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TEMPORAL PATTERNS IN POPULATION GENETIC DIVERSITY OF *PROROCENTRUM MICANS* (DINOPHYCEAE)[1]

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We describe temporal changes in the genetic structure of populations of the dinoflagellate *Prorocentrum micans* Ehrenberg over a period of 2 years at Scripps Pier (La Jolla, CA, USA). We collected 12 water samples over the course of two blooms and analyzed 166 single-cell isolates using randomly amplified polymorphic DNA analysis. Six PCR primers uncovered 27 polymorphic markers, allowing the identification of 40 unique haplotypes. Analysis of molecular variance demonstrated that >92% of the genetic variance was partitioned within water samples, providing evidence of high levels of genetic diversity and possibly sexual reproduction. Although the level of genetic diversity remained fairly stable over the sampled time interval, several populations (sampled in June 1998 and March 1999) exhibited significantly different genetic composition, demonstrating differences among bloom and nonbloom periods. About 40% of the isolates in each sample were identified as one haplotype, suggesting that a genetically distinct subgroup was a common member of the populations during the sampled periods. The composition of the remaining isolates was genetically diverse and changed over time, indicating rapid responses (days) to changing environmental conditions or extensive genetic spatial patchiness (kilometers). Within the limitations of our sampling, these two genetically distinct groups appear to exhibit different population dynamics (one stable and the other variable), suggesting that genetic diversity may be closely linked to the change in abundance of phytoplankton on ecological time scales.

Key index words: **algal blooms; dinoflagellates; genetic diversity; temporal variability**

Abbreviations: **AMOVA, analysis of molecular variance; RAPD, randomly amplified polymorphic DNA**

Harmful algal blooms are formed by many different phytoplanktonic taxa (Hallegraeff 1993), but dinoflagellates are the most common deleterious blooming organisms in the marine environment (Sournia 1995). The mechanisms responsible for the occurrence of these blooms are poorly understood and may include high growth rates (Smayda 1996), decreased grazing (Sautour and Castel 1999), and hydrographic processes (Franks 1997). Furthermore, the adaptive advantage of bloom formation is unclear: Why do some phytoplankton species form blooms, whereas others do not? It seems likely that changing environmental conditions lead a blooming species to become competitively dominant, implying that bloom species are very well adapted to their environment during these periods (Smayda 1997). Because genetic diversity is important in determining responses to changing environmental conditions (Brand 1991), the necessity of studying the genetic diversity of phytoplankton in the context of bloom formation becomes apparent.

The above realization has led to many genetic studies of blooming phytoplankton species using various phenotypes expressed by clonal isolates in the laboratory. These include growth rate (Gallagher 1982, Larsen et al. 1993), tolerance to temperature or salinity (Krawiec 1982, Brand 1984), and toxin profile (Bomber et al. 1989). Molecular techniques such as enzyme electrophoresis (Cembella and Taylor 1986) and DNA sequencing (Scholin et al. 1994) have also demonstrated genetic variation within species. Recent studies have uncovered extensive intraspecific variation within particular geographic locations using highly polymorphic genetic markers such as randomly amplified polymorphic DNA (RAPD) (Medlin et al. 1996, Bolch et al. 1999) and microsatellite DNA (Rynearson and Armbrust 2000). In general, these studies have shown that multiple single-cell isolates taken from the same water sample are genetically distinct. The next logical step is to use such methods, with larger sample sizes, to characterize the temporal genetic variation of a phytoplankton species over the course of bloom cycles.

We carried out such an analysis to begin to address some simple hypotheses. It is currently unknown if the genetic structure of a dinoflagellate species changes between a bloom and a nonbloom period. Additionally, we do not know whether temporally separated populations from the same location are different from one another, regardless of bloom stage. To address these

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questions, we used RAPD to examine *Prorocentrum micans* Ehrenberg, which forms frequent blooms off the coast of Southern California and throughout the globe (Allen 1946, Sweeney 1975, Cassie 1981, Pybus 1990).

MATERIALS AND METHODS

Sampling. We obtained seawater from surface bucket samples from Scripps Pier (La Jolla, CA, USA) on 12 dates between the spring of 1998 and the fall of 1999. To place these sampling dates in the context of phytoplankton bloom dynamics, we used conductivity-temperature-depth profiler (CTD) data (including *in vivo* fluorescence) collected throughout the year. The 12 samples were qualified as originating from bloom and nonbloom periods based on a combination of factors, including the CTD data, extracted chl *a* concentration (courtesy of J. McGowan, Scripps Institution of Oceanography), *P. micans* cell counts (bloom periods > 5 *P. micans* cells $\cdot$ mL^{-1}), and the relative abundance of *P. micans* compared with other phytoplankton. The sampling dates ranged over the periods before, during, and after two blooms, and each date was treated as a distinct population in subsequent genetic analyses.

Cell isolation and DNA extraction. Single *P. micans* cells were isolated by micropipette under a dissecting microscope within 4 h of collection and placed into separate wells of a 24-well tissue culture plate containing 0.5 mL of 0.2-μm-filtered seawater and 0.1 mL of f/2-Si medium (Guillard 1975). Cultures were grown at room temperature (approximately 22° C) and transferred into a 25-mm sterile glass tube with 10 mL f/2-Si medium after 2 weeks. All isolates were subsequently maintained at 18° C and at continuous light levels of 137 μmol photons $\cdot$ m$^{-2} \cdot$ s^{-1} (as measured by a QSL-100 photometer, Biospherical Instruments, Inc., San Diego, CA, USA) in 25 mL f/2-Si and were transferred every 3 weeks into fresh media. Of the 514 isolates that survived the culture process from the 12 water samples, 166 were analyzed with RAPD. This analysis included four populations—a 1998 early bloom, a 1998 late bloom, a 1999 prebloom, and a 1999 bloom—that were extensively examined with samples of 40, 36, 34, and 25 isolates, respectively. These populations will subsequently be referred to as the four "large" populations. We also analyzed a *P. micans* isolate obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) originally isolated in La Jolla in 1964 (CCMP 689).

Algal cells from 15 mL of each culture were pelleted by centrifugation at 200*g* for 5 min in a model CL clinical centrifuge (IEC, Needham Heights, MA, USA) and transferred to a soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA). DNA was extracted according to the manufacturer's instructions, quantified on a spectrofluorometer after staining with pico-green (Ahn et al. 1996), and stored frozen at − 20° C until amplification.

RAPD. Twenty 10-mer oligonucleotide primers (10 60% GC, 10 70% GC) were screened to identify those best able to produce clear RAPD banding patterns in the *P. micans* genome. Six primers were chosen that produced banding patterns with between four and eight repeatable clearly identifiable bands. Specific primer composition, annealing temperatures, and bands scored for each primer are reported in Table 1. RAPD amplifications were then carried out in 20-μL reactions containing 0.4 U REDtaq DNA polymerase, 1 × REDtaq PCR buffer with $MgCl_2$, 2 μM primer, 100 μM of each dNTP, and 5 ng of genomic DNA. The amplifications were carried out under the following conditions: 5 cycles of 1 min at 94° C, 1 min at 37° C, 1 min at 65° C, followed by 25 cycles of 1 min at 94° C, 1 min at 42–52° C, and 1 min at 68° C. Annealing temperatures were tested between 37 and 52° C for each primer to determine optimum conditions, and each reaction was run in duplicate. To verify the reproducibility of the results, two DNA extracts were separately prepared and amplified from each of seven isolates. The amplified products were separated on 2% agarose gels in 1 × TAE buffer, stained with ethidium bromide, and digitally photographed using a UVP digital gel documentation system (Upland, CA, USA).

RAPD data analysis. Digital images were analyzed using image-processing software (XV 3.10a on a Unix workstation, John Bradley, BrynMawr, PA, USA). Size standards (100 bp ladder, Bio-Rad, Hercules, CA, USA) were run with each gel to allow product size comparison among gels. Bands between 400 and 3000 bp were scored for presence or absence in each isolate. Scores were analyzed as binary matrices where a 1 or 0 was assigned for the presence or absence of a band, respectively. Subsequent analyses were performed with the software Arlequin 2.000 (Schneider et al. 2000).

For each of the 12 populations, we calculated two measures of population gene diversity and their sampling variances. The first measure, haplotype diversity (*H*), is defined as the probability that two haplotypes randomly chosen from a population are different. This measure is sometimes identified as "gene diversity" and is equivalent to the expected heterozygosity for diploid data (Schneider et al. 2000). In this case, the specific haplotypes of the individuals are ignored and only their relative frequencies in the populations are considered. The other measure, gene diversity (*N*), takes into account the individual genotypes (Schneider et al. 2000). This measure is sometimes referred as "nucleotide diversity" or "averaged gene diversity over loci" and is defined as the probability that two randomly chosen homologous nucleotides are different.

To determine whether RAPD loci were independent, we tested for linkage disequilibrium using 100,000 Markov chain steps and 1000 dememorization steps. We performed this analysis for all isolates as well as for the four large populations separately, for a total of five analyses. In each case, original

TABLE 1. Designation, annealing temperature, and sequence of RAPD primers used to assay *Prorocentrum micans* populations at Scripps Pier.

Primer	Annealing (°C)	Sequence	Marker bands (bp)
60–1	47	5′ CGCAGTACTC 3′	590,[a] 650, 725, 775, 900, 1050, 1600, 1700
70–2	47	5′ GGACCGACTG 3′	490, 600, 710, 850, 950, 1150
70–3	52	5′ CTGTCGGCTC 3′	550, 710, 750, 850, 900, 1350
70–4	47	5′ GGACCGCTAG 3′	390, 450,[a] 470,[a] 540, 610, 710, 850, 950
70–7	52	5′ CTATCGCCGC 3′	320, 620, 900,[a] 975, 1100
70–8	42	5′ CCGGGGTTAC 3′	580,[a] 790,[a] 825, 900, 1050, 1250, 1350, 2300

The approximate length (in base pairs) of the RAPD loci is also included.
[a]Monomorphic loci.

significance levels ($\alpha = 0.05$) were adjusted for multiple tests using the Dunn-Sidàk method (Sokal and Rohlf 1998). For example, α for pair-wise comparisons of 22 polymorphic loci was adjusted for 21 independent tests ($\alpha' = 0.00244$).

Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was conducted to examine the intraspecific diversity within populations, among populations within groups, and among groups. The populations were divided into groups for nested AMOVA according to two different schemes to test the effect of various temporal scales on the among-group variance. In the first case, populations were divided into "nonbloom" (<5 *P. micans* cells $\cdot$ mL^{-1}) and "bloom" groups (>5 *P. micans* cells $\cdot$ mL^{-1}), whereas in the second case, populations were divided into samples taken in 1998 and 1999. A third AMOVA was also conducted for only the four populations with large sample sizes (three bloom and one nonbloom) to determine whether the previous results were an artifact of the small sample sizes of most populations (3–4 isolates each versus 25–40 isolates for the large samples). To determine which large populations were different from one another, pair-wise F_{ST} values were estimated by calculating Φ_{ST}, and significance levels were obtained with 10,000 permutations of the data. An exact test of population differentiation (10,000 steps in Markov chain) was also performed as a comparison with the direct Φ_{ST} calculations. This analysis tests the random distribution of haplotypes within populations with panmixia as the null hypothesis (Schneider et al. 2000).

RESULTS

Sampling of Prorocentrum micans cultures. Two blooms were sampled during the course of this study. The 1998 bloom was moderate (Fig. 1; [*in vivo* chl *a*] $< 1.5\ \mu g \cdot L^{-1}$), and two large samples were taken during (sample PM2) and after (sample PM4) the bloom. The 1999 bloom was more substantial (Fig. 1; [*in vivo* chl *a*] $> 3\ \mu g \cdot L^{-1}$), and two large samples were taken before (sample PM7) and during (sample PM13) the bloom. Extracted chl *a* concentrations (Table 2) similarly demonstrated that the bloom in 1999 was more substantial than the one from the previous year and about twice the 18-year average extracted chl *a* concentration of $1.58\ \mu g \cdot L^{-1}$ at Scripps Pier (J. McGowan, personal communication).

A potential diversity-reducing bias in studies using algal culture isolates is the selection of more culturable strains over strains less amenable to laboratory conditions. In our study, over 70% of the *P. micans* cells isolated survived the culture process (Table 2), suggesting that culture biases were minimal. Additionally, the few samples with poor isolation survival (e.g. PM1 and PM17) exhibited RAPD gene diversities similar to those with nearly 100% survival (Table 3), suggesting that if any culture bias occurred, it did not reduce genetic diversity measures.

All isolations were performed by the same individual (Shankle), who also conducted extensive ecophysiological experiments (Shankle 2001). Based on the visual similarity of isolates and the genetic results discussed below, we are confident that all isolates were of the

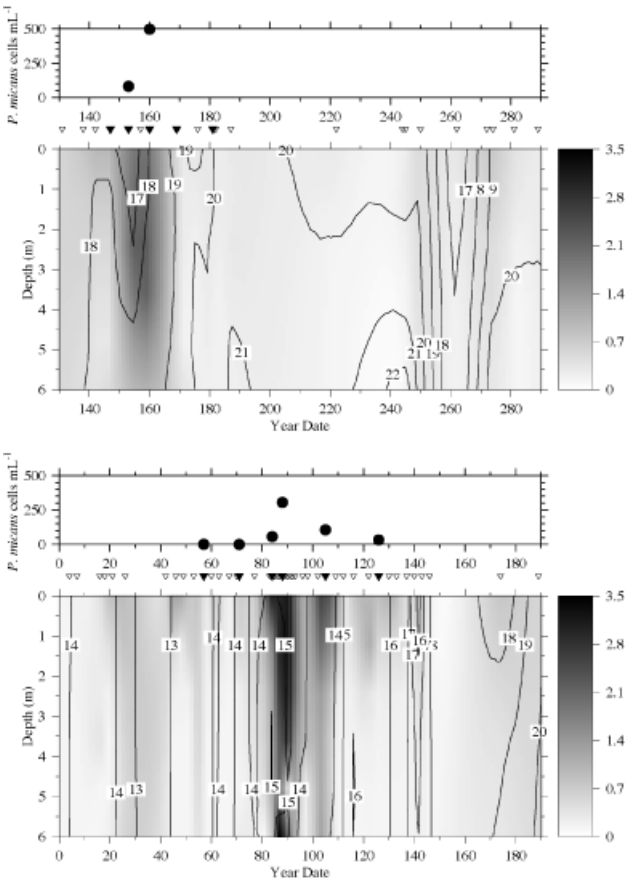

FIG. 1. Environmental conditions measured by CTD off Scripps Pier over the sampling period from 10 May 1998 to 17 October 1998 (top) and from 31 December 1998 to 9 July 1999 (bottom). The grayscale image represents *in vivo* chl *a* concentrations ($\mu g \cdot L^{-1}$) and the contours represent water temperature (°C). Closed triangles indicate the dates on which *Prorocentrum micans* populations were sampled, whereas open triangles mark the dates for which CTD data were collected. The upper panels of each plot show the *P. micans* cell concentrations versus time (see Table 2).

TABLE 2. Environmental information for samples collected at Scripps Pier.

Population	Collection date	*Prorocentrum micans* concentration (cells · mL^{-1})	Chl concentration (μg · L^{-1})[a]	Clones analyzed with RAPD	Clones initially isolated	Clones surviving isolation procedure	Bloom condition[b]
PM 1	27 May 1998	n/a	2.8	4	90	19	Y
PM 2	2 June 1998	82	2.9	40	88	62	Y
PM 3	9 June 1998	498	3.7	3	88	81	Y
PM 4	18 June 1998	n/a	1.4	36	88	79	Y
PM 5	30 June 1998	n/a	1.2	3	8	8	N
PM 6	26 February 1999	0.25	1.6	4	59	50	N
PM 7	12 March 1999	0.21	1.9	34	51	48	N
PM 9	25 March 1999	57	7.4	4	61	59	Y
PM 13	29 March 1999	306	11.6	25	64	61	Y
PM 17	15 April 1999	106	10.7	4	66	8	Y
PM 18	6 May 1999	32	4.0	3	65	30	Y
PM 19	10 September 1999	0.77	n/a	6	16	9	Y

[a]Chl data courtesy of J. McGowan (Scripps Institution of Oceanography); some dates differ from sample dates (see Fig. 1).
[b]A qualitative observation of bloom conditions is based on water discoloration. Y, bloom; N, no bloom.
n/a, not available.

same species and not the thinner narrower congener *P. triestinum* (cf. Costas 1986).

RAPD. The RAPD-PCR reactions produced good replication of bands (Fig. 2), and the six primers led to 33 bands (loci) that could be scored. Of the 33 loci, 6 were monomorphic, 9 occurred in >95% of isolates, 6 had frequencies between 5% and 82%, and the remaining 12 were present in <5%. We observed 40 haplotypes, differing by between 1 and 16 loci, among the 166 isolates: 12 haplotypes were represented by more than 1 isolate, whereas the remaining 28 were unique. One haplotype (h2) occurred with a much higher frequency than any other and comprised 43% of all isolates, including strain CCMP 689. Among the four large populations, the haplotype frequencies were similar, with the exception that haplotype 1 was present in a high frequency in only population PM13 (Table 3).

Calculated haplotype diversities (H) ranged from 0.5 to 1 (Table 3), the latter being a result of the low sample sizes in the eight smaller populations. However, the values of H from the four large populations were close to one another (0.69 ± 0.078 to 0.83 ± 0.055), although the two 1998 populations had slightly lower diversity values than the two 1999 populations (Table 3). Because of the relatively high proportion of loci present in >95% or in <5% of the individuals, average gene diversities, N, were low: 0.030–0.232. Gene diversities and their sampling variances from the four large populations were similar to those from small populations, with the exception that PM9 and PM18 exhibited higher values (Table 3). Linkage disequili-

TABLE 3. RAPD haplotype distribution for each La Jolla *Prorocentrum micans* population.

Haplotype	PM1	PM2	PM3	PM4	PM5	PM6	PM7	PM9	PM13	PM17	PM18	PM19	Total
1	3	2		1	1				7	1			15
2		21	1	19	1	2	14	1	10		1	2	72
3		1				1			1				3
4							1		1	3			5
5		5		4			6		2			1	18
6	1	1		4			3						9
7		2					1		1				4
8		2		3									5
9												1	1
10							1		1				2
11		2											2
44				2									2
Unique		3	2	3	1	1	8	3	2		2	3	28
n	4	40	3	36	3	4	34	4	25	4	3	6	166
H	0.50	0.69	1.00	0.70	1.00	0.83	0.83	1.00	0.78	0.50	1.00	0.93	0.79
H s.v.	0.265	0.078	0.272	0.076	0.272	0.222	0.055	0.177	0.063	0.265	0.272	0.122	0.031
N	0.030	0.038	0.081	0.034	0.040	0.076	0.056	0.232	0.038	0.030	0.141	0.067	0.0518
N s.v.	0.030	0.027	0.073	0.025	0.042	0.061	0.037	0.164	0.028	0.030	0.118	0.049	0.0337

Haplotypes 1–11 and 44 were present in multiple isolates. "Unique" haplotypes are those that were represented by only one isolate. n is the total number of isolates examined from each population. Haplotype diversity (H), defined as the probability of sampling two different haplotypes in a population and its sampling variance (s.v.) for the sampling process are reported. Also included is population gene diversity (N) averaged over loci and its sampling variance.

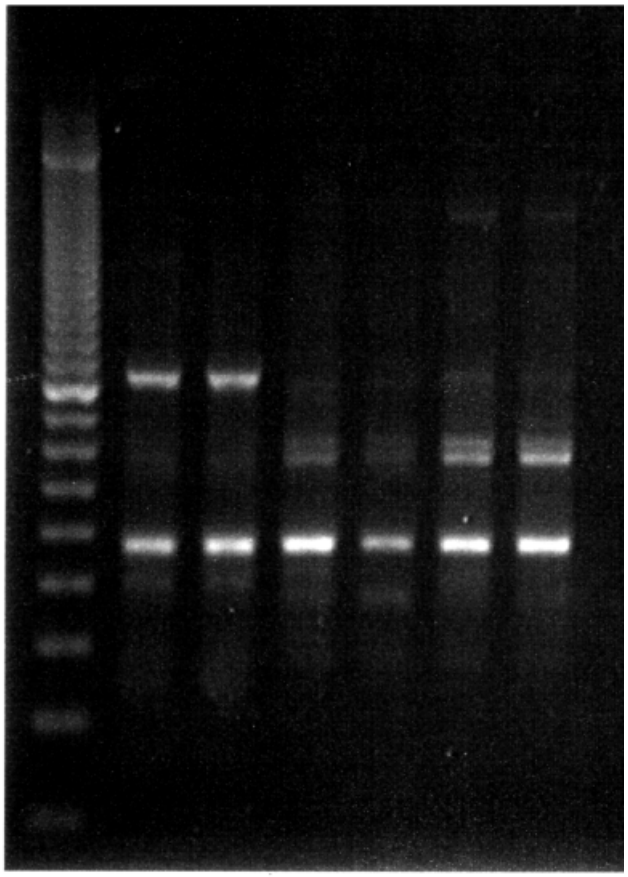

FIG. 2. Gel electrophoresis (2% agarose) demonstrating the reproducibility of RAPD on duplicate DNA extractions (lanes 2 and 3, 4 and 5, 6 and 7). Lane 1: DNA size ladder.

brium tests revealed that 26 of 231 (11.3%) loci pairs were significantly linked (whole sample, after Dunn-Sidàk corrections). Three of the four large populations considered separately revealed similar numbers (9%–13% linkage), whereas population PM13 did not exhibit any linked loci.

The three AMOVAs demonstrated that the largest portion of the total genetic variance was contributed by the within-population variance (93%–97%; Table 4). The remaining among-population variance was statistically significant for all three grouping schemes, indicating that some populations were genetically distinct. The among-group variance was not statistically significant either when grouped by sample year or when bloom and nonbloom periods were grouped. Because of the low sample size in all but four of the populations, we only calculated pair-wise Φ_{ST} values for the latter. These demonstrated that only one of the six possible pairs were statistically different at the $\alpha = 0.05$ level (Table 5): the 1998 late bloom and 1999 bloom (PM4 vs. PM13). However, the pair-wise Φ_{ST} comparison between PM7 and PM13 was significant at $\alpha = 0.10$ ($P = 0.064$), and the analysis of population differentiation using an exact test further suggested that these two populations were genetically different ($P = 0.026$; Table 5).

DISCUSSION

Based on the RAPD data, we found 1) relatively high levels of genetic diversity within temporally (and presumably spatially) separated *P. micans* populations, 2) no strong differences in the absolute level of genetic diversity within populations, 3) differences in the composition of haplotypes among populations, and 4) the dominance of one haplotype in many of the populations. We first mention some of the caveats of RAPD analysis as they relate to our study, after which we discuss the significance of these findings to the study of marine phytoplankton ecology.

Considerations. Although RAPD has been extensively used in terrestrial plant research (Huff et al. 1993, Nesbitt et al. 1995, Gugerli et al. 1999), few studies have applied it to marine macroalgae (Engelen et al. 2001, Faugeron et al. 2001) and fewer still to marine phytoplankton (Medlin et al. 1996, Bolch et al. 1998). This may be because RAPD analysis suffers from potential problems, such as PCR reproducibility, DNA contamination, and the application and interpretation of data analyses. In our case, we screened 20 potential RAPD primers and chose 6 that

TABLE 4. Results of AMOVA of RAPD data when *Prorocentrum micans* populations are grouped by sample year, by nonbloom or bloom state, or when only large sample size populations (PM2, PM4, PM7, PM13) are analyzed.

Grouping	Variance component	df	SS	Variance	Percent variation
Year	Among groups	1	1.335	−0.024	−2.74
	Among populations	10	17.745	0.088	9.79[a]
	Within populations	154	126.275	0.820	92.95[a]
	Total	165	145.355	0.884	
Bloom	Among groups	1	1.458	−0.025	−2.84
	Among populations	10	17.623	0.086	9.52[a]
	Within populations	154	126.275	0.820	93.26[a]
	Total	165	145.355	0.881	
Large samples	Among populations	3	4.273	0.022	2.92[a]
	Within populations	131	91.609	0.699	97.08[a]
	Total	134	95.881	0.721	

df, degrees of freedom; SS, sum of squares.
[a] $P < 0.05$.

TABLE 5. Pair-wise Φst (genetic distance) for pairs of populations of *Prorocentrum micans*.

Comparison	Genetic distance		Exact test *P*
	Φst	*P*	
PM 2 × PM4	−0.01021	0.711	0.432
PM 2 × PM7	0.00304	0.292	0.22
PM 2 × PM13	0.03017	0.0915	0.0929
PM 4 × PM7	−0.00144	0.404	0.201
PM 4 × PM13	0.04278	0.0421[a]	0.0013[a]
PM 7 × PM13	0.03209	0.0639	0.0263[a]

P values were calculated with permutations of the data. Results of an exact test of population differentiation are also included.

[a]Significant difference ($\alpha = 0.05$).

produced clear and repeatable banding patterns. Another concern is the potential contamination of bacterial DNA, especially when using nonaxenic dinoflagellate cultures. Using our methodology for DNA extraction from dinoflagellate cell cultures, dinoflagellates were pelleted while unattached bacteria remained in suspension (data not shown). Previous studies have shown that artificially increasing the concentration of bacterial DNA up to an 80% molar percentage of total DNA made no difference in the eukaryotic RAPD signal (Williams et al. 1993). Furthermore, studies have shown that dinoflagellate genomes (mesokaryotic) are much larger than eukaryotic genomes (Rizzo 1991), which may further dilute any signal of bacterial origin during PCR. Finally, we calculated several genetic measures to gain a broad perspective of overall genetic structure in our sampled populations and to reduce the possibility that our conclusions rely on inaccurate patterns.

Genetic diversity within populations. Although we found that many populations shared some of the same haplotypes, our results indicated high levels of genetic diversity within temporally separated populations of *P. micans*; the AMOVA demonstrated that 92% of the genetic variation was partitioned within populations. This value is similar to RAPD studies of marine phytoplankton and macroalgae that exhibit sexual reproduction: 87% in the dinoflagellate *Gymnodinium catenatum* (Bolch et al. 1998), 95% in the red alga *Mazzaella laminarioides* (Faugeron et al. 2001), and 75% in the brown alga *Sargassum polyceratium* (Engelen et al. 2001). Higher plant species that display outcrossing in reproduction have shown similar levels of within-population genetic variance (70%–95%) (Huff et al. 1993, Nesbitt et al. 1995, Gugerli et al. 1999). Bhaud et al. (1988) demonstrated sexual reproduction in *P. micans* laboratory cultures, and our data suggest that it occurs in nature as well. In addition, only 11% of locus pairs were significantly linked, which implies that recombination regularly occurs to shuffle most RAPD loci.

The calculated values of haplotype diversity (*H*) are difficult to compare with the aforementioned RAPD studies of marine algae because in those studies, most or all of the sampled individuals were genetically distinct (i.e. exhibited different multilocus haplotypes), and if *H* were calculated, it would be equal to 1. Unlike these studies, many of our isolates exhibited identical haplotypes, making the calculation of *H* significant. If we had concentrated our efforts on the development of more RAPD loci, we most likely would have differentiated more individuals. For example, Bolch et al. (1998) used 11 primers leading to 375 RAPD loci (more than our study) to differentiate 31 strains of *G. catenatum* originating from 15 blooms worldwide. However, we decided that our efforts would be better served with a greater sampling of individuals rather than loci to gain insight into *P. micans* temporal population structure. Because our sampled populations, especially the four large ones, were composed of a high number of individuals from a relatively small water sample, many of these individuals were likely to have been genetically identical because of asexual reproduction. It is also difficult to compare our indices of gene diversity (*N*) to other studies that calculated similar measures for the same reason. For example, although Medlin et al. (1996) found that all individuals of *Emiliania huxleyi* analyzed in their study exhibited distinct haplotypes and the sampled populations displayed gene diversities of 0.24 (compared with our average value of 0.052), they sampled a maximum of three individuals from each water sample. This is similar to the *N*s from some of the low sample size populations (PM9, PM18). Nevertheless, our values of haplotype and gene diversities may be useful for comparison with future studies of phytoplankton genetic diversity at small spatial or temporal scales. With a limited number of RAPD loci and isolates, we were able to detect substantial genetic diversity in *P. micans* within small water samples. This diversity may be important in regulating *P. micans* population dynamics and the response of this organism to environmental fluctuations, leading to the formation of blooms.

Genetic diversity among populations. Our data imply that the total genetic diversity of *P. micans* found at Scripps Pier did not change drastically over time and was not dependent on the organism's concentration in the water column. Although our sampling design was uneven, the gene diversities (*N*) of the small samples were similar to the ones from the large samples. The exception was population PM9, with an abnormally high *N* (0.232); three of the four isolates in this sample displayed unique haplotypes. It is likely that because of its small sample size (4), this occurred by chance. The four large populations had haplotype diversities similar to each other, even though they originated from different bloom stages. If this hypothesis is corroborated by future studies of bloom-forming dinoflagellate taxa, it could drastically change the way we view the population dynamics of these organisms.

There are several nonmutually exclusive explanations for the observed lack of change in genetic

diversity over time. One proposed mechanism of bloom formation is an increase in asexual growth rate (Smayda 1996), leading to an increase in the net growth rate of a population. This mechanism could lead to the observed results only if all strains in the population had similar net growth rates. If only one or a few strains showed increased net growth rates, the genetic diversity of a bloom population would be lowered as the faster growing strains increased their proportion in the population. It is possible that all *P. micans* individuals in our sampled populations were asexually dividing at the same growth rate and experiencing the same mortality. Thus, no diversity would have been lost. However, this possibility conflicts with previous studies demonstrating heterogeneity of laboratory growth rates within a phytoplankton species (Gallagher 1982, Larsen et al. 1994, Shankle 2001). Although laboratory growth rates may not be representative of growth rates in field populations (e.g. heterotrophic bacteria have been shown to alter phytoplankton growth rates in laboratory cultures) (Fukami et al. 1997), it seems unlikely that all strains grow at identical rates in field populations.

A second explanation for the minimal change in genetic diversity of *P. micans* over time is that sexual reproduction may be more important than previously thought, continually replenishing the genetic diversity of the population through genetic recombination. The significant linkage of only 11% of loci pairs in our study and the high within-population genetic variation imply that recombination must regularly occur. Although a resting cyst stage has not been found in *P. micans*, the La Jolla population may originate from a genetically diverse cyst bed that continually replenishes the planktonic phase. Although these hypotheses are still untested, they provide possible mechanisms by which high genetic diversities of *P. micans* and other phytoplankton species can be maintained over time.

Genetic differentiation. Although the total genetic diversity of *P. micans* did not change considerably over time, we observed some differentiation of genetic composition among temporally separated populations, for example, population PM13 (from an extensive bloom in 1999) with PM4 (late bloom in 1998) and, to a lesser extent, with PM7 (nonbloom in 1999). In addition, no linkage disequilibrium among any loci was noted in PM13, and 28% of its sampled individuals exhibited haplotype 1, otherwise relatively rare in other populations. These data support the notion that *P. micans* bloom populations were genetically distinct from nonbloom (or late bloom) populations, although they shared some haplotypes. The difference between populations PM4 and PM13 is not surprising, considering that these samples were taken during different years; stochastic year-to-year variation could explain such differences. The difference between populations PM7 and PM13 is more surprising because these populations were sampled only 17 days apart, though the former originated from a prebloom period and the latter from a *P. micans* bloom. This temporal difference is consistent with the work of Tarutani et al. (2000), who showed that the clonal composition of *Heterosigma akashiwo* (Raphidophyceae), measured by susceptibility to viral attack, changed during a bloom cycle. Viral lysis could explain the change in composition of *P. micans* detected here, although other factors such as mortality due to bacteria, zooplankton grazing, and advection of new populations may also be of consequence.

Temporal variability can also be an indication of spatial variability, as the two are tightly coupled by the advection of water past the sampling site. A rough estimate of patch sizes of haplotypes can be obtained using a canonical $10\,\mathrm{cm}\cdot\mathrm{s}^{-1}$ coastal current (approximately $10\,\mathrm{km}\cdot\mathrm{d}^{-1}$). Thus, the PM7 and PM13 populations sampled 17 days apart might have originated 170 km apart in space, suggesting that significant variations in population genetic structure of this planktonic dinoflagellate over hundreds of kilometers or less in the coastal ocean may occur. Sampling over a range of spatial scales at one time would help in distinguishing temporal from spatial variability in the dynamics of the population genetic diversity.

Dominance of haplotype 2. The clearest feature discerned through RAPD analysis of the 166 *P. micans* isolates taken from La Jolla over a 2-year time span was that the same haplotype (haplotype 2) remained dominant throughout. Whether a water sample was taken during a bloom or nonbloom period, haplotype 2 was consistently present in high frequencies. Although using more RAPD loci or more polymorphic markers, such as microsatellite DNA, would likely differentiate some haplotype 2 isolates, it is reasonable to conclude that they are more closely related to one another than to isolates exhibiting different haplotypes. Therefore, it appears that a genetically distinct subgroup of *P. micans* exhibiting haplotype 2 is a ubiquitous member of the population found at Scripps Pier. The fact that strain CCMP 689 isolated from the same location 34 years earlier also exhibits haplotype 2 further supports this hypothesis. On the other hand, the composition of the other isolates is quite variable among populations, demonstrating a relatively high level of genetic diversity. Based on these data, it appears that *P. micans* at Scripps Pier can be divided into two distinct subpopulations. The first consists of a group that remains present in the environment regardless of cell concentration or time of year (at least during the months sampled in this study). This group may exhibit phenotypes that allow it to persist in the environment, such as resistance to viral lysis or bacterial attack, high toxin content to deter grazing, or the ability to tolerate turbulent or low nutrient conditions. Alternatively, it may lack such adaptive advantages and simply be derived from a local cyst population that exhibits the same genotype. The second subpopulation, represented here by

haplotypes other than haplotype 2, is a group that varies substantially in composition over time. This group may be affected more strongly by the external factors discussed above or may originate from more distant and varied sources, leading to the temporal genetic differentiation that we observed.

Shankle (2001) showed that temperature-specific growth rates of 108 clonal strains of *P. micans* (gathered at the same time and location as the present study) remained relatively constant over more than a year in culture. In addition, the variability in growth rate among strains isolated from the same sample was constant among samples gathered at different times. However, there was no correspondence between growth rates and the genetic markers discussed above. For example, haplotype 2 had both fast- and slow-growing strains. This is possible evidence that these randomly chosen genetic markers have no ecophysiological relevance. It is also possible that the growth rates of the isolates were not phenotypic but reflected, for example, the influence of the associated bacterial populations—a hypothesis we were unable to test. Further work is required to relate genotypic variability to phenotypic variability and its ecological expression.

Our findings suggest that intraspecific groups within *P. micans* may play different ecological roles. This idea is critical to phytoplankton ecology because a vast number of studies use one or two monoclonal laboratory cultures to examine ecological questions on a species level. Future studies combining genetic fingerprinting methods and ecological or physiological experiments are necessary to test whether intraspecific genetic variability patterns are meaningful on ecological scales.

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