Bal./*A. catenella* (Whed. & Kof.) (Cembella & Taylor 1986, Hayhome *et al.* 1989), the ciguatera-toxin producing dinoflagellate *Gambierdiscus toxicus* Adac. & Fuk. (Chinain *et al.* 1997), and the freshwater dinoflagellate *Peridinium volzii* Lemm. (Hayhome *et al.* 1987). In the first study, isozyme analyses showed a high degree of enzymatic heterogeneity among isolates from the west coast of the United States with isolates from the same locality being most closely related (Cembella & Taylor 1986). A similar later analysis of east coast populations revealed a relative lack of enzymatic heterogeneity (Hayhome *et al.* 1989). These data have suggested a common origin for the east coast populations and supported a dispersal hypothesis along the east coast of the United States from Canada to Massachusetts that has been related to hydrographic events dissipating a massive red tide that occurred in 1972. *Alexandrium* species have been studied in more detail using sequence analysis of rapidly evolving genomic regions, such as the ITS and the D1/D2 region of the LSU rRNA gene. These analyses have shown that isolates of the *Alexandrium tamarense/fundyense/catenella* species complex are related by geographic origin rather than by morphological affinities (Scholin *et al.* 1995), which was originally indicated by the isozyme analysis. Hypotheses have been put forward to explain the world-wide biogeographic dispersal of ancestral populations from the Pacific. Furthermore, *Alexandrium* isolates with similar isozyme patterns from two different locations will interbreed more successfully than will isolates from the same locations but with different isozyme patterns (Sako *et al.* 1990). Isolates of *Peridinium volzii* from the same location were also found to be more closely related although quite distinct between locations. In contrast, the isolates of *Gambierdiscus toxicus* from similar geographical regions were not shown to be closely related, which suggested a multi-clonal origin (Chinain *et al.* 1997). Populations of the green freshwater alga, *Gonium pectorale* Müll, from several locations also appear to be multiclonal (Sako *et al.* 1991).

Genetic structure within bloom populations of phytoplankton using isozymes has only been studied in the marine planktonic diatom *Skeletonema costatum* (Grev.) Cleve (Gallagher 1980, 1982) and the freshwater planktonic diatom *Asterionella formosa* (Soudek & Robinson 1983). Both species are important components of the spring bloom in their respective environments. Gallagher (1980, 1982) found that spring and autumn populations were more genetically distinct than sibling species of higher plants, suggesting strong temporal distinctions in populations of *Skeletonema costatum* from the same locality. Neither the spring or autumn bloom populations of *Skeletonema* were clonal. Populations of *Asterionella formosa* Hass. from different lakes were also significantly different, but there were strong indications that there were no detectable differences between isolates of *Asterionella* taken from a single location, separated both in time and space, which suggest that populations from a single location were clonal. Given the long time lag before populations of certain diatoms (20–40 yr) become sexual, it may be reasonable to expect that clonal populations could develop (Mann 1988). A more limited study of *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle revealed that all isolates taken from a bloom were unique (Skov *et al.* 1997). A similar finding was reported for strains of the zooxanthellae algae *Symbiodinium microadriaticum Freud.* (Schoenberg &

Trench 1980) inhabiting a variety of hosts. Both of these latter studies suggest that cryptic species are likely present (see McNally *et al.* 1994).

Fingerprinting techniques

Fingerprinting techniques are only just beginning to appear in phytoplankton studies. RAPDs and AFLPs have been used to analyse bloom populations of the prymnesiophyte *Emiliania huxleyi* (Loh.) Hay & Mohl. (Barker *et al.*1995, Medlin *et al.* 1996), of the dinoflagellate *Alexandrium tamarense* (John *et al.* unpublished data) and of the diatom *Fragilaria capucina* Desm. (Lewis *et al.* 1996). Each study has found highly diverse populations within temporal and spatial scales. Multilocus fingerprinting analysis in the diatom *Skeletonema costatum* using random priming of the M13 universal primer has revealed extreme genetic variation between isolates, but reassuringly nearly identical banding patterns in the same strain separated and maintained in two laboratories apart for more than 20 years (Croyer *et al.* 1996). Both spatial and temporal differences were found in populations of the dinoflagellate *Gymnodinium catenatum* Grah. among Australian and global populations, using RAPD fingerprinting data (Boalch *et al.* 1999). Despite these differences, it was not possible to definitely define the mode of introduction into Australian waters, although it is clear from sediment records that the introduction is quite recent. AFLP banding patterns in isolates of the dinoflagellate *Alexandrium tamarense* from the Orkney Islands were correlated with toxin patterns as determined by HPLC analysis (John *et al.* unpublished data).

Microsatellites

Microsatellites have only been used in a single field study of the large planktonic diatom *Ditylum brightwellii* (West) Grun. ex V.H. (Rynearson & Amburst 1999) from both spring and autumn blooms in Puget Sound, Washington. The spring bloom was clonal or at least showed low diversity, whereas the autumn bloom appeared to contain at least six genotypes. Microsatellites have also been investigated in the coccolithophorid *Emiliania huxleyi* (Fig. 1). Most microsatellites were (GT)n repeat units and appeared as multiple bands in PCR reactions, indicating that there were likely multiple alleles involved or nested microsatellites. Simpler banding patterns were generally monomorphic (Rodriguez *et al.* unpublished data).

Genetic studies in the Antarctic

Only two studies of the population structure of Antarctic phytoplankton have been made. Despite the wide variation in the abundances of the Antarctic phytoplankton, species appear to be endemic in Antarctic waters south of the Polar Front. Wood *et al.* (1987), using quantitative genetics were able to detect sufficient genetic variability in certain morphometric characters to support rapid change (speciation) in *Thalassiosira tumida* (Jan.) Hasle given continued directional selection.

The only study devoted to the direct measurement of genetic diversity in any species of Antarctic phytoplankton is that of Lange (1997) and Lange & Medlin (unpublished data). Using variation in the ITS regions of the ribosomal operon, they examined nine *Phaeocystis antarctica* Karst. strains collected from different locations around Antarctica, one *P. pouchetii* (Har.) Lag. strain from the Greenland Sea, one *P. globosa* Scheff. strain from the North Sea, two tropical *P. globosa* strains from Surinam and Palau, and *P. jahnii* Zing. from the Mediterranean (Fig. 2a). Large sequence variations were found in this region among *P. globosa* strains from different geographical regions, supporting the earlier hypothesis that *P. globosa* is a species complex (Medlin *et al.* 1994, Vaulot *et al.* 1994). One strain of *P. antarctica* (SK 22), isolated within the Antarctic counter current (ACC) was clearly related to *P. pouchetii*. The remaining eight *P. antarctica* strains isolated from the water masses defined by the Antarctic continental boundary currents (Fig. 2b) showed a high degree of similarity. Nevertheless, molecular analyses could separate *P. antarctica* strains into at least three different populations.

Further investigations are needed in the Southern Ocean because it is a region that represents 20% of the global oceans and because it represents a fairly unique ecosystem: the largest high/nutrient, low chlorophyll region of the world oceans Bathmann *et al.* (2000). It is now well established that the Southern Ocean plays an important role in global circulation and in many biochemical cycles, such as carbon and silica. Its time of origin at *c.* 30 m.y.a. with the opening of the Drake Passage provides a specific point in time from which molecular divergences can be dated.

Discussion

The advent of molecular biological techniques has greatly enhanced our ability to analyse phytoplankton and can provide a quantitative framework through which the diversity, structure and evolution of marine phytoplankton populations can be evaluated. Temporal and spatial structuring of biodiversity below the species level can only be accessed through molecular techniques. Thus, predictive models of the dynamics of ocean ecosystems can be formulated, and the idea of functional groups in the plankton developed. Many important questions concerning population structure in the marine phytoplankton can be addressed, such as:

1) How much genetic diversity is there and over what spatial scale should we be thinking?

2) How does diversity differ in phytoplankton communities in contrasting environments, e.g., neritic vs. oceanic or eutrophic vs. oligotrophic?

3) What is the balance between spatial and temporal genetic change?

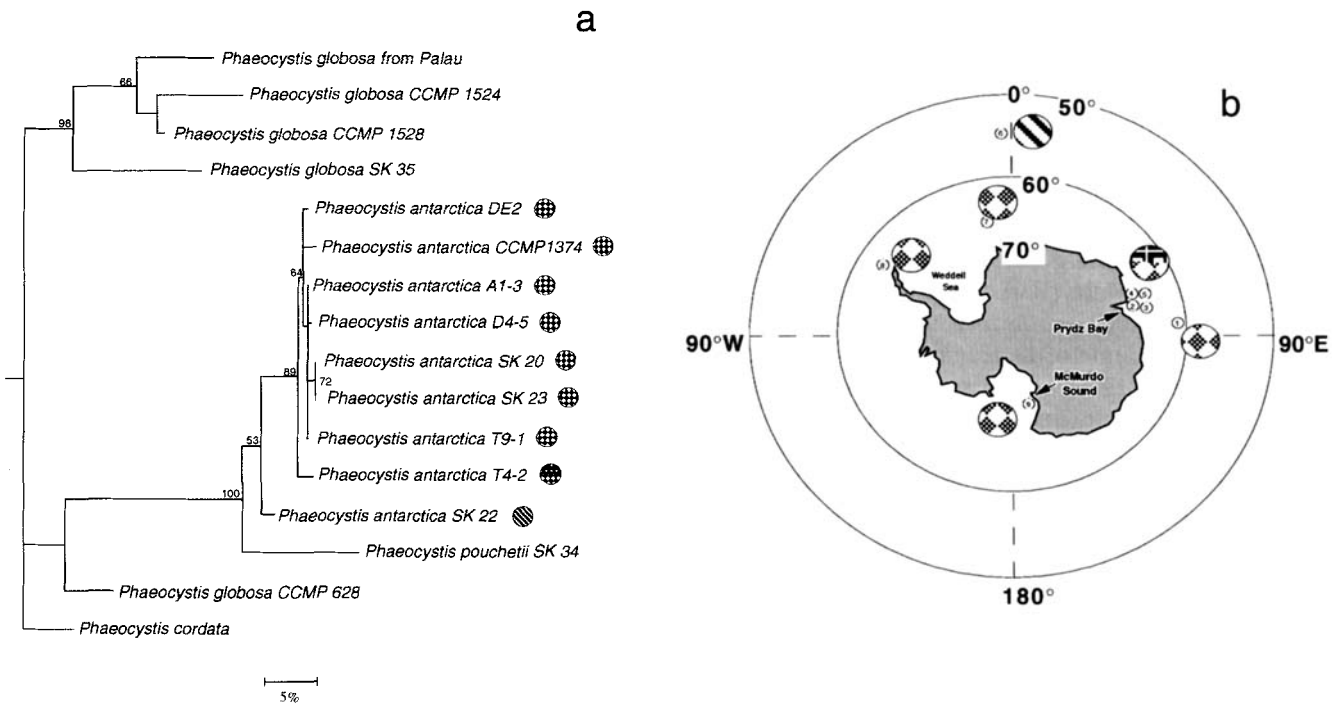

Fig. 2. a. Maximum likelihood tree of ITS1 sequences from *Phaeocystis pouchetii* (SK 34) and *P. antarctica*. Key to strains: 1 = A1–3, 2 = D4–5, 3 = DE2, 4 = T4–2, 5 = T9–1, 6 = SK 22, 7 = SK 20, 8 = SK 23, and 9 = CCMP 1374). Bootstrap values from the neighbour-joining analysis (500 replicates) are placed above the nodes. The distance corresponding to three change per 100 nucleotide positions is placed below the tree. The patterned circles beside the tree refer to the haplotype designation in our analysis and the location can be found in Fig. 1. **b.** Locations of the strains of *P. antarctica* used in this study. The location of different clades is indicated by the different patterns in the large circles. Encircled numbers beside each patterned circle indicate the strains in Fig. 1a for which the ITS1 spacer region was determined. Redrawn from Medlin *et al.* (2000).

4) Does the distribution of genetic diversity reflect ecological change?

5) Are bloom populations clonal or highly polymorphic?

6) Does the amount of genetic diversity observed depend on the species or are all bloom species naturally, highly diverse?

7) Does the ploidy level of the species influence the structure of genetic diversity?

8) Do phytoplankton with different reproductive strategies or different ploidy levels contribute differently to the genetic diversity of the ecosystem?

The impact of diminished genetic diversity at lower trophic levels and its concomitant effect on higher trophic levels is unknown. Because little or no baseline information exists for the assessment of genetic diversity in marine phytoplankton with modern molecular techniques, we need to establish baseline values for a range of phytoplankton diversities before we can make predictions about the role of anthropogenic input into the system. Without baseline values, predictive models cannot be constructed. The potential for recognizing genetic individuality is only just being realized and its use in clustering individuals into biologically meaningful groups reflecting their overall relatedness will probably increase the diversity in the marine environment by an order of magnitude (Hedgecock 1994) and will provided insights into how genetic diversity is integrated though space and time (Brand 1989).

Because population structure and genetic divergence are tightly coupled and because planktonic organisms are subjected to ever changing environmental conditions, an individual's adaptive advantage will vary through time. It is thus important to know how genetic diversity is distributed and dynamically maintained in an ecosystem. The underlying importance of having even partial answers to these questions is relevant to:

1) the establishment of population genetic models for marine organisms in general that do not fit standard models,

2) the building of better ecosystem models in which genetically diverse populations may behave in ecophysiologically different ways, thus contributing to different predictions,

3) the understanding of the role of pelagic population dynamics from a genetic perspective, and

4) the exploration of genetic diversity in phytoplankton in the context of biodiversity, i.e. the need to know how different levels of genetic diversity affect or actually

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drive changes in biodiversity – including baseline values, which are entirely unknown.

Recently, the role that bacteria and viruses play in controlling the demise of phytoplankton blooms has received increasing attention. Likewise, the population structure of these populations has also been investigated. Of the 233 marine algicidal bacteria isolated from a bloom of the red-tide forming alga *Heterosigma akashiwo* (Hada) Hada, 85 belonged to 17 different RFLP patterns of γ-Proteobacteria (Yoshinaga *et al.* 1998). The percentage of the different ribotypes changed over the course of the bloom, but all played a role in the rapid termination of the *H. akashiwo* bloom. DNA hybridisation was used to determine the genetic similarity of host specific viruses attacking *Micromomas pusilla* Butch.) Mant. & Parke (Cottrell & Suttle 1995). The genetic variation among the *M. pusilla* viruses from a single location was as large as the variation between viruses isolated from different oceans. Thus, not only are the blooms themselves multi-clonal but potential biological terminators of the blooms are also highly diverse. This suggests that each group in a given tropic level may be highly genetically diverse to accommodate potentially changing genetic diversity in the level that it controls or influences.

Summary

Despite the limited number of studies of population structure and genetic diversity in marine phytoplankton, it can be inferred that considerable genetic diversity exits on both spatial and temporal scales in the phytoplankton and in the bacteria and viral communities associated with these microalgae. Certainly, some of the diversity can be attributed to the uncovering of cryptic species. Still other differences in genetic diversity may reflect the different life cycle strategies of the various classes of marine microalgae. Only by investigating several different species in several different settings can we begin to develop some estimate of the range of diversity that we can expect. In order to zoom in with more detail within populations, the use of population-based molecular methods for single cell analysis of marine phytoplankton must be developed to enhance our ability to analyse phytoplankton in time and space. Methods that offer great potential are sorting by flow cytometry and immunobeads. Quantitative PCR may make it possible to analyse multiple banding patterns within a natural population obtained from multiplexing PCR reactions of molecular markers and hence avoiding the need to rely totally on clonal isolates.

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