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GENETIC VARIABILITY AND DIFFERENTIATION IN NICHE COMPONENTS
OF MARINE PHYTOPLANKTON SPECIES

by

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ABSTRACT

The acclimated asexual reproduction rates of many clones of
Emiliana huxlevi (82), Gephyrocapsa oceanica (19),
Cyclococcolithina leptopora (31), Prorocentrum micans (28),
Dissodinium lunula (22), Thoracosphaera heimi (20), and Gonyaulax
tamarensis (83) were measured in several light and temperature
regimes. The data were used to determine the amount of genetic
variability and the spatial patterns of genetic differentiation in
these species.

None of the species examined exist purely as clones in nature.
Statistically significant genetic variability is found even among
clones isolated from single water bottles. The amount of genetic
variability in asexual reproduction rates in individual

phytoplankton populations ranged from 3 to 13% (coefficients of variation). There is no obvious relationship between the amounts of genetic variability in the populations and the variability or predictability of the environments from which they were collected.

No genetic differentiation was found within the Sargasso Sea in any of the oceanic species, but strong genetic differentiation was found between oceanic and neritic populations of the two species (E. huxleyi and G. oceanica) found on both sides of the Gulf Stream. The spatial patterns of genetic differentiation appear to be different in these two species, however. Genetic differentiation was found between populations from the Sargasso Sea and the slope water off New England in G. oceanica. In E. huxleyi, the Sargasso Sea and slope water populations were similar, but different from the Gulf of Maine populations.

Of the three species for which populations were collected at different times of the year (E. huxleyi, C. leptopora, and T. heimi), there is evidence of significant seasonal genetic changes in only one (C. leptopora).

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Diversity is an obvious attribute of the biological world, but the way in which this diversity is apportioned among ecological and evolutionary units is not obvious or easy to determine. The concept of the species plays an important role in partitioning this diversity and in organizing thoughts and experiments in biological research, but it is not clear when the use of this concept is appropriate and when it is not. The concept of the species presently plays a central role in ecological theory. It is the spatial and temporal distribution and abundance of species which many ecologists try to explain.

Hutchinson's n-dimensional niche hyperspace concept (Hutchinson, 1957) is often considered quite useful in attempting to explain the distribution of a species. This niche hyperspace maps components of fitness with respect to environmental variables. It represents the genetic potential of the individual organism in a spectrum of environments. A two-dimensional slice through the niche hyperspace which relates a component of fitness to an environmental variable will be called an environmental fitness curve in this thesis.

The study of phytoplankton ecology has been greatly facilitated by the fact that many components of the niche hyperspace of phytoplankton can be measured under controlled laboratory conditions

rather easily (relative to most organisms). The component of fitness measured almost exclusively in phytoplankton studies has been the asexual reproduction rate. But the common practice has been to study components of the niche hyperspace of a single clone of a species and extrapolate this to the entire population or species. To what extent an individual's niche hyperspace is representative of all the individuals of a population or species, however, is open to debate. This has been a subject of primary interest to population geneticists and evolutionary biologists for a long time.

Three general questions can be asked. First, to what extent have different populations of the same species differentiated genetically, resulting in spatially or temporally separated populations with different composite niche hyperspaces? Second, how much genetic variability in components of the niche hyperspace exists within a single population? Third, how rapidly can a population or species evolve in response to environmental changes? These are the questions addressed in this thesis.

Genetic differentiation

Whether the species or the population should be considered as the primary ecological and evolutionary unit depends upon the amount of gene flow occurring and how much genetic differentiation has developed. Genetic differentiation among geographically separated

populations is now well known in terrestrial plants. The early work by Turesson, Gregor, Clausen, Keck, and Hiesey and more recent work on genetic differentiation in terrestrial plant species has been reviewed by Morley (1959), Mayr (1963), Heslop-Harrison (1964), Hiesey and Milner (1965), Jain and Bradshaw (1966), Stebbins (1970), Langlet (1971), Bradshaw (1972), and Snayden (1973).

The development of genetic differentiation by diversifying selection is opposed, to some degree, by dispersal and gene flow. The relative roles of selection pressure and gene flow in shaping geographic genetic patterns are not well understood (see Mayr, 1963; Ehrlich and Raven, 1969; Endler, 1973; Sokal, 1974; and Levin, 1979 for divergent views on this subject). Recent studies have demonstrated that very little dispersal and gene flow actually occurs in many terrestrial plants, despite the initial impression one gets that seeds and pollen travel long distances (see reviews by Ehrlich and Raven, 1959; Bradshaw, 1972; and Levin and Kerster, 1974). Therefore, it is not known whether the genetic differentiation observed in terrestrial plants is more the result of reduced dispersal and gene flow or the result of strong selective forces acting on populations with adequate genetic variability for diversifying selection to act upon.

Despite all the data on genetic differentiation in terrestrial plants, very little is known about genetic differentiation in marine phytoplankton species. Day (1963), Hart (1963), and McGowan (1971)

have argued that little if any genetic differentiation should be expected in marine phytoplankton species because they are subjected to such large amounts of advection and potential gene flow over large geographical areas. This would be true only if gene flow closely follows advection patterns and if gene flow really is important in preventing genetic differentiation from developing. The hypothesis that little genetic differentiation exists in marine phytoplankton needs testing. Places in the ocean such as the Antarctic Convergence, Gulf Stream, and Kuroshio Current are rather strong barriers to dispersal and separate quite different environmental regimes. It is not hard to envision genetic differentiation developing in species which live on both sides of these barriers. Indeed, this seems to be the case for Thalassiosira pseudonana.

Genetic differences between three clones of this species, one from an estuary on Long Island, one from the slope water off New England? and one from the Sargasso Sea, have been demonstrated in their reproduction rates at various temperatures and salinities (Guillard and Ryther, 1962), capacity for using various organic nitrogen sources (Guillard, 1963), vitamin B12 specificity (Guillard, 1968), half-saturation constants for nitrate uptake (Carpenter and Guillard, 1971), sensitivity to polychlorinated biphenyls (Fisher, et al., 1973), maximum uptake rates of silicic acid (Nelson, et al., 1976), electrophoretic mobility of isozymes

(Murphy and Guillard, 1976), sensitivity to exotic chemicals (Fisher, 1977), diel cell division periodicity (Nelson and Brand, 1979), and sensitivity to a complex industrial waste product (Murphy and Belastock, 1980). Despite large genetic differences between neritic and oceanic clones, very little or no genetic differences can be detected between neritic clones isolated from such widely separated places as New York, Virginia, Germany, Australia, and the Virgin Islands (Brand, et al., 1980). More genetic variability was found among five clones of T. pseudonana from one water bottle collected in a warm core eddy than there was among the five neritic clones from widely different parts of the world (Brand, et al., 1980). Thus, how spacial patterns of genetic differentiation relate to the spatial patterns of dispersal and environmental factors is not at all obvious. Detailed geographic genetic studies on more species must be carried out to determine these spatial genetic patterns.

The data on the genetic structure of Thalassiosira pseudonana may not be representative of marine phytoplankton in general. In many ways, T. pseudonana is not a typical marine phytoplankton species. First of all, it appears not to be a dominant species in the ocean although it can form extensive blooms in certain estuaries (Guillard and Ryther, 1962). Nevertheless, it is cosmopolitan, being found in the open ocean, coastal waters, estuaries, lakes and rivers (Guillard and Ryther, 1962; Hasle and Heimdal, 1970;

Hargraves and Levandowsky, 1971; Niemi and Hallfors, 1974; Lowe and Busch, 1975; Makarova, 1976; Hasle, 1976, 1975; Fryxell and Hasle, 1977; Belcher and Swale, 1977). Very few species are found in such a wide range of habitats. Few marine species are ever found in freshwater lakes and rivers.

A second problem is that it is possible that *T. pseudonana* is really a cluster of sibling species, which have diversified into drastically different habitats. This question has been addressed by Murphy and Guillard (1976). Their electrophoretic data indicate that *T. pseudonana* is differentiated into at least two distinct populations (coastal and oceanic) with complex hybridization occurring when they mix. Thus, the electrophoretic data, while not proof, support the idea that *T. pseudonana* is either one species with strong genetic differentiation or two hybridizing semispecies.

Third, the Gulf Stream is one of the strongest barriers in the ocean and separates very different environmental regimes. The Gulf Stream is also a relatively old feature of the ocean (Berggren and Hollister, 1977; Kaneps, 1979). Therefore, it is not surprising to find genetic differentiation developed in *T. pseudonana* across the Gulf Stream because of the large environmental differences across it and the long time period over which differentiation could have developed.

Although one can argue that *T. pseudonana* is not a typical marine phytoplankton species, unpublished data of Guillard and

Hulburt (presented in Guillard and Kilham, 1977) show genetic differences between clones from the Woods Hole area and tropical Atlantic coastal waters in four other diatom species. Genetic differences between temperate and tropical clones have also been observed in Phaeocystis poucheti (Guillard and Hellebust, 1971) and Biddulphia aurita (Underhill, 1977). Other genetic differences known to occur between clones of marine phytoplankton species are in heavy metal tolerance (Jensen, et al., 1974; Rraek, et al., 1976), bioluminescence (Swift, et al., 1973; Schmidt, et al., 1978) and reproduction rates (Braarud, 1951; Swift, et al., 1973). Therefore, the possibility exists that genetic differentiation has developed in many or most phytoplankton species.

The data available at the present time on clonal differences in marine phytoplankton species are inadequate for determining what proportion of marine phytoplankton species have developed genetic differentiation and on what spatial scales this can occur because too few clones have been examined. Neither did these past studies consider the genetic variability within each population sampled, making it impossible to know whether the clonal differences observed indicate genetic differentiation or merely genetic variability within natural populations.

One purpose of this thesis was to determine how much genetic differentiation exists in other species of phytoplankton, especially dinoflagellates and coccolithophores, because essentially all the

work done so far has been on diatoms. Furthermore, the sampling was designed to test for the occurrence of genetic differentiation on smaller spatial scales than have heretofore been examined.

Genetic variability

How much genetic variability exists within a single population is a more difficult question. The amount of genetic variability within interbreeding populations has been an important concern for population geneticists and evolutionary biologists ever since Chetverikov (1926), Fisher (1930), Wright (1931), and Haldane (1932) discussed the potential relationship between genetic variability and evolutionary change. Two divergent views on the amount and role of genetic variability in natural populations have developed and persisted to the present time. Deriving largely from the mutational studies pioneered by Muller, one theory has been that most natural populations are genetically quite homogeneous. It holds that most of the genetic variability within populations is largely the result of deleterious mutation pressure. Only ever so rarely does an advantageous mutation appear which is selected for in the population. This view of genetic variability in natural populations leads to the conclusion that evolution is slow and dependant upon rare, random, mutational events (see Muller, 1950, 1958; and Kimura and Ohta, 1971a for a presentation of this hypothesis). An alternative hypothesis is that extensive balanced polymorphism

exists within natural populations. This hypothesis developed subsequent to the findings by Dobzhansky and others of chromosomal polymorphisms maintained in natural populations of Drosophila (reviewed by Dobzhansky, 1970 and Parsons and McKenzie, 1972). Supporters of this hypothesis argue that large amounts of genetic variability are maintained in natural populations by balancing selection. This large pool of genetic variability may then allow genetic adaptation to environmental changes to occur rather rapidly. This second view on the amount of genetic variability and its role in natural populations leads to the conclusion that evolution can be rapid, perhaps on an ecological time scale, allowing populations to track their environment genetically to some extent.

Biologists in different disciplines tend to adopt different views on how much genetic variability exists in natural populations (either explicitly or implicitly). Most physiological ecologists implicitly assume the classical view of the species---that there is little genetic variability and that each species has essentially one niche. This allows them to study the physiology of a single organism and extrapolate to the entire species. This concept is used in most theories concerning Biogeography, species diversity, and competition. This view is also implicitly held by paleoecologists who further assume that the niche of a species remains rather constant over geological time, thus allowing the use

of fossils as indicators of past environments. Sachs, et al. (1977) have reviewed the techniques and assumptions of this method.

On the other hand, most (but not all) population geneticists and evolutionary biologists believe that much genetic variability is hidden under the apparent morphological uniformity of a species. Many theories in evolutionary ecology are optimization theories, which assume that most natural populations have adequate genetic information and evolutionary potential for evolving the "optimal strategy" for a particular environment (see Lewontin, 1978; and Maynard Smith, 1978a for a discussion). How easily or how far populations are able to evolve along the "adaptive topography" (see Lewontin and White, 1960; Turner, 1967; and Wallace, 1968b for a discussion of this concept) towards an "adaptive peak" or "optimal strategy" is unknown.

All individuals are different from each other to some degree, of course. But it is unclear and difficult to determine how much of the variability observed is caused by environmental and developmental differences and how much is the result of genetic differences. Genetic differences between individuals from the same population are known. It is upon these differences that Mendelian genetics has been developed (although many Mendelian geneticists have relied upon artificially induced mutations to provide the genetic differences they work with). But the differences studied by Mendelian geneticists are usually in characteristics (often

morphological) of obscure adaptive significance. The adaptive characteristics for which polymorphisms are known (reviewed by Ford, 1971 and Clarke, 1975, 1979) are usually controlled by a single gene or only a few genes. It is not clear whether similar amounts of genetic variability can be expected in highly polygenic characteristics such as temperature fitness curves. Segregational load could be a significant deterrent to extensive genetic variability in highly polygenic characteristics.

Furthermore, classical Mendelian genetic analysis can detect only genetic loci that are polymorphic. It is just as important to ask what proportion of all the genes in an organism are monomorphic within a population. An understanding of the genetic potential of a population requires a knowledge of the proportion of genes that are polymorphic, the number of alleles at a locus, their distance to fixation, the potential interactions between genes, and the degree to which different alleles result in different fitnesses.

Part of this problem was solved in 1966, when the technique of gel electrophoresis was used on enzyme extracts of individuals to detect approximately one-fourth of the amino acid differences in particular enzymes between individuals and determine what proportion of the genes that code for enzymes are polymorphic (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Harris, 1966). This technique has since been widely used by many population geneticists (see Lewontin, 1974a and Ayala, 1976 for a review). The technique

is limited by the fact that it can observe variability in only structural genes and can detect only about one-fourth of all the amino acid substitutions possible (King and Wilson, 1975; Lewontin, 1974a; Selander, 1976). Nevertheless, the electrophoretic data have shown that tremendous amounts of genetic variability exist within natural populations at the molecular level.

But these data have not really answered the question of how much genetic variability there is in adaptive characteristics and components of fitness. It is possible that most of the electrophoretically detectable differences in amino acid sequences in enzymes do not result in differences in fitness (Kimura, 1968, 1969; King and Jukes, 1969; Kimura and Ohta, 1971a, 1971b; Jungck, 1971). If this is the case, the hypothesis that the population or species is genetically quite uniform in adaptive characteristics and fitness components could still be true. The rigorous determination of whether or not many different electromorphs have significantly different fitness values has proven to be very difficult (Lewontin, 1974a; Ewens and Feldman, 1976; Avise, 1977). Thus the question still remains--how much genetic variability exists within a population in adaptive characteristics and components of fitness?

The initial discovery of large amounts of electrophoretic polymorphism in natural populations led to a problem with the concept of genetic load (Lewontin and Hubby, 1966). This problem was partially solved when Sved, *et al.* (1967), King (1967), Milkman

(1967), and Wallace (1968a, 1970) pointed out that absolute fitness values are meaningless and that epistatic interactions are likely (including threshold responses). Furthermore, Wallace (1968a, 1975) pointed out that at least some genetic load is balanced by ecological' load. The "cost" of evolutionary potential is an important consideration, but it must be weighed against the "cost" of not evolving. While the concepts of relative fitness of most probable genotypes, threshold responses, and genetic load balanced by ecological load reduce the problem of enormous genetic load to some degree, the problem of overall genetic variance in fitness values remains. No matter what the absolute fitness values are, a large variance in fitness would not be expected, because it would result in either biologically unrealistic low values at one end of the range or absurdly high values at the other end. On the other hand, if the variance is quite small, then selection coefficients are small and genetic change is necessarily slow. In small populations with extensive polymorphism but only small selection coefficients, genetic drift could become quite important. Random drift to fixation could make the maintenance of advantageous alleles difficult. A knowledge of the genetic variance in fitness values in natural populations is of great importance. A knowledge of the number of genes in the genome of a population that are polymorphic is not sufficient.

Even if we could be sure that a substantial fraction of the

electrophoretic differences do result in differences in fitness, we cannot be sure if any of it is additive and what kind of adaptive genetic changes the genetic variability will allow. It is quite likely that populations can have genetic variability which will allow them to evolve in response to one type of environmental change, but not another. Surely some characteristics can evolve more rapidly than others. The electrophoretic data do not tell us much about specific adaptations to the environment.

This points to a dilemma in trying to determine how much genetic variability exists within natural populations. The basic problem is to separate the genetic and environmental causes of phenotypic variability. Most population geneticists have attempted to solve this problem by examining the molecular end of the ontogenetic process, because genetic differences between individuals in molecular characteristics such as nucleotide and amino acid sequences are influenced little by environmental factors. But the characteristics of most direct importance for adaptation to the environment (components of fitness) are also the ones most influenced by environmental variables during the ontogenetic process. Thus, the characteristics of most adaptive significance and of most interest to ecologists are the most difficult ones in which to measure genetic variability.

Furthermore, theory predicts (Robertson, 1955, 1968; Salthe, 1975) and the experience of animal breeders indicates (Robertson,

1955; Falconer, 1960) that much more of the genetic variability is additive in characteristics having little relation to fitness than in characteristics contributing much to fitness. Selection always tends to reduce additive genetic variability (Ewens, 1976).

Therefore, the measurement of the amount of genetic variability in a characteristic with little direct relationship to fitness may not be a very good indicator of the amount of additive genetic variability in important components of fitness. Also, unless it is known whether normalizing or diversifying selection is the primary force on a character, one does not know whether the existence of genetic variability in the characteristic is evidence for or against its relevance to fitness.

There are two ways to measure genetic variability in ecologically relevant characteristics. The first is to observe the response to selection of a characteristic. In fact, the data on response to selection is the best evidence at the present time for the hypothesis of large amounts of genetic variability in natural populations (Robertson, 1953; Lewontin, 1974a). It is an indirect measure, in that genetic variability is estimated from the selection response by using Fisher's Fundamental Theorem of Natural Selection. While it estimates only the additive component of genetic variability, it does estimate the potential for genetic change in a population--of great interest to population geneticists and evolutionary biologists. Unfortunately, most response to

selection experiments have been on morphological characteristics of obscure adaptive significance or on domesticated populations, which are perhaps more variable than natural populations (Schwanitz, 1959; Wright, 1978).

The second method is to attempt to separate directly the genetic and environmental influences on phenotypic variability in adaptive characteristics. This is the approach taken in this thesis. The problem of separating environmental and genetic influences upon phenotypic variability is avoided by measuring components of fitness of all the different individuals in exactly the same environment. A number of other requirements must also be met, however, to confirm that the differences in fitness that are observed in the same environment are indeed genetic in origin.

The first requirement is that the organisms must be fully acclimated to the experimental environment to assure that any differences observed are not simply the result of the organisms being in different stages of acclimation. It was found in this thesis that phytoplankton take approximately five to twenty asexual generations to acclimate (see Methods section). Thus, only organisms capable of asexual reproduction can be analyzed by this method for genetic variability, since the same genotype must be maintained for a number of generations to achieve complete acclimation. The same genotype must be maintained and its fitness measured for a number of generations to confirm that, in addition,

the genotype reproduces at a constant rate in a constant environment once acclimation has been achieved. This is important because not all organisms do so. It has been found that diatoms do not reproduce at constant rates over time (see Methods section). Complete acclimation and constant reproduction rates were achieved with the coccolithophores and dinoflagellates examined in this thesis.

A second requirement is the obvious necessity of replicate measurements to assure that the observed differences between genotypes in fitness are statistically significant. Again, this requires that one be able to replicate the genotype. This has been a major problem in Drosophila studies (Lewontin, 1974a). Sequential replicates were obtained on all reproduction measurements reported in this thesis, so the errors involved in the measurements are known.

Third, stochastic processes at the individual level must be dealt with. It has been demonstrated in bacteria (Kelly and Rahn, 1932), mammalian cell cultures (Minor and Smith, 1974), Euglena gracilis (Cook and Cook, 1962), Tetrahymena geleii (Prescott, 1959), and diatoms (S. Chisholm, personal communication) that the daughter cells resulting from a mitotic division which are presumably genetically identical and in basically the same environment do not reproduce at the same rate. The cause of this is difficult, perhaps impossible, to determine because proper replicates cannot be set up. Possible explanations for genetically identical daughter cells

having different reproduction rates include microenvironmental differences, an unequal division of cytoplasm, organelles, or other somatic materials, somatic mutations, or stochastic developmental processes (see discussion by Spudich and Kochland, 1976). Whatever the cause, it is clear that a genotype does not have a single fitness value in any given environment. Instead, the fitness of a genotype should be considered to have a probability distribution. This means that the average fitness of many identical genotypes must be measured to determine if there is a significant difference in fitness among genotypes. Fortunately, this is easily done with phytoplankton. The average reproduction rates of clonal cultures containing millions of individuals are measured in this thesis.

Although not a requirement, it is desirable to measure the fitness of a genotype in more than one environment. The magnitude of genetic differences in fitness could easily be different in different environments. The extent to which the relative fitnesses of different genotypes are different in different environments (the genotype-environment interaction component of the additive biometrical model as described by Falconer, 1960 and Mather and Jinks, 1971) may indicate the adaptive significance of the genetic variability and how it is maintained. Haldane (1946), Lewontin (1974b), and Gregorius (1977) have discussed the important implications of this in genetic analysis. In this thesis, the reproduction rates of clones at two different temperatures are

reported. Unfortunately, time limitations have not allowed a greater range of environmental conditions to be used.

For the reasons presented above, it is obviously necessary to replicate the genotype in order to measure the amount of genetic variability present in natural populations in ecologically relevant adaptive characteristics. It is impossible to measure this in species which reproduce only sexually because the genotype cannot be replicated. Therefore, phytoplankton that have the capability of asexual reproduction offer an advantage in the study of genetic variability that species, such as Drosophila, commonly studied by population geneticists, do not have.

Up to the present time, there have been only two studies (Gallagher, 1978; Murphy, unpublished) on electrophoretic variability within natural populations of phytoplankton. Gallagher's study on Skeletonema costatum reveals electrophoretic variability comparable to that found in most other organisms (Lewontin, 1974a and Selander, 1976). The data of Gallagher and Murphy clearly demonstrate that at least some diatom species do not exist as single clones in nature. There have been no studies on the amount of genetic variability within a single population in reproduction rates or other components of fitness in phytoplankton.

In this thesis, the magnitude of genetic differences in reproduction rates among many clones of a species isolated from one water bottle was measured in a number of species to determine the

amount of genetic variability present in natural populations of phytoplankton.

Rates of genetic change

The fossil record is the primary evidence we have that evolution occurs. It reveals changes, however, only in the morphology of hard parts that can be fossilized. There need not be any congruence between the evolutionary rates of external morphology and environmentally relevant adaptations such as components of the niche hyperspace (Sokal, 1974; Salthe, 1975). It is not known on what time scales populations are able to track their variable environment genetically. The best documented cases of rapid genetic changes in populations are those associated with human activities--industrial melanism (Kettlewell, 1958), resistance to pesticides in insects (Georghiou, 1972) and the domestication of useful plants and animals. In most cases: these rapid genetic changes are the result of novel selective forces. One might expect higher amounts of additive genetic variability in characteristics that have not been under strong selection in the past (analogous to the situation for characteristics not closely linked to components of fitness). The asexual reproduction rates of phytoplankton within a certain range of environmental conditions, however, have probably always been under strong selection. Therefore, one might expect to find much less additive genetic variability and evolutionary potential in

environmental fitness curves than in the characteristics which have usually been observed to evolve on short time scales.

In summary, it is not known and it is difficult to predict how rapidly important components of the niche hyperspace of phytoplankton populations might evolve, given the appropriate selective pressures. Fisher's Fundamental Theorem of Natural Selection is not useful in predicting evolutionary change from genetic variability if the variability is largely non-additive. It is also incapable of predicting how far genetic changes can proceed. The study of evolutionary rates in phytoplankton offers advantages not available with most organisms. First, as discussed earlier, many components of the niche of a single genotype can be measured in phytoplankton rather easily. Second, the generation time of phytoplankton is on the order of a day. This allows many generations to be observed within reasonable research time and makes seasonal genetic changes more likely. Because genotypes of phytoplankton can be maintained clonally through time, individuals from different seasons can be maintained and analyzed simultaneously.

Summary

At the present time, no data exist that allow us to predict how much genetic variability in niche components exists in phytoplankton populations, how much genetic differentiation has developed on various spatial scales, or how fast phytoplankton populations can

evolve different niche hyperspaces in response to environmental changes. This thesis is an attempt to address these questions directly in phytoplankton and to consider their ecological and evolutionary implications.

CHAPTER 2

METHODS

METHODS

Clone isolation and culture collection

Seawater from which phytoplankton cells were isolated was obtained either from Niskin bottles on standard hydrocasts or from PVC collection tubes tossed from the ship while underway. The phytoplankton cells in this seawater were then concentrated by gentle filtration (a vacuum of 5 psi. or less) with a 8 micron membrane filter. Large phytoplankton cells were collected with 32 or 64 micron mesh plankton nets towed at approximately one knot (ship drift).

Within several hours of collecting the water (or occasionally overnight), single cells were isolated by micropipette under a compound microscope from the concentrated plankton samples into individual test tubes (16mm x 100mm borosilicate tubes), each containing approximately 5 ml of f/20-Si medium (the preparation of this medium will be described later). Because only 10 to 50% of the isolations were successful and it is not known if part of the differential survival of the isolates has a genetic basis, it is possible that the measurements of genetic variability in natural populations of phytoplankton are underestimates. Because lack of successful isolations was due primarily to contaminating species dominating the cultures and only occasionally due to nothing at all growing in isolation tubes, it seems unlikely that the differential

survival has a genetic basis.

The clonal cultures which developed from these single cell isolations were maintained in constant environment rooms at 20°C, with a 14:10 hr light-dark cycle and a light intensity of approximately 0.02 ly/min provided by cool-white fluorescent lights.

In exception to these general procedures, most of the clones of Gonyaulax tamarensis were established from cells which had germinated from cysts maintained by Dr. Donald Anderson or Dr. David Wall. These clonal cultures were then maintained in a culture room at 16°C (all other environmental conditions the same as for the rest of the culture collection).

The culture collection was maintained by transferring an inoculum of each clone to fresh f/20-Si medium approximately once every two weeks. These cultures were clonal but not axenic. Although bacteria were never abundant enough to be observable under a light microscope, enrichment of the cultures with 0.1% Bacto-peptone usually yielded visible bacterial growth. The clonal cultures used in this study and pertinent data with respect to the time and place of their isolation are presented in tables in the Results section. All the clones from one water sample are referred to as a population in this thesis, even if the population consists of only one clone.

Taxonomy

Scanning electron micrographs were taken of each clone of Emiliana huxleyi, Gephyrocapsa oceanica, Cyclococcolithina leptopora, Prorocentrum micans and Thoracosphaera heimi. Light microscope photographs were taken of each clone of Dissodinium lunula and Gonyaulax tamarensis. Representative photographs of each of these species are shown in Figures 1 through 7.

The correct identification of the species was confirmed by comparing my scanning electron micrographs with those in the following publications: Emiliana huxleyi- Hay, ~~et al.~~ (1967), McIntyre and Be (1967), Okada and McIntyre (1977); Cephyrocapsa oceanica- McIntyre ~~et al.~~ (1965), McIntyre and Be (1967), Okada and McIntyre (1977); Cyclococcolithina leptopora- McIntyre and Be (1967); Prorocentrum micans- Dodge (1975); Thoracosphaera heimi- Futterer (1976), Jafar (1980). My light microscopic observations of Dissodinium lunula were compared with those of Swift and Durbin (1971). The identification of Gonyaulax tamarensis was made by comparison with the photographs of Turpin ~~et al.~~ (1978) and D. Anderson (unpublished). The systematics proposed for G. tamarensis by Schmidt and Loeblich (1979) is adopted in this thesis.

Culture media

The culture media were prepared by filtering 33 ppt. salinity seawater from Vineyard Sound through a glass fiber filter and then

Figure 1.

Scanning electron micrograph of Emiliana huxleyi. Scale = 1 micron.

Figure 2.

Scanning electron micrograph of Gephyrocapsa oceanica. Scale = 1
micron.

Figure 3.

Scanning electron micrograph of Cyclococcolithina leptopora. Scale
= 5 microns.

Figure 4.

Scanning electron micrograph of Prorocentrum micans. Scale = 10
microns ■

Figure 5.

Light microscope photograph of Dissodinium lunula. Scale = 100
microns.

Figure 6.

Scanning electron micrograph of Thoracosphaera heimi. Scale = 5
microns.

Figure 7.

Light microscope photograph of Gonyaulax ~~tamarensis~~. Scale = 10
microns.

autoclaving in one liter teflon bottles (Nalge Co.) for fifteen minutes at 121°C and 15 psi. After being cooled for at least 24 hours, the seawater was enriched with nutrients to provide f/2, nutrient concentrations (Guillard, 1975), with the exception that silicic acid was added only for the culture of diatoms. f/2 medium without silicic acid is denoted as f/2-Si. All experiments described in this thesis were conducted in f/2-Si medium. Medium with nutrient concentrations only one-tenth that of f/2 is denoted as f/20. Each nutrient stock was autoclaved in a separate teflon bottle except that iron and EDTA were in one stock solution as Ferric Sequestrene (Geigy Chemical Corp.), all the other trace metals (Cu, Zn, Co, Mn, Mo) were together in another stock solution, and the vitamins were autoclaved together in borosilicate glass tubes. Once made, the media were stored in sterile two liter polycarbonate bottles (Nalge Co.).

The culture media were made in this manner for several reasons. First, the dissolution of large amounts of silicic acid from borosilicate vessels and the precipitation of various nutrients during autoclaving is eliminated. Second, I have had much higher success in isolating single cells of certain species into clonal culture using this type of medium rather than medium prepared using standard procedures. Third, the initial lags usually seen in batch cultures at low cell densities are reduced or eliminated. Fourth, some species reproduce at slightly higher rates when grown in medium

prepared this way.

All culture tubes were cleaned with detergent, then 3N HCP, then with several rinses of distilled water. They were then capped with polypropylene caps and autoclaved with distilled water in them. The water was then poured out and the medium was dispensed with an autoclaved dispenser (Brinkmann Dispensette) into the tubes using sterile techniques. The culture tubes used in experiments were allowed to equilibrate thermally in the constant environment devices before inoculation.

Constant environment devices

In order to measure the acclimated reproduction rates of a large number of phytoplankton clones in many different steady state environments, several constant environment devices were constructed. A diagram of one such device is shown in Fig. 8. It consists of a plexiglass tray, three feet wide, four feet long, and four inches deep, which is illuminated from below by a three by four foot light bank holding eighteen 40W Sylvania cool-white fluorescent lights which provide a light intensity of approximately 0.23 ly/min as measured by a calibrated Eppley black and white pyranometer. The ballasts for the fluorescent lights are on long cords so that they may be placed away from the water bath, reducing heat transfer to the plexiglass water tray. Aluminum screening is placed between the light bank and plexiglass tray to reduce the light intensity to the

Figure 8.

Schematic diagram of a constant environment device.

Figure 8.

desired level for each experiment. Fans blow across the air space between the light bank and plexiglass tray to lessen heat transfer to the plexiglass water tray. A timer is included in the light bank circuit so that light:dark cycles can be provided. A heating-cooling water bath is used to provide a reservoir of constant temperature water which is continually circulated through the plexiglass tray in a layer approximately one and one-half inches deep. The water is pumped by submersible pumps from the heating-cooling water bath to the plexiglass tray. The water then drains back to the heating-cooling water bath through ports in the plexiglass tray. Approximately 10 gallons of water per minute are pumped through the system. Thus the average residence time of the water within the plexiglass tray is less than a minute. This provides a very uniform temperature regime. The 25mm x 150mm Pyrex culture tubes with 20 ml. of culture media and capped with polypropylene caps are then placed within these trays in coated wire racks that hold them at a 45° angle. About 300 culture tubes can be maintained in each device at a uniform temperature and light intensity. While the heating-cooling water bath may fluctuate approximately 0.2°C over the course of several months, the temperature within the culture tubes differs among tubes at any one time by less than 0.05°C. The positions of the culture tubes in the constant environment apparatus are changed each day.

Measurement of reproduction rates

A method was needed that was sufficiently rapid to allow the reproduction rates of hundreds of clonal cultures to be measured in a single experiment, yet also accurate and precise enough to allow for the detection of small genetic differences between clones from the same population.

The widely used batch culture technique was considered inadequate for such a study because it does not provide sufficient acclimation time. Different stages of acclimation could be interpreted erroneously as clonal differences which, of course, would not be truly genetic in origin. Continuous cultures or chemostats are inadequate for such a study because they allow one to measure only the nutritional environment produced by a culture with a given reproduction rate and not the reproduction rate resulting from a particular environment; genetic differences can only be confirmed if different clones are compared in identical environments. Turbidostats are capable of doing this but are difficult and expensive to set up and one obviously could not set up enough turbidostats to analyze hundreds of clones at a time. Therefore, continuous batch culture was chosen as the best method for detecting genetic differences in reproduction rates between clones of phytoplankton. This method is basically the same as the semi-continuous culture method used by Paasche (1975). The essential feature of the continuous batch culture method is the

transfer of an inoculum from one batch culture to a new batch culture well before the culture population depletes the nutrients in the medium. Transfers are usually made when a culture approaches approximately one-tenth the density at which nutrient limitation begins. The only non-steady state factors in this type of culture regime are cell density, excreted metabolites and possibly light intensity (depending upon the density one allows the cultures to attain). By analyzing statistically the reproduction rates of the sequential cultures, it is possible to determine whether or not these factors cause non-steady state reproduction rates within a given range of cell densities. I found these factors not to be important over a reasonably wide range of cell densities.

The continuous batch culture technique requires that some measure of cell abundance be measured often (usually once a day) for the calculation of exponential reproduction rates. A rapid and precise method of estimating cell abundance was necessary in order to monitor hundreds of cultures in simultaneous experiments. The use of counting chambers under a microscope is definitely too slow and imprecise (and tedious). The use of a particle counter is feasible only for those phytoplankton species within a certain size range that do not form chains, have long appendages, or produce particles such as the empty thecae of dinoflagellates or the empty calcareous spheres of *Thoracosphaera*. I found the best method to be the measurement of in vivo fluorescence. In vivo fluorescence has

been successfully used in the past to monitor the growth of phytoplankton cultures by Hannan and Patouillet (1972), Thomas, et al. (1974), Tunzi, et al. (1974), and Paasche (1977). An additional advantage of this method is that subsampling is not required if culture tubes that fit directly into a fluorometer are used. The feasibility, precision, and accuracy of this method depend critically upon the success of maintaining the phytoplankton in a physiological steady state, because in vivo fluorescence per cell can vary with environmental conditions (Kiefer, 1973a, 1973b; Loftus and Seliger, 1975; Slovacek and Hannan, 1977). Thomas, et al. (1974) found discrepancies between reproduction rates calculated from cell counts and those calculated from fluorescence readings in short term batch cultures. This emphasizes the importance of achieving complete acclimation for the accurate measurement of reproduction rates using in vivo fluorescence. Control experiments, in which cell counts were compared with in vivo fluorescence, demonstrated that the method of continuous batch culture provides a constant environment in which the exponential increase in in vivo fluorescence accurately reflects the exponential increase in cell numbers. No significant differences (at the 95% confidence level) were found between reproduction rates calculated from cell counts and those calculated from fluorescence measurements in five cultures each of Emiliania huxleyi, Gephyrocapsa oceanica, Cyclcoccolithina leptopora, and Prorocentrum micans. Light:dark cycles can be used

as long as the *in vivo* fluorescence measurements are made at the same time in each light:dark cycle.

Once a day, at the same time each day, the culture tubes were taken out of the constant environment device, dried with a paper towel, mixed on a vortexer, and inserted directly into a Turner 10-000R fluorometer (Turner Designs, Mountain View, Ca. 94043) adapted for 25m x 150mm cuvettes and equipped with an infrared sensitive photomultiplier for the measurement of *in vivo* chlorophyll fluorescence. The filters used were as follows: 10-050 Corning color specification 5-60 for excitation, 10-052 Corning color specification 3-56 for reference, and 10-051 Corning color specification 2-64 for emission. Much time is saved by measuring the *in vivo* fluorescence of the entire culture rather than subsampling. It takes only about 30 seconds to measure the fluorescence of each culture. Control experiments have shown that the brief insertion of the culture into the fluorometer each day does not affect its reproduction rate. When the cultures were being grown in relatively bright light (greater than 0.1 ly/min), the tubes were held in dim light (around 0.01 ly/min) for 15 minutes before being measured in the fluorometer. This allows the fast component of fluorescence change (Kiefer, 1973b, Loftus and Seliger, 1975) to occur, thus yielding a fairly constant fluorescence value. The tubes, while outside the constant environment device, were kept in a tray of appropriate temperature water to avoid temperature

change. For most experiments, sequential batch cultures were maintained for one to three months in order to achieve a steady state and accumulate enough replicated data.

The fluorometer was calibrated by measuring the fluorescence of a sequence of two-fold dilutions of dense cultures down to a cell density that is fluorometrically undetectable. Log-log plots of fluorescence vs. cell concentration for all species tested this way have the same slope. This slope, however, is slightly different from that expected of a two-fold dilution sequence. Part of this is probably due to self-absorption. Differences between fluorometers indicate inaccuracies within the fluorometers as well. The ratio of the expected slope to measured slope was used as the calibration factor (my fluorometers have calibration factors ranging from 1.05 to 1.3).

Statistical analysis

Fluorescence vs. time for the sequential replicates was plotted on semi-logarithmic paper. Acclimation was judged to be complete when the slopes of successive replicates were approximately the same. Statistical analysis was then performed on all these acclimated replicates. First, the exponential reproduction rate of each sequential replicate was determined by the least squares method of linear regression (Draper and Smith, 1966). The reproduction rates of all the acclimated sequential replicates of a single

clone-environment combination were then used to calculate a mean reproduction rate and a variance. From these statistics, the coefficient of variation and 95% confidence limits were also calculated.

Precision of the reproduction rate measurements

In order to demonstrate the precision of the method, the between-sequential replicates variance of each clone-environment combination was calculated using data from several experiments. The variances for many clones of each species in one experimental environment were then averaged. From this mean variance, the coefficient of variation was calculated. These mean coefficients of variation for each species-environment combination are presented in Table 1. These coefficients of variation represent the sum of errors (including measurement and experimental errors) introduced over the course of a one to three month experiment in measuring the acclimated reproduction rates of individual clones. Thus, the method allows one to detect rather small genetic differences in the asexual reproduction rates of different clones.

Constancy of phytoplankton reproduction rates

When a culture line is taken from the culture collection and begun in the constant environment device, it usually takes from five to twenty generations (about one to three weeks) before acclimation

Table 1.

Summary statistics showing the number of clones averaged, the mean reproduction rate, the average error coefficient of variation, and the average ratio of the actual variance divided by the predicted variance.

TABLE 1

Species	Environmental conditions Temp. (°C)	Light (ly/min)	Length of exp (days)	No. of clones	Mean divisions per day	Within clone coefficient of variation	Ratio-actual to predicted variance
<u>Cyclococcolithina leptopora</u> (Murray & Blackman) Wilcoxon	20	0.023	52	31	0.928	2.08%	0.80
"	24	0.023	45	32	0.835	2.34%	2.88
<u>Dissodinium lunula</u> (Schutt) Klebs	24	0.023	102	22	0.345	3.65%	1.45
<u>Emiliana huxleyi</u> (Lohm.) Hay & Mohler (ex-Coccolithus huxleyi)	16	0.23	23	82	1.529	3.50%	1.17
"	26	0.23	46	82	2.395	3.77%	1.37
<u>Gephyrocapsa oceanica</u> Kampt	16	0.023	45	19	1.215	2.38%	0.58
<u>Gonyaulax tamarensis</u> Lebour	16	0.023	48	83	0.552	4.25%	1.74
<u>Prorocentrum micans</u> Ehrenberg	22	0.23	41	28	0.869	3.28%	1.50
"	26	0.23	38	28	0.988	2.94%	0.93
<u>Thoracosphaera heimi</u> (Lohm.) Kampt.	20	0.023	53	20	0.577	2.02%	1.57
<u>Thalassiosira pseudonana</u> (Hustedt) Hasle & Heimdal (ex Cyclotella nana)	12	0.23	22	20	0.885	15.66%	5.19
"	16	0.23	24	20	1.686	7.70%	3.83
"	20	0.23	22	20	2.600	6.36%	6.83
"	24	0.23	21	20	3.279	6.29%	7.97

and a constant reproduction rate is achieved. This emphasizes the importance of maintaining long term steady states for the measurement of acclimated reproduction rates.

The constancy of the exponential reproduction rates over time can be tested by one-way analysis of variance. Because of the time and expense of doing this for thousands of clone-environment combinations, only simple visual analysis was used to determine if the semi-logarithmic plots of the sequential replicates are parallel. To demonstrate the constancy (or lack thereof) of the acclimated reproduction rates in the constant environment devices, the ratio of the actual to predicted between-replicates variance was calculated for each clone-environment combination. The predicted between-replicate variance was calculated from the average within-replicate variance from the regression lines. The ratios for each clone within one species in one experimental environment were then averaged. The mean ratios for each species-environment combination are presented in Table 1. A ratio much larger than one indicates that their reproduction rates changed during the experiment.

Surveys of large numbers of clones of coccolithophores and dinoflagellates, using the techniques just described, have shown that, within the precision of the method, they grow at constant rates for several months when in a constant environment. Because of the large number of degrees of freedom, the ratio of actual to

predicted between-replicate variance should be extremely close to one. Why they differ from one as much as they do is not known.

It appears that most diatom cultures do not reproduce at constant rates. The data on Thalassiosira pseudonana presented in Table 1 demonstrate this quite well. A survey of approximately twenty other diatom species indicated that they too do not reproduce at constant rates (most were less constant than T. pseudonana). The reproduction rates of diatoms fluctuated both up and down over the course of weeks to months. While the possibility of subtle environmental changes within the constant environment devices cannot be ruled out, this explanation seems unlikely because coccolithophores and dinoflagellates appear to reproduce at constant rates in the same devices. Other explanations seem more likely.

One possible reason is that most planktonic diatoms are diploid and homothallic, and thus genetic recombination can occur in clonal culture. This has been demonstrated electrophoretically in Thalassiosira pseudonana and Skeletonema costatum (Murphy, 1978). Selection among the resulting mixture of genotypes may then occur, possibly resulting in a higher reproduction rate of the culture. Natural selection, however, cannot explain the shifts to lower reproduction rates also often seen in diatom cultures grown under constant environmental conditions. A second, more likely explanation, is cell size changes within the diatom cultures. It is known that cell size changes occur in diatom cultures, and that

different size diatom cells reproduce at different rates (Paasche, 1973; Durbin, 1977).

Because diatoms do not exhibit constant reproduction rates over time and because they can recombine in "clonal" culture, they are not good experimental organisms for studying the amount of genetic variability present in natural populations and were therefore not examined in this thesis.

Control experiment

The control experiment described below was conducted to test for several potential problems. First, do the constant environment devices really provide a uniform environment? Second, are all the cells in a clonal culture really genetically identical in their overall reproductive physiology? Is mutation pressure high enough so that a significant fraction of the cells in a clonal culture have reproduction rates different from the parent clones, thus allowing selection to occur in "clonal" cultures? Third, do low numbers of bacteria have *an* effect on phytoplankton reproduction rates? These questions all need to be answered before one can be assured that the differences observed in the reproduction rates of the different clones are truly genetic in origin.

Three clones (92, 96, 299) of Emiliania huxleyi were used in the experiment. Twelve cells from each of these three clonal cultures were isolated by micropipette into new clonal cultures. In

addition, penicillin G (to a concentration of 100 mg/l) and streptomycin (to a concentration of 50 mg/l) were added to actively growing subcultures of the three original clonal cultures. The next day, single cells were washed and isolated from these cultures with antibiotics into **new** clonal cultures. Once they had developed into dense cultures, they were tested for the presence of bacteria by adding 0.1% Bacto-peptone. Five axenic subclones of each of the three original clones were then used in the control experiment, along with the twelve other subclonal cultures previously isolated.

The seventeen subclonal cultures of each of the three original clones were then grown in the constant environment devices using the same methods as were used in all the other experiments. One-way analysis of variance was used to determine if there were significant differences among the seventeen subclones of each genotype. The results are shown in Table 2. They show no significant differences among the subclonal cultures. This indicates that the constant environment devices do provide a uniform environment, that mutation pressure is not the major direct cause of the observed genetic variability, and that the presence of low numbers of bacteria do not have a significant effect on the exponential reproduction rates of the clonal cultures. The reproduction rates measured in this experiment do not differ significantly with those measured on the same clones eleven months earlier (see Table 4).

Table 2

Mean reproduction rates and ANOVA tables showing degrees of freedom, sum of squares, mean squares, F-ratios and P values for each of three clones of Emiliana huxleyi from which seventeen subclones were isolated and examined. A P value less than 0.05 would have indicated significant differences among subclones.

Table 2

Clone 92: 2.625 divisions per day

	df	SS	MS	F-ratio	
Between	16	0.0758	0.00474	1.016	P=0.44
Within	128	0.597	0.00466		
Total	144	0.573			

Clone 95: 2.099 divisions per day

	df	SS	MS	F-ratio	
Between	16	0.0211	0.00132	0.784	P=0.70
Within	98	0.165	0.00168		
Total	114	0.186			

Clone 299: 1.732 divisions per day

	df	SS	MS	F-ratio	
Between	16	0.0139	0.000869	1.156	P=0.32
Within	72	0.0541	0.000751		
Total	88	0.058			

CHAPTER 3

RESULTS

RESULTS

Emiliana huxleyi

The isolation locations of the 82 clones of *E. huxleyi* are listed in Tables 3 and 4 and are shown with respect to hydrography detected by satellite imagery in Figs. 9, 10, and 11. Populations A, B, and C were collected before rigorous sampling and recording procedures were adopted for this research. Therefore, the exact locations of their isolations are not known. They are included here because they provide a basis for testing whether or not clonal cultures change genetically over the course of several years. These clones were grown for 23 days at 16°C and for 46 days at 26°C in a 14:10 LD photoperiod with a light intensity of 0.23 ly/min. The reproduction rates at 16°C and 26°C, along with the 95% confidence intervals, are shown in Tables 3 and 4. The mean errors (coefficients of variation) in these measurements were 3.50% at 16°C and 3.772 at 26°C. The ratio of the reproduction rate at 26°C divided by the reproduction rate at 16°C was calculated for each clone. These ratios are presented in Table 5.

Because of the low numbers of clones in the individual populations, populations F and L from slope water and population K from the edge of a warm core eddy are grouped together into what will be called the neritic group. Clones from the Sargasso Sea and Gulf Stream are grouped together into those isolated in May (pops.

D, E, and G) and those isolated in September and October (pops. M, N, P, Q, R, S, T, and U). These will be referred to as the spring and fall oceanic groups, respectively. Populations H, I, and J were isolated in the Gulf of Maine near Georges Bank and will be called the cold water group. Histograms for these groups of the reproduction rates at 16°C and 26°C and the 25/16 ratio are shown in Figs. 12, 13, and 14, respectively.

One-way analysis of variance showed that there is a significant difference among the four groups at 16°C (F ratio of 3.753 with 3 and 69 degrees of freedom and a P value of 0.0147), at 26°C (F ratio of 10.451 and a P value less than 0.0001), and in the 26/16 ratio (F ratio of 7.755 and a P value of 0.00021). Least significant difference tests and Scieffe tests indicated that there are no significant differences among the fall oceanic, spring oceanic, and neritic groups, but that the Gulf of Maine group is significantly different from the other three at the 0.05 level at 16°C, 26°C, and in the 26/16 ratio.

Despite the presence of genetic variability in probably most or all the populations of E. huxleyi (see the largest populations, D, N, P, and R in Tables 3, 4, and 5), there is no indication of a seasonal genetic shift between the spring and fall oceanic groups (compare histograms in Figs. 12, 13, and 14). This indicates that the genetic variability present is largely non-additive or else not responsive to those environmental factors which change on a seasonal

cycle. A comparison of the neritic group and the oceanic groups also reveals no genetic differentiation between them. The cold water group from the Gulf of Maine, however, is definitely genetically different from the other groups (compare histograms in Figs. 12, 13, and 14). They, in general, reproduce more rapidly than the warm water clones at 16°C and slower at 26°C. This difference is most clearly seen in the 26/16 ratio. The overlap is due largely to the two clones isolated in the Northeast Channel between Georges Bank and Browns Bank (pop. I) where the water was 4°C warmer than in the Gulf of Maine where populations H and J were collected. The water in the channel may have been entrained from a nearby warm core eddy. The satellite imagery for the week before the isolation of population I was made is shown in Fig. 15. It shows slope water and part of a warm core eddy being entrained into the channel.

The data indicate that genetic differentiation has not developed in *E. huxleyi* across the Gulf Stream but has developed between the Gulf of Maine and the warmer waters further south. It is possible that the genetic differences observed in the cold water group are the result of rapid selection in expatriate populations from the Sargasso Sea but it seems more parsimonious to consider the genetic differences to be the result of genetic differentiation that has probably developed over a longer period of time. This would mean that most of the Gulf of Maine cells are part of an indigenous

population with its own population dynamics, and not simply the result of advection.

There is no evidence among the 82 clones examined of genetic differentiation within the species in the morphology of the coccoliths. Micropaleontologists have found geographic differences in coccolith morphology in *E. huxleyi* (Okada and McIntyre, 1977). These differences are probably the result of environmental influences on morphology or of genetic differentiation on spatial scales larger than were examined in this thesis.

An examination of the clones in populations A and B, isolated in 1975 and 1976, respectively, shows that they are not different from the rest of the populations, isolated in 1977. This is further evidence that genetic change in clonal culture is not a problem with haploid species.

Figure 9.

Hydrography as detected by satellite imagery and the locations of populations D, E, F, and G of Emiliana huxleyi isolated on OCEANUS 25 (May 18-May 23, 1977).

MAY
18, 1977

Figure 10.

Hydrography as detected by satellite imagery and the locations of populations K, L, M, N, P, Q, R, S, T, and U of Emiliana huxleyi isolated on OCEANUS 35 (Oct. 18-Oct. 24, 1977).

OCTOBER
19, 1977

Figure 11.

Hydrography as detected by satellite imagery and the locations of populations H, I, and J of Emiliana huxleyi isolated on ALBATROSS 77-09 (Sept. 19-Sept. 25).

SEPTEMBER
21, 1977

Table 3.

Mean reproduction rate of each clone of Emiliana huxleyi at 16°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 3

Emiliana huxleyi

Environmental conditions: 16°C, 33 ppt., 0.23 ly/min., 14:10 LD

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	August, 1975	western North Atlantic	
43	1.529	6	1.457-1.601
Pop. B	12-16-76	western North Atlantic	
46	1.501	5	1.445-1.558
47	1.434	5	1.385-1.484
54	1.600	5	1.549-1.650
63	1.649	7	1.599-1.699
67	1.522	7	1.478-1.566
68	1.627	7	1.566-1.687
69	1.201	4	1.085-1.318
Pop. C	Jan., 1977	western North Atlantic	
82	1.401	5	1.348-1.454
Pop. D	5-21-77	36°58'N, 67°42'W	Sargasso Sea
86	1.602	5	1.564-1.639
87	1.463	5	1.429-1.497
88	1.706	5	1.664-1.747
89	1.573	4	1.487-1.658
90	1.583	6	1.530-1.635
91	1.631	6	1.524-1.737
92	1.593	4	1.456-1.731
93	1.541	7	1.512-1.570
94	1.237	5	1.214-1.260
95	1.576	6	1.508-1.645
96	1.662	6	1.607-1.717
106	1.658	5	1.584-1.733
111	1.351	4	1.306-1.397
113	1.618	4	1.556-1.680
119	1.584	6	1.555-1.613

Table 3 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. E	5-19-77	36°56'N, 67°37'W	Sargasso Sea
97	1.523	6	1.439-1.606
110	1.331	4	1.210-1.453
Pop. F	5-18-77	39°40'N, 69°44'W	warm core eddy
114	1.454	5	1.385-1.522
115	1.885	8	1.810-1.960
116	1.470	3	1.294-1.646
Pop. G	5-22-77	37°02'N, 67°26'W	Sargasso Sea
112	1.594	5	1.522-1.667
Pop. H	9-20-77	42°10'N, 66°22'W	Gulf of Maine
334	1.827	4	1.704-1.950
Pop. I	9-22-77	42°23'N, 65°58'W	Northeastern Channel
299	1.525	3	1.378-1.671
309	1.571	5	1.464-1.677
Pop. J	9-24-77	42°08'N, 68°01'W	Gulf of Maine
266	1.978	7	1.927-2.030
267	1.706	7	1.653-1.758
Pop. K	10-18-77	39°12'N, 69°19'W	warm core eddy
355	1.405	5	1.374-1.435

Table 3 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. L	10-18-77	39°50'N, 69°50'W	slope water
373	1.489	5	1.409-1.569
374	1.642	4	1.574-1.710
375	1.561	6	1.520-1.602
543	1.483	4	1.437-1.529
577	1.536	3	1.463-1.609
617	1.726	3	1.690-1.762
Pop. M	10-19-77	37°01'N, 67°33'W	Gulf Stream
371	1.490	4	1.436-1.544
Pop. N	10-21-77	36°39'N, 67°54.5'W	Sargasso Sea
361	1.461	5	1.323-1.600
362	1.577	5	1.530-1.624
528	1.554	5	1.484-1.623
530	1.521	5	1.434-1.609
531	1.522	5	1.465-1.579
534	1.493	6	1.459-1.528
535	1.458	4	1.376-1.540
536	0.944	3	0.833-1.054
545	1.514	5	1.501-1.527
Pop. P	10-20-77	36°49'N, 67°27'W	Sargasso Sea
363	1.609	6	1.554-1.664
525	1.682	6	1.630-1.734
526	1.473	5	1.417-1.529
527	1.219	3	1.118-1.320
537	1.557	5	1.491-1.622
538	1.451	5	1.371-1.530
559	1.601	5	1.527-1.675
566	1.544	3	1.537-1.552
578	1.473	6	1.437-1.510
598	1.705	3	1.643-1.767
602	1.486	5	1.434-1.538

Table 3 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. Q	10-22-77	36°44'N, 69°20'W	Sargasso Sea
364	1.620	6	1.581-1.659
541	1.700	5	1.584-1.814
Pop. R	10-22-77	36°18'N, 69°28'W	Sargasso Sea
367	1.444	5	1.400-1.487
540	1.472	4	1.422-1.523
557	1.531	6	1.481-1.580
558	1.291	6	1.240-1.342
564	1.664	5	1.562-1.767
572	1.641	4	1.564-1.717
587	1.613	6	1.552-1.674
588	1.197	5	1.171-1.223
596	1.232	4	1.177-1.286
Pop. S	10-22-77	36°50'N, 68°12'W	Sargasso Sea
532	1.573	5	1.523-1.622
533	1.472	4	1.391-1.554
560	1.655	5	1.583-1.727
561	1.591	6	1.537-1.646
565	1.523	5	1.461-1.585
Pop. T	10-22-77	36°49'N, 68°01'W	Sargasso Sea
579	1.518	4	1.442-1.593
Pop. U	10-21-77	36°39'N, 67°54'W	Sargasso Sea
529	1.444	6	1.413-1.474
571	1.341	6	1.302-1.380

Table 4.

Mean reproduction rate of each clone of Emiliana huxleyi at 26°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 4

Emiliana huxleyi

Environmental conditions: 26°C, 33 ppt., 0.23 ly/min., 14:10 LD

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	August, 1975	western North Atlantic	
43	2.505	13	2.458-2.552
Pop. B	12-16-76	western North Atlantic	
46	2.220	6	2.154-2.286
47	2.191	11	2.145-2.237
54	2.500	9	2.449-2.550
63	2.478	11	2.422-2.534
67	2.518	9	2.448-2.590
68	2.467	11	2.390-2.544
69	2.054	10	1.979-2.129
Pop. C	Jan., 1977	western North Atlantic	
82	2.194	11	2.142-2.246
Pop. D	5-21-77	36°58'N, 67°42'W	Sargasso Sea
86	2.500	12	2.412-2.587
87	2.484	13	2.425-2.542
88	2.303	9	2.206-2.400
89	2.553	9	2.515-2.591
90	2.432	10	2.364-2.499
91	2.500	12	2.438-2.562
92	2.550	12	2.461-2.640
93	2.123	10	2.059-2.186
94	2.368	16	2.316-2.419
95	2.261	9	2.118-2.403
96	2.063	10	2.014-2.111
106	2.451	12	2.393-2.508
111	2.280	6	2.106-2.454
113	2.555	14	2.503-2.606
119	2.360	9	2.311-2.409

Table 4 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. E	5-19-77	36°56'N, 67°37'W	Sargasso Sea
97	2.515	9	2.457-2.572
110	2.475	15	2.423-2.526
Pop. F	5-18-77	39°40'N, 69°44'W	warm core eddy
114	2.471	11	2.380-2.561
115	2.277	10	2.225-2.329
116	2.383	10	2.323-2.444
Pop. G	5-22-77	37°02'N, 67°26'W	Sargasso Sea
112	2.525	10	2.439-2.611
Pop. H	9-20-77	42°10'N, 66°22'W	Gulf of Maine
334	2.019	7	1.963-2.074
Pop. I	9-22-77	42°23'N, 65°58'W	Northeastern Channel
299	1.715	8	1.653-1.777
309	2.287	14	2.219-2.354
Pop. J	9-24-77	42°08'N, 68°01'W	Gulf of Maine
266	2.168	11	2.115-2.221
267	1.963	10	1.922-2.004
Pop. K	10-18-77	39°12'N, 69°19'W	warm core eddy
355	2.572	8	2.506-2.638

Table 4 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. L	10-18-77	39°50'N, 69°50'W	slope water
373	2.002	9	1.929-2.074
374	2.479	12	2.436-2.523
375	2.447	11	2.391-2.504
543	2.550	9	2.501-2.600
577	2.670	8	2.630-2.710
617	2.369	5	2.288-2.450
Pop. M	10-19-77	37°01'N, 67°33'W	Gulf Stream
371	2.474	9	2.403-2.545
Pop. N	10-21-77	36°39'N, 67°54.5'W	Sargasso Sea
361	2.496	11	2.437-2.555
362	2.396	9	2.358-2.433
528	2.163	10	2.111-2.215
530	2.365	13	2.306-2.424
531	2.355	14	2.305-2.406
534	2.492	13	2.463-2.522
535	2.584	8	2.512-2.657
536	2.525	8	2.470-2.580
545	2.433	9	2.366-2.500
Pop. P	10-20-77	36°49'N, 67°27'W	Sargasso Sea
363	2.628	8	2.589-2.666
525	2.359	10	2.292-2.425
526	2.455	14	2.410-2.500
527	1.931	8	1.883-1.979
537	2.564	7	2.502-2.625
538	2.348	12	2.297-2.400
559	2.560	11	2.509-2.610
566	2.497	7	2.429-2.566
578	2.545	7	2.476-2.613
598	2.242	8	2.184-2.299
602	2.649	12	2.547-2.750

Table 4 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. Q	10-22-77	36°44'N, 69°20'W	Sargasso Sea
364	2.545	8	2.496-2.594
541	2.412	10	2.330-2.494
Pop. R	10-22-77	36°18'N, 69°28'W	Sargasso Sea
367	2.578	4	2.527-2.629
540	2.393	12	2.335-2.450
557	2.419	7	2.387-2.451
558	2.576	5	2.450-2.702
564	2.544	8	2.476-2.612
572	2.457	5	2.348-2.565
587	2.271	5	2.225-2.317
588	2.516	10	2.455-2.576
596	2.400	11	2.350-2.451
Pop. S	10-22-77	36°50'N, 68°12'W	Sargasso Sea
532	2.591	7	2.536-2.647
533	1.858	11	1.778-1.939
560	2.649	6	2.526-2.772
561	2.557	11	2.492-2.622
565	2.433	12	2.372-2.495
Pop. T	10-22-77	36°49'N, 68°01'W	Sargasso Sea
579	2.413	9	2.330-2.497
Pop. U	10-21-77	36°39'N, 67°54'W	Sargasso Sea
529	2.379	14	2.314-2.444
571	2.568	10	2.525-2.612

Table 5.

The ratio of the reproduction rate at 26°C divided by the rate at 16°C for each clone of Emiliana huxleyi.

1/10
1/10
1/10
1/10

Table 5

Emiliana huxleyi

Clone no.	26/16 ratio	
Pop. A	Aug., 1975	western North Atlantic
43	1.64	
Pop. B	12-15-76	western North Atlantic
46	1.48	
47	1.53	
54	1.56	
63	1.50	
67	1.66	
68	1.52	
69	1.71	
Pop. C	Jan., 1977	western North Atlantic
82	1.56	
Pop. D	5-21-77	36°58'N, 67°42'W Sargasso Sea
86	1.56	
87	1.70	
88	1.35	
89	1.62	
90	1.54	
91	1.53	
92	1.60	
93	1.38	
94	1.91	
95	1.43	
96	1.24	
106	1.48	
111	1.69	
113	1.57	
119	1.49	

Table 5 (continued)

Clone no.	26/16 ratio	
Pop. E 5-19-77	36°56'N, 67°37'W	Sargasso Sea
97	1.65	
110	1.86	
Pop. F 5-18-77	39°40'N, 69°44'W	warm core eddy
114	1.70	
115	1.21	
116	1.62	
Pop. G 5-22-77	37°02'N, 67°26'W	Sargasso Sea
112	1.59	
Pop. H 9-20-77	42°10'N, 66°22'W	Gulf of Maine
334	1.10	
Pop. I 9-22-77	42°23'N, 65°58'W	Northeast Channel
299	1.13	
309	1.46	
Pop. J 9-24-77	42°08'N, 68°01'W	Gulf of Maine
266	1.10	
267	1.15	
Pop. K 10-18-77	39°12'N, 69°19'W	warm core eddy
355	1.84	

Table 5 (continued)

Clone no.	26/16 ratio		
Pop. L	10-18-77	39°50'N, 69°50'W	slope water
373		1.34	
374		1.51	
375		1.57	
543		1.72	
577		1.73	
617		1.37	
Pop. M	10-19-77	37°01'N, 67°33'W	Gulf Stream
371		1.66	
Pop. N	10-21-77	36°39'N, 67°54.5'W	Sargasso Sea
361		1.71	
362		1.52	
528		1.39	
530		1.56	
531		1.55	
534		1.67	
535		1.77	
536		2.69	
545		1.61	
Pop. P	10-20-77	36°49'N, 67°27'W	Sargasso Sea
363		1.63	
525		1.40	
526		1.67	
527		1.58	
537		1.64	
538		1.62	
559		1.60	
566		1.62	
578		1.73	
598		1.32	
602		1.78	

Table 5 (continued)

Clone no.	26/16 ratio		
Pop. Q	10-22-77	36°44'N, 69°20'W	Sargasso Sea
364	1.57		
541	1.42		
Pop. R	10-22-77	36°18'N, 69°28'W	Sargasso Sea
367	1.79		
540	1.63		
557	1.58		
558	2.00		
564	1.53		
572	1.50		
587	1.41		
588	2.10		
596	1.95		
Pop. S	10-22-77	36°50'N, 68°12'W	Sargasso Sea
532	1.65		
533	1.27		
560	1.61		
561	1.61		
565	1.60		
Pop. T	10-22-77	36°49'N, 68°01'W	Sargasso Sea
579	1.59		
Pop. U	10-21-77	36°39'N, 67°54'W	Sargasso Sea
529	1.65		
571	1.92		

Figure 12.

Histograms of the reproduction rates of Emiliana huxleyi clones at 16°C. The groups are the spring oceanics (D+E+G), fall oceanics (M+N+P+Q+R+S+T+U), slope water clones (F+K+L), and those from the Gulf of Maine (H+I+J).

DIVISIONS PER DAY

Figure 13.

Histograms of the reproduction rates of Emiliana huxleyi clones at 26°C. The groups are the spring oceanic~(D+E+G), fall oceanic~(M+N+P+Q+R+S+T+U), slope water clones (F+K+L), and those from the Gulf of Maine (H+I+J).

DIVISIONS PER DAY

Figure 14.

Histograms of the ratio of the reproduction rate at 26°C divided by the reproduction rate at 16°C of Emiliana huxleyi clones. The groups are the spring oceanics (D+E+G), fall oceanics (M+N+P+Q+R+S+T+U), slope water clones (F+K+L), and those from the Gulf of Maine (H+I+J).

RATIO 26/16

Figure 15.

Hydrography as detected by satellite imagery one week before populations H, I, and J of Emiliana huxleyi were isolated.

SEPTEMBER
14, 1977

Gephyrocapsa oceanica

Twenty clones of G. oceanica were isolated in the western North Atlantic. The locations of their isolations are listed in Table 6. These locations are shown with respect to hydrography as detected by satellite imagery in Figs. 16, 17, and 18. Population D, not shown in the hydrography figures, was isolated in the southern Sargasso Sea at 19°N latitude. The acclimated reproduction rates of these clones were measured at a temperature of 16°C, a light intensity of 0.023 ly/min, and in a 14:10 photoperiod. The cultures were maintained in this environment for 45 days. The average error (coefficient of variation) of these measurements is 2.38%. The reproduction rate measurement and 95% confidence interval for each clone is shown in Table 6. Fig. 19 shows histograms of the reproduction rates of each population.

Population A (the only one with enough clones to yield statistically significant data) has significant genetic variability within it (see Table 6). Thus, this species does not exist as simply one huge clonal family in nature. A comparison of populations A and D, from the northern and southern Sargasso Sea, respectively, reveals no genetic differentiation (see Fig. 19). Populations B and C from a warm core eddy and the slope water, respectively, are somewhat different (for reasons discussed below). A comparison of the oceanic group (A+D) and the neritic group (B+C) reveals clear genetic differentiation between them (see Fig. 20).

One-way analysis of variance showed that the difference between these two groups is highly significant (F ratio of 18.750 with 1 and 16 degrees of freedom and a P value of 0.0005). The overlap between the two groups is due to two clones (360, 562) within the warm core eddy (pop. B) which are similar to the Sargasso Sea clones. This is not unexpected because much of the water within the warm core eddy originated from the Sargasso Sea. The population within the warm core eddy is probably a mixture of genotypes from both north and south of the Gulf Stream. Whether or not interbreeding between the populations is occurring is not known.

The most likely explanation of the data is that genetic differentiation has developed between populations of G. oceanica across the Gulf Stream. Because the neritic clones were collected in October (pops. B and C) and the oceanic clones (pops. A and D) were collected in February and March, the hypothesis that the genetic differences observed between the neritic and oceanic populations are the result of seasonal genetic changes cannot be ruled out. It is also possible that the genetic differences seen are the result of rapid genetic changes in expatriate populations from the Sargasso Sea as they are carried into the slope region and its different environment. Because Hulburt and MacKenzie (1971) and Marshall (1976) have found G. oceanica in the shelf and slope waters elsewhere off the eastern coast of the United States, it seems most likely that the populations of G. oceanica in the slope water are

indigenous rather than expatriate. It seems most parsimonious at the present time to consider the genetic differences observed between the oceanic and slope water populations to be the result of genetic differentiation, with the Gulf Stream being the ecological boundary and dispersal barrier.

Although one would expect clones in the Gulf Stream to be similar to those from the Sargasso Sea, the single clone in population E from the Gulf Stream is similar to those in the slope water north of the Gulf Stream. The most likely explanation is that it was entrained into the Gulf Stream from slope water.

There is no indication of genetic differentiation in the morphology of the species despite the genetic differentiation in its reproductive physiology.

Figure 16.

Hydrography as detected by satellite imagery and the location of population A of Gephyrocapsa oceanica isolated on OCEANUS 40 (Feb. 20-Mar. 4, 1978).

MARCH
1, 1978

Figure 17.

Hydrography as detected by satellite imagery and the locations of populations B and C of Gephyrocapsa oceanica isolated on OCEANUS 35 (Oct. 18-Oct. 24, 1977).

OCTOBER
19, 1977

Figure 18.

Hydrography as detected by satellite imagery and the location of population E of Gephyrocapsa oceanica isolated on OCEANUS 48 (June 27-July 10, 1978).

Table 6.

Mean reproduction rate of each clone of Gephyrocapsa oceanica at 16°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 6

Gephyrocapsa oceanica

Environmental conditions: 16°C, 33 ppt., 0.023 ly/min., 14:10 ID

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	3-3-78	35°00.5'N, 68°00'W	Sargasso Sea
641	1.123	8	1.099-1.147
669	1.216	3	1.196-1.236
674	1.216	7	1.183-1.249
683	1.174	5	1.134-1.214
688	1.168	5	1.141-1.195
707	1.139	7	1.119-1.159
713	1.090	5	1.041-1.139
715	1.191	6	1.170-1.212
743	0.886	5	0.869-0.904
Pop. B	10-19-77	39°10.7'N, 69°16'W	edge of warm core eddy
360	1.201	6	1.172-1.231
562	1.154	4	1.102-1.206
567	1.440	6	1.415-1.464
Pop. C	10-23-77	38°00'N, 71°00'W	slope water
555	1.375	7	1.355-1.395
556	1.372	5	1.296-1.448
585	1.406	6	1.375-1.438
586	1.389	4	1.370-1.407
Pop. D	2-21-78	19°19.5'N, 65°32.3'W	Sargasso Sea
643	1.067	5	1.013-1.122
701	1.095	6	1.090-1.100
Pop. E	6-30-78	38°20'N, 65°57.5'W	Gulf Stream
805	1.377	8	1.360-1.393

Figure 19.

Histograms of the reproduction rates at 16°C for **the** individual populations of Gephyrocapsa oceanica.

DIVISIONS PER DAY

NO. OF CLONES

Figure 20.

Histograms of the reproduction rates at 16°C of the oceanic (A+D)
and neritic (B+C) groups of Gephyrocapsa oceanica.

A+D

B+C

Cyclococcolithina leptopora

The isolation locations and reproduction rate data for 31 clones of Cyclococcolithina leptopora are shown in Tables 7, 8, and 9. All these clones were isolated in the Sargasso Sea. The locations of the individual populations isolated in the winter and summer are shown in Figs. 21 and 22, respectively. An examination of satellite imagery maps indicated that none of these clones came from cold core eddies or other anomalous waters. These clones were grown for 52 days at 20°C and for 45 days at 24°C. The cultures were grown in a 14:10 LD cycle of 0.023 ly/min light. The mean coefficient of variation for the error in these measurements was 2.08% at 20°C and 2.34% at 24°C.

Genetic variability within the populations is detectable (see pop. F, the only population with more than eight clones, in Tables 7, 8, and 9), demonstrating that C. leptopora does not exist simply as a clone. There is no indication of genetic differentiation between populations within the Sargasso Sea (although there are too few clones in most of the populations to test this hypothesis critically). Therefore, the populations were grouped into those that were isolated in the winter and those isolated in the summer. Figures 23, 24, and 25 show histograms of the winter and summer group reproduction rates at 20°C and 24°C and the ratios of the reproduction rates at 24°C divided by those at 20°C. A comparison of the histograms of the two groups (winter and summer)

reveals genetic differences between them. This is not evident in the 24°C data, but is visible in the 20°C data and most clear in the histograms of the 24/20 ratio. One-way analysis of variance showed that the differences are significant at 20°C (F ratio of 17.013 with 1 and 29 degrees of freedom and a P value of 0.0003) and in the 24/20 ratio (F ratio of 17.443 and a P value of 0.0002), but not at 24°C (F ratio of 0.450 and a P value of 0.508). This is evidence of a seasonal genetic change in the populations. This change is not large and there is much overlap between the two groups. The change in the 24/20 ratio is in the opposite direction from what would be expected if temperature was the major selective force causing the seasonal genetic change. The genetic change in the slope of the temperature-fitness curve is presumably **the** result of pleiotropic interaction with some other characteristic under seasonal selection, although there is no direct evidence for this.

No morphological distinctions could be made between the winter and summer clones.

Figure 21.

Locations of the winter populations of Cyclcoccolithina leptopora (A, B, C, D, and E). Population A was isolated on OCEANUS 35 (Oct. 18-Oct. 24, 1977). Populations B, C, D, and E were isolated on OCEANUS 40 (Feb. 20-Mar. 4, 1978).

Figure 22.

Locations of the summer populations of Cyclococcolithina leptopora
(D, G, H, I, J, and K) isolated on OCEANUS 48 (~ m 27-July 10,
1978).

Table 7.

Mean reproduction rate of each clone of Cyclococcolithina leptopora at 20°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 7

Cyclococcolithina leptopora

Environmental conditions: 20°C, 33 ppt., 0.023 ly/min., 14:10 LD

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	10-21-77	36°39'N, 67°54.5'W	Sargasso Sea
600	0.899	8	0.887-0.912
Pop. B	3-1-78	25°33'N, 66°14'W	Sargasso Sea
650	0.850	6	0.838-0.862
678	0.924	6	0.892-0.956
679	0.689	6	0.676-0.702
680	0.955	8	0.945-0.964
681	0.928	8	0.917-0.939
Pop. C	3-3-78	35°00.5'N, 68°00'W	Sargasso Sea
691	0.713	4	0.695-0.731
Pop. D	2-20-78	18°47'N, 65°28'W	Sargasso Sea
689	0.939	8	0.924-0.955
Pop. E	2-28-78	21°49'N, 65°20.5'W	Sargasso Sea
723	0.625	6	0.600-0.649

Table 7 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. F	7-2-78	32°05.3'N, 65°03.6'W	Challenger Bank
809	0.953	8	0.947-0.960
811	0.882	7	0.864-0.901
816	0.975	4	0.954-0.996
817	0.996	5	0.983-1.010
818	0.981	8	0.961-1.001
821	0.926	5	0.886-0.965
891	0.944	6	0.936-0.952
892	0.994	6	0.973-1.015
896	0.935	8	0.917-0.954
897	0.980	5	0.973-0.987
898	0.998	5	0.963-1.034
899	0.887	7	0.874-0.901
900	0.961	8	0.938-0.984
Pop. G	6-30-78	35°20'N, 65°57.5'W	Sargasso Sea
812	0.900	9	0.887-0.913
813	1.069	7	1.052-1.085
Pop. H	7-1-78	33°45'N, 67°30'W	Sargasso Sea
795	1.000	7	0.986-1.013
815	0.989	6	0.969-1.009
819	0.890	4	0.868-0.913
Pop. I	7-6-78	33°44'N, 71°00'W	Sargasso Sea
784	1.000	4	0.984-1.015
826	0.949	6	0.922-0.976
Yop. J	7-4-78	33°45'N, 67°30'W	Sargasso Sea
814	1.036	7	1.008-1.065
Pop. K	7-5-78	33°45'N, 67°29'W	Sargasso Sea
825	0.936	7	0.923-0.949

Table 8.

Mean reproduction rate of each clone of Cyclococcolithina leptopora at 24°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 8

Cyclococcolithina leptopora

Environmental conditions: 24°C, 33 ppt., 0.023 ly/min., 14:10 LD

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	10-21-77	36°39'N, 67°54.5'W	Sargasso Sea
600	0.853	6	0.839-0.867
Pop. B	3-1-78	25°33'N, 66°14'W	Sargasso Sea
650	0.906	7	0.887-0.925
678	0.870	6	0.860-0.881
679	0.755	7	0.725-0.785
680	0.842	5	0.814-0.871
681	0.851	6	0.838-0.864
Pop. C	3-3-78	35°00.5'N, 68°00'W	Sargasso Sea
691	0.781	7	0.772-0.791
Pop. D	2-20-78	18°47'N, 65°28'W	Sargasso Sea
689	0.901	5	0.888-0.914
Pop. E	2-28-78	21°49'N, 65°20.5'W	Sargasso Sea
723	0.569	4	0.545-0.594

Table 8 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. F	7-2-78	32°05.3'N, 65°03.6'W	Challenger Bank
809	0.860	5	0.845-0.875
811	0.837	7	0.824-0.849
816	0.868	5	0.852-0.884
817	0.828	6	0.813-0.842
818	0.826	5	0.822-0.831
821	0.835	4	0.828-0.842
891	0.880	6	0.870-0.891
892	0.838	5	0.819-0.857
896	0.830	6	0.819-0.841
897	0.880	6	0.861-0.899
898	0.758	5	0.739-0.778
899	0.668	3	0.569-0.767
900	0.841	3	0.778-0.904
Pop. G	6-30-78	35°20'N, 65°57.5'W	Sargasso Sea
812	0.853	5	0.834-0.872
813	0.875	5	0.862-0.888
Pop. H	7-1-78	33°45'N, 67°30'W	Sargasso Sea
795	0.818	5	0.807-0.830
815	0.871	5	0.853-0.890
819	0.838	5	0.827-0.849
Pop. I	7-6-78	33°44'N, 71°00'W	Sargasso Sea
784	0.804	2	0.501-1.106
826	0.909	6	0.897-0.920
Pop. J	7-4-78	33°45'N, 67°30'W	Sargasso Sea
814	0.802	6	0.744-0.859
Pop. K	7-5-78	33°45'N, 67°29'W	Sargasso Sea
825	0.799	3	0.767-0.832

Table 9.

The ratio of the reproduction rate at 24°C divided by the rate at 20°C for each clone of Cyclococcolithina leptopora.

Table 9

Cyclococcolithina leptopora

Clone no.	24/20 ratio	
Pop. A 1.0-21-77	36°39'N, 67°54.5'W	Sargasso Sea
600	0.95	
Pop. B 3-1-78	25°33'N, 66°14'W	Sargasso Sea
650	1.07	
678	0.94	
679	1.10	
680	0.88	
681	0.92	
Pop C 3-3-78	35°00.5'N, 68°00'W	Sargasso Sea
691	1.10	
Pop. D 2-20-78	18°47'N, 65°28'W	Sargasso Sea
689	0.96	
Pop. E 2-25-78	21°49'N, 65°20.5'W	Sargasso Sea
723	0.91	

Table 9 (continued)

Clone no.	24/20 ratio		
Pop. F	7-2-78	32°05.3'N, 65°03.6'W	Challenger Bank
809	0.90		
811	0.95		
816	0.89		
817	0.83		
818	0.84		
821	0.90		
891	0.93		
892	0.84		
896	0.89		
897	0.90		
898	0.76		
899	0.75		
900	0.88		
Fop. G	6-30-78	35°20'N, 65°57.5'W	Sargasso Sea
812	0.95		
813	0.82		
Pop. H	7-1-78	33°45'N, 67°30'W	Sargasso Sea
795	0.82		
815	0.88		
819	0.94		
Pop. I	7-6-78	33°45'N, 71°00'W	Sargasso Sea
784	0.80		
826	0.96		
Pop. J	7-4-78	33°45'N, 67°30'W	Sargasso Sea
814	0.77		
Pop. K	7-5-78	33°45'N, 67°29'W	Sargasso Sea
825	0.85		

Figure 23.

Histograms of the reproduction rates at 20°C of the winter
(A+B+C+D+E) and summer (F+G+H+I+J+K) groups of Cyclococcolithina
leptopora.

Figure 24.

Histograms of the reproduction rates at 24°C of the winter
(A+B+C+D+E) and summer (F+G+H+I+J+K) groups of Cyclococcolithina
leptopora.

NO. OF CLONES

DIVISIONS PER DAY

Figure 25.

Histograms of the ratio of the reproduction rate at 24°C divided by the reproduction rate at 20°C of the winter (A+B+C+D+E) and summer (F+G+H+I+J+K) groups of Cyclococcolithina leptopora.

A+B+C+D+E

F+G+H+I+J+K

RATIO 24/20

Prorocentrum micans

Twenty-eight clones of *P. micans* were isolated from the locations listed in Tables 10 and 11 and shown with respect to satellite detected hydrography in Fig. 26. The reproduction rates and 95% confidence intervals of these clones grown at 22°C for 41 days and at 26°C for 38 days are shown in Tables 10 and 11, respectively. The 26/22 ratios are listed in Table 12. These clones were grown in a 14:10 ID photoperiod with a light intensity of 0.23 ly/min. The mean errors (coefficients of variation) for these experiments were 3.28% at 22°C and 2.94% at 26°C. The clones were grouped into those populations isolated on Georges Bank (pops. A, C, and D) and those isolated in the Gulf of Maine just north of Georges Bank (pops. B and E). The water in the Gulf of Maine was approximately 2°C colder than the water on Georges Bank. The histograms of the reproduction rates of these two groups at 22°C and 26°C and the 26/22 ratios are shown in Figs. 27, 28, and 29.

No statistically significant genetic variability appears in the reproduction rates at 22°C (Fig. 27), but significant genetic variability is apparent in the 26°C data (Fig. 28) for the Gulf of Maine populations (pops. B and E). A comparison of the two groups (Georges Bank and Gulf of Maine) reveals no major differences between them at 22°C or 26°C. The Georges Bank group appears rather uniform while the Gulf of Maine group exhibits a range of

reproduction rates at 26⁰C with the spread toward lower reproduction rates than found in the Georges Bank group. A small difference is seen between the two groups in the 26/22 ratio (one-way analysis of variance F ratio of 4.228 and a P value of 0.050). The Gulf of Maine group is more variable and has a somewhat lower ratio.

The lower 26/22 ratio of the Gulf of Maine group (Fig. 29) could be interpreted as genetic differentiation in temperature adaptation, because the water on Georges Bank was approximately two degrees warmer than the Gulf of Maine at the time of clone isolation. The possibility of the pattern being the result of a pleiotropic interaction with some other characteristic under selection cannot be ruled out, however.

The most likely explanation is that the absence of statistically significant genetic variability in the Georges Bank group is the result of the absence of most sexual reproduction and recombination. The greater genetic variability in the Gulf of Maine may reflect more sexual reproduction and recombination in the less productive environment. Most of the water on Georges Bank derives from the Gulf of Maine (Bumpus, 1976). Because of the large amount of vertical mixing and influx of nutrients on Georges Bank and the resultant high productivity, Prorocentrum micans may be reproducing primarily asexually in the presumably more favorable environmental regime. Only the most rapidly reproducing clones would remain in

abundance on Georges Bank. **This** is usually thought to be the general tendency in phytoplankton. Williams (1975) has given theoretical reasons **why** organisms might reproduce primarily asexually in environments favorable for rapid reproduction and sexually in less favorable environments.

Figure 26.

Hydrography as detected by satellite imagery and the locations of populations A, B, C, D, and E of Prorocentrum micans isolated on ALBATROSS 77-09 (Sept. 19-Sept. 25, 1977).

SEPTEMBER
21, 1977

Table 10.

Mean reproduction rate of each clone of Prorocentrum micans at 22°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 10

Prorocentrum micans

Environmental conditions: 22°C, 33 ppt., 0.23 ly/min., 14:10 ID

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	9-20-77	41°16.3'N, 68°17'W	Georges Bank
310	0.917	4	0.907-0.927
326	0.828	5	0.795-0.862
Pop. B	9-24-77	42°06'N, 68°04'W	Gulf of Maine
312	0.850	5	0.755-0.946
313	0.913	3	0.841-0.985
314	0.891	4	0.806-0.976
315	0.857	4	0.779-0.934
316	0.871	3	0.843-0.899
317	0.854	4	0.780-0.927
318	0.917	4	0.900-0.934
321	0.872	4	0.855-0.899
322	0.842	3	0.773-0.912
323	0.893	5	0.857-0.928
346	0.922	4	0.897-0.947
576	0.851	4	0.806-0.897
Pop. C	9-20-77	41°13'N, 67°57'W	Georges Bank
327	0.842	4	0.828-0.856
328	0.834	2	0.692-0.976
329	0.856	4	0.824-0.887
Pop. D	9-20-77	41°48'N, 66°43'W	Georges Bank
330	0.813	4	0.798-0.829
331	0.867	4	0.816-0.918
332	0.848	3	0.783-0.912
333	0.808	4	0.787-0.829

Table 10 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. E	9-24-77	41°39'N, 68°39'W	Gulf of Maine
336	0.849	3	0.818-0.880
337	0.883	4	0.847-0.919
338	0.915	4	0.886-0.943
340	0.854	3	0.809-0.899
341	0.896	4	0.873-0.918
343	0.880	4	0.884-0.915
345	0.915	4	0.898-0.932

Table 11.

Mean reproduction rate of each clone of Prorocentrum micans at 26°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 11

Prorocentrum micans

Enviroumental conditions: 26°C, 33 ppt., 0.23 ly/min., 14:10 LD

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	9-20-77	41°16.3'N, 68°17'W	Georges Bank
310	1.014	4	0.989-1.040
326	0.976	5	0.949-1.002
Pop. B	9-24-77	42°06'N, 68°04'W	Gulf of Maine
312	1.008	5	0.974-1.042
313	1.004	5	0.963-1.046
314	0.953	4	0.916-0.991
315	1.008	6	0.992-1.025
316	1.054	4	1.039-1.069
317	1.019	5	0.970-1.068
318	1.068	5	1.025-1.110
321	1.025	4	1.005-1.046
322	0.647	4	0.631-0.664
323	1.041	5	0.967-1.115
346	1.105	6	1.069-1.141
576	0.859	5	0.839-0.879
Pop. C	9-20-77	41°13'N, 67°57'W	Georges Bank
327	1.019	7	0.995-1.043
328	0.983	5	0.959-1.006
329	1.006	6	0.964-1.048
Pop. D	9-20-77	41°48'N, 66°43'W	Georges Bank
330	1.022	7	0.999-1.045
331	1.027	6	1.006-1.048
332	1.064	4	1.032-1.096
333	0.959	5	0.928-0.990

Table 11 (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. E	9-24-77	41°39'N, 68°39'W	Gulf of Maine
336	1.042	4	0.994-1.089
337	0.888	5	0.846-0.926
338	1.030	7	1.005-1.054
340	0.909	3	0.839-0.980
341	1.038	3	0.892-1.185
343	0.848	4	0.829-0.866
345	1.064	6	1.049-1.079

Table 12.

The ratio of the reproduction rate at 26°C divided by the rate at 22°C for each clone of Prorocentrum micans.

Table 12

Prorocentrum micans

Clone no.	26/16 ratio	
Pop. A 9-20-77	41°16.3'N, 68°17'W	Georges Bank
310	1.11	
326	1.18	
Pop. B 9-24-77	42°06'N, 68°04'W	Gulf of Maine
312	1.19	
313	1.10	
314	1.07	
315	1.18	
316	1.21	
317	1.19	
318	1.17	
321	1.18	
322	0.77	
323	1.17	
346	1.20	
576	1.20	
Pop. C 9-20-77	41°13'N, 67°57'W	Georges Bank
327	1.21	
328	1.18	
329	1.18	
Pop. D 9-20-77	41°48'N, 66°43'W	Georges Bank
330	1.28	
331	1.19	
332	1.26	
333	1.19	

Table 12 (continued)

Clone no.	26/16 ratio		
Fop. E	9-24-77	41°39'N, 68°39'W	Gulf of Maine
336	1.23		
337	1.00		
338	1.13		
340	1.06		
341	1.16		
343	0.96		
345	1.16		

Figure 27.

Histograms of the reproduction rates at 22°C of the Georges Bank (A+C+D) and Gulf of Maine (B+E) groups of Prorocentrum micans.

Figure 28.

Histograms of the reproduction rates at 26⁰C of the Georges Bank (A+C+D) and Gulf of Maine (B+E) groups of Prorocentrum micans.

ave. 95 %
confidence interval

A+C+D

NO. OF CLONES

B+E

DIVISIONS PER DAY

Figure 29.

Histograms of the ratio of the reproduction rate at 26°C divided by the reproduction rate at 22°C of the Georges Bank (A+C+D) and Gulf of Maine (B+E) groups of Prorocentrum micans.

NO. OF CONES

A+C+D

B+E

Dissodinium lunula

The locations of the isolations of the 22 clones of D. lunula, all from the Sargasso Sea, are listed in Table 13 and shown in Fig. 30. Satellite imagery of sea surface temperature does not reveal any anomalous water where these clones were isolated. These clones were grown at 24°C in a 14:10 LD photoperiod with an illumination of 0.023 ly/min for 102 days. The reproduction rates and 95% confidence intervals for these clones are presented in Table 13. The average error in the reproduction rate measurements was 3.65% (coefficient of variation). Histograms of the reproduction rates for the three groups are shown in Fig. 31.

Significant genetic variability is found within population A (the only one with enough clones to examine), indicating that D. lunula is not purely clonal in nature (see Table 13). The histograms in Fig. 31 show that group B+C (from near the Gulf Stream) is significantly different from group D+E+F+G (from near Bermuda), but there is no a priori hydrographic reason to group them this way. The only logical grouping would be A+B+C (near the Gulf Stream) and D+E+F+G (near Bermuda). These two groups are not significantly different because population A is similar to group D+E+F+G. Why the three clones in group B+C have higher reproduction rates than the others is not known. Unseen hydrographic events or environmental factors may be involved, but the most parsimonious explanation at the present time is that of an accidental skew in the

random sampling of the clones in nature.

The range of reproduction rates found in *D. lunula* in this study is similar to that found by Swift, ~~et al.~~ (1973). Unlike their findings, however, all my clones were bioluminescent.

Figure 30.

Locations of populations A, B, C, D, E, F, and G of Dissodinium
lunula. isolated on OCEANUS 48 (June 27-July 10, 1978).

Table 13.

Mean reproduction rate of each clone of Dissodinium lunula at 24°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 13

Dissodinium lunula

Environmental conditions: 24°C, 33 ppt., 0.023 ly/min., 14:10 ID

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	7-7-78	36°02'N, 70°57.5'W	Sargasso Sea
785	0.317	6	0.306-0.328
787	0.337	5	0.327-0.347
823	0.335	7	0.325-0.344
824	0.355	7	0.348-0.362
829	0.349	4	0.335-0.364
861	0.336	6	0.323-0.348
876	0.360	5	0.312-0.407
879	0.285	5	0.278-0.292
880	0.370	5	0.363-0.377
881	0.318	4	0.297-0.339
882	0.352	6	0.344-0.359
884	0.366	5	0.347-0.385
885	0.351	4	0.347-0.355
886	0.346	5	0.342-0.350
Pop. B	7-6-78	33°44'N, 71°00'W	Sargasso Sea
859	0.408	5	0.392-0.424
Pop. C	7-7-78	36°52'N, 70°58'W	Sargasso Sea
807	0.369	4	0.360-0.379
863	0.388	4	0.376-0.400
Pop. D	7-5-78	33°45'N, 67°28'W	Sargasso Sea
783	0.304	4	0.282-0.325
831	0.351	5	0.342-0.361
Pop. E	7-4-78	33°45'N, 67°30'W	Sargasso Sea
832	0.338	5	0.331-0.345
Pop. F	6-30-78	33°44'N, 67°29'W	Sargasso Sea
893	0.309	3	0.273-0.345
Pop. G	7-3-78	33°45'N, 67°29'W	Sargasso Sea
966	0.357	5	0.344-0.370

Figure 31.

Histograms of the reproduction rates at 24°C of three groups of Dissodinium lunula clones.

Thoracosphaera heimi.

The twenty clones of T. heimi examined were all isolated in the Sargasso Sea (the locations of these isolations are shown in **Fig. 32**). Satellite imagery revealed no anomalous water where these clones were isolated. The reproduction rates of these clones, acclimated for 53 days at 20°C in a 14:10 LD photoperiod of 0.023 ly/min light, are shown in Table 14 with the 95% confidence intervals. The average error associated with these measurements was 2.02% (coefficient of variation).

Because there were no obvious differences between individual populations (most were too small to compare with any statistical significance), the populations were grouped into those isolated in October, 1977; those isolated in March, 1978; and those isolated in July, 1978. Histograms of the reproduction rates of the clones in each of these three groups are presented in **Fig. 33**. There is no evidence of genetic differentiation between populations within the Sargasso Sea or of seasonal genetic changes in Thoracosphaera heimi. One-way analysis of variance yielded an F ratio of 1.406 with 2 and 17 degrees of freedom and a P value of 0.272. Least significant difference and Scheffe tests at the 0.05 level showed no significant differences between any of the groups. Statistically significant genetic variability does exist within the populations, however (see pops. D and F).

Figure 32.

Locations of populations A, B, C, D, E, F, and G of Thoracosphaera heimi. Populations A, B, and C were isolated on OCEANUS 35 (Oct. 18-Oct. 24, 1977). Populations D and E were isolated on OCEANUS 40 (Feb. 20-Mar. 4, 1978). Populations F and G were isolated on OCEANUS 48 (June 27-July 10, 1978).

Table 14.

Mean reproduction rate of each clone of Thoracosphaera heimi at 20°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 14

Thoracosphaera heimi

Environmental conditions: 20°C, 33 ppt., 0.023 ly/min., 14:10 LD

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A	10-21-77	36°39'N, 67°54.5'W	Sargasso Sea
606	0.557	5	0.542-0.572
607	0.549	8	0.539-0.560
618	0.523	8	0.514-0.532
Pop. B	10-22-77	36°44.2'N, 69°20'W	Sargasso Sea
603	0.587	7	0.578-0.595
Pop. C	10-22-77	36°11'N, 69°34.5'W	Sargasso Sea
608	0.601	7	0.591-0.611
Pop. D	3-3-78	35°00.5'N, 68°00'W	Sargasso Sea
708	0.583	4	0.569-0.597
710	0.594	7	0.576-0.612
711	0.588	8	0.582-0.593
712	0.562	8	0.552-0.572
714	0.583	9	0.574-0.593
730	0.570	8	0.566-0.574
Pop. E	3-1-78	27°07.5'N, 66°40'W	Sargasso Sea
724	0.572	6	0.559-0.585
Pop. F	7-3-78	33°45'N, 67°30'W	Sargasso Sea
800	0.556	7	0.554-0.567
801	0.590	6	0.572-0.609
802	0.555	9	0.548-0.562
836	0.615	7	0.601-0.629
843	0.595	4	0.580-0.611
Pop. G	7-7-78	36°09.5'N, 70°58.2'W	Sargasso Sea
974	0.581	5	0.577-0.585
975	0.602	7	0.590-0.614
976	0.574	5	0.565-0.584

Figure 33.

Histograms of the reproduction rates at 20°C of the summer (F+G), fall (A+B+C), and winter (D+E) groups of Thoracosphaera heimi.

NO. OF CLONES

DIVISIONS PER DAY

Gonyaulax tamarensis

The isolation locations and reproduction rates of the 83 clones of G. tamarensis are listed in Table 15. These clones were grown at 16°C in a 14:10 LD photoperiod of 0.023 ly/min light for 48 days. The average error in the reproduction rate measurements was a coefficient of variation of 4.25%.

The histogram (see Fig. 34) for population D, isolated from cells which had germinated from cysts collected in Perch Pond by Dr. Donald Anderson, shows statistically significant genetic variability within the population. The group A+C, also shown in Fig. 34, is a collection of cells which were isolated into clonal culture from non-clonal cultures which had been maintained by Dr. David Wall for approximately one month before I made the clonal isolations from them. Each of these non-clonal cultures originated from three to ten cells from Perch Pond. Most of the clones in this small group (A+C) have lower reproduction rates than the clones in population D, from the same pond. One possible explanation is a year to year genetic change in the Perch Pond population. Two other explanations are perhaps more likely, however. The first potential explanation is that the non-clonal populations changed genetically during the two months before they were cloned, due to the selection pressures they experienced in the laboratory. A second, less likely explanation is that recombination within the original non-clonal cultures led to inbreeding depression because of the small number of

cells from which the non-clonal cultures originally developed.

Inbreeding depression is almost universally found in a wide variety of organisms (Dobzhansky, 1970; Wright, 1977; Roughgarden, 1979).

Because of the high degree of turbulent mixing to which phytoplankton are subjected, phytoplankton are probably rarely, if ever, burdened with inbreeding depression in nature. The genetic system of phytoplankton may be more susceptible to inbreeding depression than that of most organisms because there is **less** selection pressure for protection against inbreeding depression.

Whether or not substantial genetic changes occurred in the non-clonal cultures is not known conclusively, but it is a possibility which must be considered. This emphasizes the importance of establishing clonal cultures at sea without enrichment in order to obtain an unbiased sample of the natural **population**.

The reproduction rates of the clones isolated elsewhere (pops. B, E, F, and G) tend to be lower than those in population D. Because most of them also began non-clonally, it is unclear whether these differences are the result of real populational differences, laboratory selection, or inbreeding depression.

Table 15.

Mean reproduction rate of each clone of Gonyaulax ~~tamarensis~~ at 16°C. Also shown are the isolation locations, number of replicates involved, and the 95% confidence intervals for the reproduction rate measurements.

Table 15

Gonyaulax tamarensis

Environmental conditions: 16°C, 33 ppt., 0.023 ly/min., 14:10 LD

Clone no.	Divisions per day	No. of replicates	95% confidence interval
Pop. A 9-14-76 Perch Pond, Falmouth, Massachusetts isolated from cysts collected by D. Wall			
445	0.493	5	0.464-0.521
446	0.536	5	0.482-0.590
447	0.260	4	0.216-0.305
Pop. B 5-29-76 Mill Pond, Orleans, Massachusetts isolated from cysts collected by D. Wall			
448	0.190	3	0.155-0.224
Pop. C spring, 1977, Perch Pond, Falmouth, Massachusetts isolated from cysts collected by D. Wall			
449	0.434	6	0.399-0.469
Pop. D spring, 1978, Perch Pond, Palmouth, Massachusetts isolated from cysts collected by D. Anderson			
458	0.524	6	0.513-0.536
459	0.597	4	0.578-0.615
460	0.624	3	0.611-0.637
461	0.535	6	0.522-0.548
462	0.588	4	0.549-0.628
463	0.546	4	0.531-0.561
464	0.515	4	0.394-0.636
465	0.541	4	0.518-0.563
466	0.610	5	0.594-0.625
467	0.524	5	0.515-0.533
468	0.547	5	0.491-0.604
469	0.537	5	0.455-0.618
470	0.564	5	0.541-0.587
471	0.584	4	0.489-0.680

Table 15 (continued)

Pop. D (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
473	0.627	3	0.612-0.643
474	0.539	6	0.528-0.551
475	0.591	5	0.574-0.609
476	0.578	3	0.542-0.613
477	0.553	6	0.542-0.564
478	0.573	4	0.507-0.640
479	0.549	6	0.542-0.557
480	0.609	4	0.596-0.622
481	0.563	6	0.541-0.584
482	0.608	3	0.592-0.624
483	0.559	6	0.546-0.571
484	0.510	4	0.484-0.536
485	0.577	5	0.549-0.606
487	0.564	4	0.535-0.593
488	0.567	6	0.541-0.593
489	0.571	5	0.569-0.574
490	0.578	6	0.571-0.585
491	0.581	4	0.561-0.600
492	0.657	4	0.605-0.709
494	0.590	4	0.572-0.608
495	0.561	4	0.541-0.581
496	0.580	4	0.556-0.603
497	0.623	5	0.604-0.643
498	0.452	4	0.441-0.463
499	0.543	4	0.532-0.554
500	0.590	4	0.573-0.608
501	0.578	3	0.522-0.634
502	0.595	5	0.569-0.620
507	0.511	2	0.431-0.591
512	0.624	3	0.551-0.697
513	0.659	6	0.639-0.680
514	0.612	4	0.594-0.629
515	0.632	6	0.611-0.653
516	0.596	6	0.579-0.613
517	0.650	4	0.633-0.668
518	0.568	4	0.560-0.575
519	0.576	5	0.555-0.597
520	0.609	4	0.597-0.621
522	0.623	4	0.605-0.642
523	0.548	4	0.529-0.567

Table 15 (continued)

Pop. D (continued)

Clone no.	Divisions per day	No. of replicates	95% confidence interval
524	0.554	4	0.532-0.576
1051	0.194	3	0.025-0.363
1052	0.633	3	0.544-0.722
1055	0.508	4	0.475-0.541
1056	0.565	4	0.548-0.582
1057	0.504	3	0.460-0.548
1058	0.605	5	0.593-0.617
1059	0.601	5	0.590-0.612
1060	0.573	5	0.567-0.579
1061	0.632	6	0.608-0.656
1062	0.565	4	0.551-0.579
1063	0.601	6	0.549-0.654
1064	0.383	3	0.344-0.422
1065	0.547	4	0.520-0.574
1066	0.583	3	0.561-0.604
1067	0.591	4	0.550-0.633
1068	0.559	5	0.545-0.573
1070	0.537	3	0.508-0.567
1072	0.568	5	0.559-0.576
1073	0.545	4	0.521-0.570
1074	0.534	3	0.503-0.566

Pop. E Sept., 1972, Gloucester, Ma.
culture first isolated by C. Martin

429	0.389	3	0.378-0.401
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Pop. F spring, 1977 Mill Pond, Orleans, Ma.
clone isolated by D. Anderson

GTMP	0.558	4	0.547-0.569
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Pop. G Aug., 1973 Saanich Inlet, Vancouver Island, British Columbia
non-clonal culture isolated by R. Waters

NEPCC-71	0.445	3	0.411-0.479
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Figure 34.

Histograms of the reproduction rates at 16°C of the clonal (D) and aclonal (A+C) groups of Gonyaulax tamarensis from Perch Pond.

CHAPTER 4

DISCUSSION

DISCUSSION

Clonal stability

The data available indicate that clonal cultures of coccolithophores and dinoflagellates do not change genetically over time. A comparison of the reproduction rates of clones 92, 96, and 299 of Emiliana huxleyi measured in the experiment conducted on all the clones of E. huxleyi (see Table 4) and the reproduction rates measured in the control experiment conducted eleven months later (see Table 2) reveals no significant differences. A comparison of the clones of E. huxleyi isolated from the western North Atlantic in 1975 and 1976 (pops. A and B) with those isolated in 1977 (pops. C, D, E, F, G, K, L, M, N, P, Q, R, S, T, and U) shows no significant differences (see Tables 3, 4, and 5). Thus, it seems safe to assume that haploid coccolithophores and dinoflagellates do not change genetically in their reproduction rates in clonal culture (over the course of a year or two anyway).

While clonal cultures of coccolithophores and dinoflagellates appear to be genetically quite stable, the importance of the cultures being truly clonal must be strongly emphasized. Murphy (1978) has demonstrated that clonal cultures of diatoms can change genetically through time. The most likely explanation for this is that diatoms are diploid and most are homothallic. Thus they can

recombine in culture, producing many new genotypes. Selection within these now non-clonal cultures can potentially lead to substantial genetic changes. While this cannot happen in clonal cultures of coccolithophores and dinoflagellates because they are haploid, genetic changes could certainly occur in non-clonal cultures. Circumstantial evidence for this in Gonyaulax tamarensis was presented. This emphasizes the importance of isolating cells from nature as clones immediately to avoid selection and inbreeding. Essentially all past isolations of phytoplankton cultures have been enrichment cultures which were made clonal only at some later date, if at all. It is not known how representative these cultures are of the genotypes originally present in the water.

Genetic variability

In essentially all cases where at least eight clones were isolated **from** one water bottle, statistically significant genetic variability was detected. This implies that most marine phytoplankton either reproduce sexually at least part of the time or consist of many parallel clonal lines. The electrophoretic variability found by Gallagher (1978) and Murphy (unpublished) in other phytoplankton species indicates that genetic recombination occurs often. Table 16 gives the range and coefficient of variation for the asexual reproduction rates in each (water bottle) population

Table 16.

The **range** of reproduction rates and the coefficients of variation
among clones in all populations with more than **eight** clones.

Table 1.6

Species	Pop.	Experimental temperature	Range of reproduction rates	Coefficient of variation
<u>E. huxleyi</u>	D	16	1.237-1.706	7.9%
"	D	26	2.063-2.555	6.52
"	N	16	0.944-1.577	13.4%
"	N	26	2.163-2.584	5.1%
"	P	16	1.219-1.705	8.7%
"	P	26	1.931-2.649	8.6%
"	R	16	1.197-1.664	12.2%
"	R	26	2.392-2.578	3.1%
<u>G. oceanica</u>	A	16	0.886-1.216	9.0%
<u>C. leptopora</u>	F	20	0.882-0.998	4.1%
"	F	24	0.668-0.880	6.9%
<u>P. micans</u>	B	22	0.842-0.922	3.2%
"	B	26	0.647-1.105	12.4%
<u>D. lunula</u>	A	24	0.285-0.370	6.6%
<u>G. tamarensis</u>	D	16	0.194-0.659	10.4%

with more than eight clones. The amount of genetic variability in asexual reproduction rate observed in most of the populations (see Table 16) was around 3-13% (coefficient of variation). The two populations that had greater amounts of genetic variability (pop. I of *E. huxleyi* and pop. B of *G. oceanica*) were from hydrographic regimes where different populations from more than one water mass were probably being mixed together. This emphasizes the importance of knowing the hydrographic history of a "population". It should be remembered that the amount of genetic variability observed may be an underestimate because only 10-50% of the single cell isolations were successful. It is possible (but I think unlikely) that some genotypes are less successful than others in surviving as single cell isolations. Because of the statistical limits of the method, it is impossible to determine from the data whether the species examined exist as only a few large clonal families or as "Mendelian" populations with almost every individual genetically different. The relative proportions of cells in nature that are the result of asexual or sexual reproduction and the size of clonal families resulting from asexual reproduction are not known. Electrophoretic data are needed to help answer these questions.

A small fraction of the cells in the populations have substantially lower reproduction rates. They seem to lie outside the "normal" distribution of fitness values. It is not known if they are the result of some sort of genetic load or have some

adaptive advantage.

The genetic variability examined in this thesis is in components of fitness of entire genotypes. This says nothing about individual genes. It is not known whether only a few or many genes are responsible for the genetic differences detected among the clones. Furthermore, it is not known what the relationship is between genetic variability in components of fitness and the amount of gene polymorphism within a population.

Whether species and populations in more variable or unpredictable environments have more or less genetic variability than those in more constant environments has been of great theoretical interest (see discussions by Levins, 1968; Beardmore, 1970; Grassle, 1972; Selander and Kaufman, 1973; Ayala, *et al.* 1975; Bryant, 1976; Hedrick, *et al.* 1976; Soule, 1976; Valentine, 1976; and Grassle and Grassle, 1977). While some electrophoretic studies have tended to support one view or the other, in general, there has been no clear trend in favor of any particular hypothesis (Lewontin, 1974a, Soule, 1976). Ayala and Valentine (1979) found higher levels of electrophoretic variability in a tropical euphausiid species than in a boreal euphausiid species, but it is not known what the relationship is between electrophoretic variability and genetic variability in components of fitness. The data presented in this thesis show no obvious differences in the amount of genetic variability in fitness between species or populations, even though

species from oceanic, neritic, and estuarine waters, and populations of the same species from both oceanic and neritic waters were examined. This cannot be taken to be strong evidence that there is no environmental pattern, however. Several problems arise in trying to discern any general pattern from so few data.

First of all, the amount of genetic variability in reproduction rates depends upon where in the niche hyperspace the reproduction rates are measured. One indication of this is the genetic variability seen in populations B and E of Prorocentrum micans at 22°C and 26°C. More genetic variability in reproduction rates is apparent at 26°C than at 22°C (see Figs. 27 and 28). The location within the niche hyperspace where the reproduction rates are measured may be as important as the environment from which the population was derived in determining the amount of genetic variability observed.

A second problem with making generalizations about the amount of genetic variability observed in natural populations of phytoplankton is that sexual reproduction is probably somewhat sporadic in nature. It is possible that different amounts of genetic variability could occur in those populations that have recently undergone extensive sexual reproduction than in those populations reproducing primarily asexually. The relative amounts of sexual and asexual reproduction that occur in phytoplankton populations in different environmental situations and the conditions under which

one reproductive mode or the other prevails are not known at the present time. Therefore, it is possible that the differences observed in the amount of genetic variability observed could be explained simply by the amount of sexual reproduction occurring in the populations when the clones were isolated from them. Depending upon whether normalizing, directional, or diversifying selection is predominating, more or less genetic variability could be found in a population reproducing primarily sexually than in one reproducing asexually.

A third problem with trying to relate genetic variability with environmental variability or predictability is that the amount of environmental variability or predictability perceived by humans may not reflect at all the amount experienced by phytoplankton cells. Many parameters of the physical environment can probably be considered to be more variable and unpredictable in neritic waters than oceanic waters, but this may not be true for biological parameters. Because of the higher species diversity of the oceanic communities, species interactions may be more variable and unpredictable. Before anything definite can be said about this consideration, the details of species interactions must be better understood and the relative importance that physical and biological factors have in influencing the ecology of phytoplankton species must be known. It is generally thought that species interactions among phytoplankton are mediated through physical factors such as

nutrients and light, but this is not known conclusively. We cannot, a priori, say that the oceanic environment really is more constant and predictable than the neritic environment. The best way to avoid this problem is to examine the amount of genetic variability in particular environmental fitness curves and the variability and predictability of the corresponding environmental factor. This approach must be used with caution because of the potential importance of genetic linkage and pleiotropic interactions between the environmental fitness curve of interest and other characteristics.

In conclusion, there is no evidence of a relationship between environmental variability or predictability and genetic variability, but there are really too few data at the present time to test this hypothesis adequately. The only case in which a distinct difference between populations in amount of genetic variability appeared was in Prorocentrum micans. Populations B and E from the Gulf of Maine had more genetic variability in their reproduction rates at 26°C than did populations A, C, and D from Georges Bank. Probably the most parsimonious explanation at the present time is that of much more sexual reproduction occurring in the Gulf of Maine than on Georges Bank because of the more favorable environmental conditions on Georges Bank.

What maintains the amount of genetic variability present in most marine phytoplankton populations? Several explanations, not

mutually exclusive, are possible.

The possibility of high mutation pressure being the immediate cause of the genetic variability observed can probably be ruled out because of the results of the control experiment in which no genetic differences were found among individual cells of clonal cultures. This does not, however, rule out the possibility that the genetic variability is the result: of mutants (generated by a low mutation pressure) that are maintained in long-term coexistence by some other mechanism. It is not known if the different genotypes are the direct products of mutations or recombination.

A second explanation for the maintenance of the observed genetic variability could be heterosis. How important this mechanism is in maintaining genetic variability in natural populations is unknown. Lewontin, *et al.* (1978) and Clarke (1979) cast doubt on the idea that simple heterosis is the major mechanism. Heterosis is somewhat unlikely (but not impossible) in the case of coccolithophore and dinoflagellate species that are haploid in the vegetative state with only the zygote being diploid.

Another potential mechanism is temporal environmental variability. This hypothesis has received much attention (Levins, 1965; Beardmore, 1970; Gillespie, 1975; Valentine, 1976; Soule, 1976; Bryant, 1976; Felsenstein, 1976; Hedrick, 1976; and Hedrick, *et al.*, 1976), but the requirements for maintaining substantial genetic variability this way alone are quite rigorous (Haldane and

Jayakar, 1963). If this mechanism were important, one would expect to find higher amounts of genetic variability in populations living in temporally more variable environments. There is no positive evidence of this in my data, but there are really too few data available to test the hypothesis adequately (for reasons discussed earlier).

Another hypothesis is that the genetic variability is the result of the physical mixing and recombination among genotypes from different populations that have differentiated genetically (see discussions by Soule, 1971 and Felsenstein, 1976). There is no evidence for this hypothesis in my data, because no genetic differentiation was found within water bodies such as the Sargasso Sea. Physical mixing probably is an important factor only in generating the larger amounts of genetic variability observed in those populations isolated from warm core eddies (pop. I of *E. huxleyi* and pop. B of *G. oceanica*), where populations from different water bodies were being mixed together. Brand, et al. (1980) also found what appeared to be an unusually large amount of genetic variability in a population of the diatom *Thalassiosira pseudonana* isolated in a warm core eddy. This variability was attributed to the physical mixing of genetically different neritic and oceanic populations and to reduced reproduction rates resulting from the hybridization of populations with very different gene pools. It is doubtful that this explanation is adequate for most of the genetic

variability observed in phytoplankton populations, however.

Marginal overdominance (discussed by Wallace, 1968b), in which heterozygotes are not necessarily more fit in any given environment, but are more fit overall in the whole range of environments experienced by the population, is another mechanism by which polymorphism can be maintained in natural populations. This mechanism is also based upon environmental variability. The importance of this mechanism is not known.

Another mechanism which could be important in maintaining genetic variability is what I shall term semi-neutral genetic variability. It is probably best explained in graphic form as shown in Fig. 35. Over most of the environmental range, the different genotypes have the same fitnesses, thus genetic changes and allele fixation can occur only by genetic drift. Under certain environmental conditions, however, different genotypes have different fitnesses and selection can occur. This type of genetic variability is hidden from natural selection most of the time, so substantial amounts of genetic variability can be maintained within a population rather easily. The kind of data presented in this thesis could, in principle, determine whether or not this type of variability exists in phytoplankton populations, but more data are needed than have been collected to date. Reproduction rate data are needed all the way to the end of an environmental fitness curve to test the hypothesis.

Figure 35.

Hypothetical environmental fitness curves for two genotypes.

15

COMPONENT OF FITNESS

ENVIRONMENTAL FACTOR

Frequency-dependent selection is another candidate as a mechanism for maintaining genetic variability. **Clarke** (1979) has argued that this may be the most important mechanism for at least certain types of polymorphisms. Diseases, parasites, and grazers may tend to attack the most abundant genotype. Again, there are no data on phytoplankton with which to test this hypothesis at the present time.

Another explanation that has been proposed to explain genetic variability in populations of other types of organisms is that of spatial environmental heterogeneity and niche diversity (see discussions by Levene, 1953; Van Valen, 1965; Gillespie, 1974, 1975; Taylor, 1976; Felsenstein, 1976; Hedrick, *et al.*, 1976; Taylor and Powell, 1977; Steiner, 1977; Powell and Taylor, 1979). The occupation of different microhabitats by different genotypes makes the maintenance of genetic variability much easier. There is evidence for this hypothesis in a number of organisms (see Powell and Taylor, 1979), but it is not clear whether it is applicable to phytoplankton. It is precisely because there are no obvious microhabitats for phytoplankton that Hutchinson (1961) proposed the "paradox of the plankton". Whether the observed species diversity of phytoplankton communities is partly the result of microhabitats is not known at the present time.

Hypotheses about the maintenance of genetic variability, that rely upon environmental variability or niche diversity, are similar

to those hypotheses proposed to explain the "paradox of the plankton" (Hutchinson, 1961; Richerson, ~~et al.~~, 1970; Williams, 1971; Tilman, 1977; Turpin and Harrison, 1979). Only one has been verified experimentally (Tilman, 1977) and this mechanism can probably maintain no more than perhaps five species in coexistence. Whether similar mechanisms are responsible for maintaining both species diversity within phytoplankton communities and genetic variability within populations is not known. One indication that this is not the case is the clear trend toward higher species diversity in oceanic environments than in neritic environments (Hulburt, 1963, 1970; Margalef, 1967), but no evidence of higher amounts of genetic variability in oceanic populations than in neritic populations. Indeed, if the hypotheses that species diversity and genetic variability are maintained by environmental variability are correct, the immediate prediction would be that both species diversity and genetic variability would be higher in neritic waters than in oceanic waters, assuming our perception that neritic waters are more variable is correct. This prediction is clearly false for species diversity and appears to be false for genetic variability as well. The explanation cannot be so simple.

The mechanisms I have just listed as being potentially important in maintaining genetic variability are not necessarily mutually exclusive. Indeed, I have stated them imprecisely enough that they necessarily overlap in some cases. There is no need to go into any

more detail because too little is known at the present time to test the hypotheses rigorously and distinguish among them.

It is not known if the genetic variability present in phytoplankton populations is adaptive or merely some type of genetic load. One cannot prove that the genetic variability is not adaptive, unless reproduction rates over the entire niche hyperspace are measured to confirm that a genotype has a lower fitness under all circumstances. Furthermore, it is not known whether the variability might be adaptive for current environmental changes or a hedge against unpredictable long-range environmental changes as suggested by Williams (1975) and Maynard Smith (1978b). The very fact that sexual recombination occurs in most species of plants and animals suggests that there must be some advantage to recombination and genetic variability, especially given the 50% genetic cost of meiosis (Williams, 1975; Maynard Smith, 1978b). At the present time, it is not known whether sexual reproduction can be maintained by individual selection or if some sort of group selection is necessary (Williams, 1975; Maynard Smith, 1978b). The benefits of sexual recombination and genetic variability have never been rigorously demonstrated.

The type of data collected in this thesis is not precisely what is needed to test ideas concerning the costs and advantages of sexual recombination because the cells collected in the ocean are not direct products of recombination, but are the result of both

recombination and clonal propagation. Thus, while there is no evidence in my data of sisyphean fitness, as hypothesized by Williams (1975), we cannot rule out entirely the remote possibility that the cells collected in nature are the surviving sisyphean genotypes and the majority of the genotypes resulting from recombination are rarely observed. It seems most parsimonious to conclude, however, that sisyphean fitness is not part of the genetic structure of phytoplankton populations.

Genetic differentiation

Several cases of genetic differentiation are obvious in the data. The spatial genetic patterns observed must be interpreted with caution for several reasons, however. First, the absence of detectable genetic differentiation in any given set of data is not proof that differentiation is not present. It is always possible that genetic differentiation could be found if reproduction rates in another part of the niche hyperspace were measured. Second, it is possible that the genetic differences seen between populations are the result of rapid selection in expatriate populations subjected to a new environmental regime. A knowledge of the history of the populations is needed to distinguish between the hypotheses of long term genetic differentiation between independent populations and rapid selection in advected populations. Rapid selection is a viable explanation only if the populations have the genetic

potential for such rapid genetic change. No evidence has been found for rapid genetic changes in phytoplankton populations. Despite these potential problems, the data seem to indicate that the spatial patterns of genetic differentiation are not the same in different species.

Gephyrocapsa oceanica appears to consist of at least two genetically different populations, one in the Sargasso Sea and one in the slope water, with the Gulf Stream probably serving as the dispersal barrier and environmental boundary between them. There is evidence for the mixing of these two populations in a warm core eddy (see pop. B in Fig. 19). The spatial pattern of genetic differentiation across the Gulf Stream appears similar to that found by Brand, et al. (1980) for the diatom Thalassiosira pseudonana. Whether or not interbreeding is occurring between the two populations of G. oceanica, as has been suggested for T. pseudonana (Murphy and Guillard, 1976; Brand, et al., 1980), is not known.

Emiliana huxleyi is also found on both sides of the Gulf Stream, but there is no evidence for genetic differentiation across it as was found in G. oceanica and T. pseudonana. The group collected from two warm core eddies and the slope water (F+K+L) and the two groups from the Sargasso Sea (D+E+G and M+N+P+Q+R+S+T+U) do not differ significantly. There is, however, strong evidence for genetic differentiation between the Gulf of Maine group (H+I+J) and the rest of the populations collected further south (see Figs. 12,

13, and 14). The Gulf of Maine group is clearly adapted for colder temperatures, reproducing more rapidly at 16°C and more slowly at 26°C than the populations from the Sargasso Sea and slope water.

The different spatial patterns of genetic differentiation in G. oceanica and E. huxleyi demonstrate that a knowledge of only the environmental and hydrographic regime is inadequate for predicting the spatial genetic structure of phytoplankton species. This is especially well demonstrated by the comparison of E. huxleyi and G. oceanica because they are closely related species that have somewhat similar global biogeographies. The fossil record indicates that E. huxleyi speciated from Gephyrocapsa approximately 250,000 years ago (McIntyre, 1970). It appears that E. huxleyi and G. oceanica have evolved different types of adaptations to the same temperature regime. G. oceanica has developed genetic differentiation (an adaptation at the population level), while E. huxleyi has evolved a wider temperature niche width (Brand, unpublished) at the individual level. A similar pattern has been found in Drosophila species (Levins, 1969).

No evidence was found for genetic differentiation among populations of Emiliana huxleyi, Cyclcoccolithina leptopora, Thoracosphaera heimi, and Dissodinium lunula within the Sargasso Sea. It is not known whether gene flow or the uniformity of the environment is most important for the lack of detectable genetic differentiation within the Sargasso Sea. These populations were

isolated in the western part of the Sargasso Sea where advective and diffusive processes are most energetic. It remains to be seen if genetic differentiation or clines develop across the broad expanse of the central gyres where much less advection takes place. Similarly, the amount of genetic differentiation that has developed between oceans is not known.

The only evidence for genetic differences between populations near each other in the same water mass is found in the differences between populations of Prorocentrum micans in the Gulf of Maine and those on George Bank. The differences are small and most likely result from asexual reproduction predominating in populations over Georges Bank where conditions are conducive to rapid reproduction (and where essentially no genetic variability was found) and a greater amount of sexual reproduction occurring (and correspondingly more genetic variability) in the Gulf of Maine.

Rates of genetic change

Populations of E. huxleyi, C. leptopora, and T. heimi were isolated at several times during the seasonal cycle. This allows us to test the hypothesis that phytoplankton populations have the genetic potential for evolving on a time scale of months. A shift in the genetic composition of C. leptopora from winter to summer is apparent, but no shift is seen in E. huxleyi or T. heimi. It is not known what the selective forces might be behind the seasonal genetic

change in *C. leptopora*. Gallagher (1978) found a clear difference between winter and summer populations of *Skeletonema costatum* in the electrophoretic mobility of enzymes. The presence of two distinct groups with almost no intermediate genotypes indicates that *S. costatum* is probably genetically differentiated on a seasonal basis rather than evolving seasonally.

Evidence was found for seasonal genetic change in *C. leptopora*, but not in *E. huxleyi* and *T. heimi*, despite significant amounts of genetic variability in all three species. It is not known whether seasonal genetic changes occur in only a few phytoplankton species or occur in most, but are simply difficult to detect. Several factors make the detection of rapid genetic changes difficult or at least unlikely in the data presented in this thesis.

First of all, genetic variability within populations tends to obscure any overall differences that may exist between two populations. Very large numbers of clones are needed to be assured of statistical significance when only small genetic changes occur. Probably the best way of detecting genetic differentiation is to examine the ratio of reproduction rates in two different environmental regimes. In this thesis, only the ratio of reproduction rates at different temperatures was examined.

Second, there is reason to believe that genetic changes in adaptation to temperature are much slower than in other types of adaptations. It seems quite likely that temperature adaptation

involves a very large number of genes. Rapid genetic change would then probably result in a rather large segregational load. Genetic adaptations that require changes at only a few loci can probably evolve more rapidly. This idea needs to be tested, which is possible with the methods developed for this thesis.

The data on all the species taken together indicate that while small genetic changes may occur on the order of months, most phytoplankton populations probably do not have the genetic potential to evolve very different niche hyperspaces quickly. The time scale on which phytoplankton can evolve significantly different niche hyperspaces remains unknown.

Given the amount of genetic variability that is observed in natural populations of phytoplankton, the observation of only small genetic changes on short time scales indicates that: the genetic variability is largely non-additive, or else not responsive to environmental factors that change seasonally. This indicates that a simple knowledge about only how much genetic variability exists in populations does not allow us to predict the potential rates and direction of genetic changes in response to environmental changes.

CHAPTER 5

CONCLUSIONS

CONCLUSIONS

Clonal cultures of coccolithophores and dinoflagellates were sufficiently stable genetically to permit the detection of genetic differences among clones.

Genetic differences among clones were found in all seven species examined. Thus, the concept of a phytoplankton species as a solely asexually reproducing clone with all individuals genetically the same is wrong. Despite the genetic variability present in populations, however, a single clone is, in most cases, reasonably representative of most of the cells in a population because the genetic differences among cells in reproduction rates are only of the order of 10%. Two exceptions, which call for caution, were found, however. First, a small percentage of the cells in a population appear to have reduced reproduction rates that lie outside the "normal" range of reproduction rates within a population. Two or three cells from a population should probably be examined to avoid the possibility of drawing erroneous conclusions about a population from data based upon one of these rare cells with reduced reproductive capacity. Second, a single clone is not representative of a population in hydrographic regimes where two or more populations that have differentiated genetically are mixed together or are hybridizing. Such situations have been found in a

warm core eddy population of *T. pseudonana* (Brand, et al., 1980) and in population I of *E. huxleyi* and population B of *G. oceanica* (this thesis).

Populations and species from estuaries, coastal waters, and the Sargasso Sea were examined. No relationship was found between the amount of genetic variability within the populations and the variability or predictability of their environments.

Individual clones, while usually reasonably representative of the individuals within a single population, are not necessarily representative of the individuals of the entire species. Although no strong genetic differentiation was found within water masses, significant genetic differentiation was found within all species for which populations from more than one water mass were examined. Therefore, it seems quite likely that most species that live in more than one water mass are differentiated genetically. Differentiation between populations from the slope water off New England and the Sargasso Sea has been found in *T. pseudonana* (Brand, et al., 1980) and *G. oceanica* (this thesis) but not in *E. huxleyi* (this thesis). Genetic differentiation in *E. huxleyi* was found between the Gulf of Maine and the populations further south in warmer waters. Thus, the genetic systems of different species appear to respond to the same spatial environmental patterns in different ways. Neritic clones of *T. pseudonana* collected from around the world were found to be surprisingly similar (Brand, et al., 1980). These data indicate

that patterns of spatial genetic differentiation will not be easily predictable.

The population dynamics of species, such as *T. pseudonana*, *E. huxleyi*, and *G. oceanica*, which are subdivided genetically into different populations, must be conceptualized in a somewhat different manner than has been done previously. Genetically different populations are largely independent of each other in their ecological behavior. The population, not the species, must be considered the ecological unit. Rather than advection from a central population providing the biomass found in peripheral populations (the classical oceanographic viewpoint), the advection should probably be considered as a loss to the parent population, which does not contribute significant biomass to the peripheral population. The advected cells (carried either direction) are probably less well adapted to the new environmental regime than those in the indigenous population. The "peripheral" populations must maintain themselves independently. In addition to grazing and sinking out of the photic zone, advection and diffusion out of the habitat in which the population can be maintained should be considered an important source of loss to phytoplankton populations. The genetic differentiation of species into local populations makes losses by advection and diffusion an even more important problem than has previously been thought. Presumably, the "cost" of increased advective loss is balanced by the advantage of

better adaptations to the local environment resulting from greater genetic differentiation.

It is clear that, in addition to the widening of the physiological tolerance of the individual organisms, the development of genetic differentiation is an important mechanism by which phytoplankton species can expand their biogeographic range.

It remains unclear at the present time how much and how quickly phytoplankton populations can change genetically in response to environmental changes. It appears that at least some species have the potential for seasonal genetic changes, but it is also clear that most of the genetic variability in asexual reproduction rates is non-additive, or else not responsive to seasonal selection for other reasons. The physiological tolerance of individual organisms appears to be a more important characteristic than the potential of populations for genetic change that allows phytoplankton populations to survive the environmental fluctuations that occur on the order of weeks to years.

The presence of genetic variability and differentiation in phytoplankton populations and species implies more than simply the fact that the characteristics of a single clone cannot always be extrapolated to an entire species. It forces us to consider the spatial and temporal distribution and abundance of species as being the result of both environmental factors and genetic processes. For example, the recent migration of the "red tide" dinoflagellate

Conyaulax tamarensis southward along the eastern North American coastline could be the result of a changed environmental regime or of genetic changes within the populations that allow them to live in the more southern environments. As another example, the decline in abundance of certain species in warm and cold core eddies could be attributed to the deterioration of the environment within the eddies, but one cannot rule out the possibility that part of the decline also is due to reduced fitness resulting from hybridization of semispecies with gene pools that are not coadapted.

This thesis has not given an explanation for any particular ecological phenomenon observed in phytoplankton populations in the ocean. It has simply demonstrated that, in addition to environmental factors, genetic processes are plausible, alternative (not exclusive) explanations for the ecological patterns observed. Both environmental and genetic factors must be considered in attempting to explain ecological processes.

CHAPTER 6

POSTSCRIPT

POSTSCRIPT

In addition to environmental factors, the adaptations that populations and species are able to evolve are important in determining ecological behavior. To what extent the evolution of these adaptations is directed by environmental patterns and to what extent it is restricted and shaped by genetic constraints is not known. That only a small amount of genetic change occurs on the order of months gives us no answer to the question of how far populations can evolve toward an "optimal strategy". It does not tell us to what extent natural selection is capable of producing ecologically relevant adaptations and to what extent the adaptive potential of the populations and species is restricted by genetic potential, pleiotropic metabolic interactions, and other forms of phyletic inertia (see discussion by Gould and Lewontin, 1979).

Studies higher up the phylogenetic hierarchy may indicate the potential limits of natural selection for developing environmentally relevant adaptations. Data on genetic variability present in natural populations provide us with basic information on how representative a single organism is of all the organisms in a population, but they may tell us very little about long term evolutionary potential, especially if genetic linkage, epistasis, pleiotropism, and non-additive genetic variability are important.

Other approaches may be more useful.

An examination of the amount of genetic differentiation with respect to habitat differences probably gives an indication of how far natural selection can change populations genetically in response to different environmental regimes on somewhat longer time scales. Habitat related patterns and phylogenetic patterns in various characteristics of organisms, including environmental fitness curves, should indicate the extent to which natural selection drives the evolution of ecologically relevant adaptations and the extent to which the evolutionary potential of populations is restricted by their genetic potential, pleiotropic interactions, and other types of phyletic inertia. They should indicate what types of adaptations are relatively easy to evolve and what types are not. Very few such data have been collected on phytoplankton, but those available show that this approach could provide useful insight.

Phylogenetic trends have been found in the maximum potential reproduction rate of phytoplankton (Chan, 1978; Brand and Guillard, 1980) and the diel periodicity of cell division of phytoplankton (Nelson and Brand, 1979). Thus, data now exist on the amount of genetic variability within individual populations of phytoplankton and on genetic constancy within phytoplankton phyla. Clearly, data on genetic constancy and variability at levels in between these two extremes, such as species clusters, genera, and families, would help elucidate the extent to which the evolution of various

characteristics and environmental fitness curves is influenced by the environment through natural selection, and the extent to which evolutionary patterns are restricted by genetic potential. Such information is needed for an understanding of what types of adaptations can evolve rapidly and what types are difficult or slow to evolve. The hierarchical levels at which phylogenetic and habitat related trends are found should indicate to some extent the time scales upon which various characteristics can evolve. Only by searching for both diversity and uniformity in components of the niche hyperspace and other characteristics along the whole phylogenetic hierarchy from the individual to entire phyla can we begin to understand the interaction of ecological and evolutionary processes.

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