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GENETIC VARIATION AMONG STRAINS OF THE TOXIC DINOFLAGELLATE *GYMNODINIUM CATENATUM* (DINOPHYCEAE)[1]

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The toxic dinoflagellate *Gymnodinium catenatum* Graham has formed recurrent toxic blooms in southeastern Tasmanian waters since its discovery in the area in 1986. Current evidence suggests that this species might have been introduced to Tasmania prior to 1973, possibly in cargo vessel ballast water carried from populations in Japan or Spain, followed by recent dispersal to mainland Australia. To examine this hypothesis, cultured strains from *G. catenatum* populations in Australia, Spain, Portugal, and Japan were examined using allozymes and randomly amplified polymorphic DNA (RAPD). Allozyme screening detected very limited polymorphism and was not useful for population comparisons; however, Australian, Spanish, Portuguese, and Japanese strains showed considerable RAPD diversity, and all strains examined represented unique genotypes. Multidimensional scaling analysis (MDS) of RAPD genetic distances between strains showed clear separation of strains into three nonoverlapping regional clusters: Australia, Japan, and Spain/Portugal. Analysis of genetic distances between strains from the three regional populations indicated that Australian strains were almost equally related to both the Spanish/Portuguese population and the Japanese population. Analysis of molecular variance (AMOVA) found that genetic variation was partitioned mainly within populations (87%) compared to the variation between the regions (8%) and between populations within regions (5%). The potential source population for Tasmania's introduced *G. catenatum* remains equivocal; however, strains from the recently discovered mainland Australian population (Port Lincoln, South Australia, 1996) clustered with Tasmanian strains, supporting the notion of a secondary relocation of Tasmanian *G. catenatum* populations to the mainland via a shipping vector. Geographic and temporal clustering of strains was evident among the Tasmanian strains, indicating that genetic exchange between neighboring estuaries is limited and that Tasmanian *G. catenatum* blooms are composed of localized, estuary-bound subpopulations.

Key index words: **allozyme; ballast water; dinoflagellate; DNA; genetic variation; *Gymnodinium catenatum;* isozyme; multidimensional scaling; population genetics**

Abbreviations: **AMOVA, analysis of molecular variance; MDS, multidimensional scaling**

The known global distribution of the chain-forming, toxic dinoflagellate *Gymnodinium catenatum* Graham has increased rapidly over the last decade. First described from the Gulf of California in 1943 (Graham 1943), it was subsequently reported from Argentina in 1961 (Balech 1964) and Japan in 1967 (Hada 1967, as *Gymnodinium* sp. A3). *Gymnodinium catenatum* was first linked with paralytic shellfish poisoning (PSP) outbreaks in Mexico in 1979 (Mee et al. 1986) and was retrospectively identified as the causative organism of PSP episodes in Spain in 1976 (Estrada et al. 1984). Further PSP episodes in Tasmania in 1986 (Hallegraeff et al. 1989), Portugal (Franca and Almeida 1989), Japan (Ikeda et al. 1989), and Venezuela (La Barbera-Sanchez et al. 1993) have now been clearly attributed to *G. catenatum.* This dramatic increase in global distribution might be the result of increased recognition of a cryptic species present as part of the ''hidden flora'' but might also represent recent dispersal to new areas (Hallegraeff 1993).

In Australia, *Gymnodinium catenatum* was first recognized in 1986 in southeastern Tasmania, where it was responsible for the first definitive Australian cases of human PSP intoxication and the closure of commercial shellfish farms (Hallegraeff and Sumner 1986). Recurrent blooms have since caused regular farm closures for periods of up to 6 months (Hallegraeff et al. 1989, 1995). Examination of historical plankton samples has indicated that *G. catenatum* has been present in southern Tasmania since at least 1980; however, surveys for the distinctive resting cysts in dated (^{210}Pb, ^{137}Cs) sediment cores indicate that the cyst was not present in the area before 1973 (McMinn et al. 1997). These core data, combined with the unusual disjunct global distri-

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bution of *G. catenatum* during the 1980s, led to the hypothesis that this species was introduced to Tasmania prior to 1972 (McMinn et al. 1997, Hallegraeff and Fraga 1998).

Two potential introduction vectors have been proposed: (1) via ship's ballast water discharges from populations in Japan/Korea (wood-chip carriers visiting Triabunna, Eastern Tasmania, since 1971) or from Spain/Portugal (fruit export vessels visiting the Huon Estuary during the 1960s) or (2) associated with the introduction of the Pacific oyster from Japan into Pittwater in southeastern Tasmania in 1943 (McMinn et al. 1997). The feasibility of ballast water dispersal for *G. catenatum* was demonstrated by an extensive Australia-wide survey of ship ballast water samples that confirmed that 5% to 6% of all ships contained viable toxic dinoflagellate cysts of both *Alexandrium* spp. and *G. catenatum* (Hallegraeff and Bolch 1992). Despite the strong circumstantial evidence of introduction, the probable source population for Tasmanian *G. catenatum* remains unclear.

Molecular genetic methods that can discriminate between strains of *G. catenatum* might provide additional data to further support or refute the recent Australian introduction hypothesis and to assist in tracing the global dispersal of this species. In addition, molecular methods can provide a useful insight into the level of genetic diversity, the level of population clonality, and the genetic structure of populations of coastal dinoflagellates.

Few genetic studies of phytoplankton have been conducted at the population level (e.g. Gallagher 1980, the diatom *Skeletonema costatum;* Medlin et al. 1996, the coccolithophorid *Emiliania huxleyi*), and even fewer have examined marine dinoflagellates. To date, studies have focused on regional and intercontinental variation. The comparison of ribosomal RNA gene sequences (rDNA) of dinoflagellates has been successful in discriminating geographic groups of *Alexandrium tamarense* and *A. catenella* (e.g. Adachi et al. 1994, 1996, Scholin et al. 1995), but preliminary studies with a few strains of *G. catenatum* from different global populations have shown very limited variation (Adachi et al. 1997).

Recent studies have shown that a PCR-based DNA fingerprinting technique called randomly amplified polymorphic DNA (RAPD) is able to discriminate individual strains of *G. catenatum* (Adachi et al. 1997, Bolch et al. 1998). Using allozymes and RAPD-PCR, the present study examines genetic diversity between strains of *G. catenatum* from Tasmania and three other likely source populations: Japan, Spain, and Portugal. In addition, we examine genetic variation within and between estuarine Tasmanian populations isolated during the previous decade to aid understanding of the population structure and dynamics of this economically important toxic dinoflagellate.

MATERIALS AND METHODS

Culturing. The origin and isolation details of dinoflagellate strains and additional *G. catenatum* strains isolated from Japan, Spain, and Portugal are shown in Table 1. Dinoflagellate strains were isolated by micropipette from plankton samples or from germinated cysts isolated from sediment samples collected from several locations in southeastern Tasmanian and mainland Australian waters (Fig. 1): the Derwent River Estuary (DE); the Huon River Estuary (HU); Hastings Bay (HA); Spring Bay, Triabunna (TRA); and Port Lincoln, South Australia (PTL). All Australian strains were established from single cells or single chains. All *G. catenatum* strain names carry a GC prefix, which has been omitted for simplicity. Stock cultures were maintained in 40 mL of GSe medium (28 $g \cdot kg^{-1}$ salinity) (Blackburn et al. 1989) in 50-mL Erlenmeyer flasks at 17° C and 80 $\mu mol\ PAR \cdot m^{-2} \cdot s^{-1}$ and a 12:12 h LD (light:dark) cycle. The two gymnodinoid species with similar morphological features, *Gyrodinium uncatenum* Hulbert and *Gyrodinium impudicum* Fraga (e.g. similar apical groove type and chain-forming habit in *G. impudicum*), were included in the analyses for comparative purposes.

Allozymes. Cultures of selected *G. catenatum* strains (Table 1) were grown to late logarithmic phase and 100 mL harvested by centrifugation and washed in Tris-HCl buffer (pH 8.0). The cell mass was sonicated on ice twice for 10 s. Cell extracts were used immediately for allozyme studies. Two electrophoretic systems were used as described by Bolch et al. (1993). The following six enzymes were found to have poor activity or were not clearly resolved and were not examined further: amino peptidase (Leu-Gly-Gly substrate, PEP1), amino peptidase (Leu-Tyr substrate, PEP2), glycerol-3-phosphate dehydrogenase (G3PDH), fumarate hydratase (FH), isocitrate dehydrogenase (IDH), and malic enzyme (ME). Eleven enzymes produced visible, well-resolved and consistent banding patterns: acid phosphatase (ACP), aldolase (ALD), amino peptidase (Phe-Ala-Leu substrate, PEP3), esterase (α-naphthyl acetate substrate, EST), esterase-D (umbeliferyl acetate substrate, ESTD), glucose-6-phosphate dehydrogenase (G6PDH), glucose phosphate isomerase (GPI), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and superoxide dismutase (SOD). All enzymes were repeated at least twice with independent cell extractions to confirm electrophoretic mobility variation.

DNA extraction. Cultures were grown to late logarithmic phase under the conditions described and 15 mL harvested by centrifugation at 2000 × *g* for 5 min. Growth media were decanted and the cells resuspended in 400 μL of sterile distilled water to lyse the cells. The lysate was transferred to a 1.5-mL centrifuge tube, 50 μL of 10% SDS were added, and this was gently mixed by pipette. Then 50 μL of 100 mM STE buffer (100 mM Tris, 100 mM EDTA, 100 mM NaCl, pH 8.0) were added (Scholin and Anderson 1994). Cell lysates were extracted twice with 500 μL of Tris-buffered phenol:chloroform:isoamyl alcohol (25:24:1) and once with 500 μL of chloroform:isoamyl alcohol (24:1). DNA was precipitated by the addition of 1/10 volume of 3M sodium acetate (pH 5.0) and two volumes of cold ethanol. DNA was pelleted by centrifugation, air-dried, redissolved in 200 μL of TE buffer, and reprecipitated by the addition of 200 μL of NaCl-1.6M NaCl, 13% (w/v) polyethylene-glycol 8000 (PEG). The PEG-precipitated DNA was repelleted by centrifugation, washed with 70% ethanol, and dried and resuspended in 200 μL of distilled water. Coextracted polysacharrides were removed by precipitation in the presence of 2M NaCl (Fang et al. 1992). Extracts were stored at −20° C until used for PCR.

PCR and agarose gel electrophoresis. DNA aliquots were checked for quality and purity by gel electrophoresis and diluted to an approximate concentration of 5 $ng \cdot \mu L^{-1}$ (by gel standard comparison) for RAPD analyses. Initial screening for suitable primers was carried out using strains JP01, HU15, DE9305, and SP04. A total of 160 random 10-mer primers (sets OPB, G, H, I, J, K, P, and Q; Operon Technologies, Almeida, California) were screened. Primers producing clear, reproducible, polymorphic banding patterns were used for subsequent analyses (Table 2). Replicate reactions of various primer/strain combinations were

TABLE 1. Origin and isolation details of dinoflagellate strains and additional *Gymnodinium catenatum* strains isolated from Japan, Spain, and Portugal. ND indicates status uncertain.

Strain name	Date collected	Source	Isolator	Clonal	Allozyme	RAPD
Gymnodinium catenatum						
Australia						
DE02	23 January 1986	Derwent Estuary, Tasmania	S. Blackburn	+	+	
DE05	8 January 1987	Derwent Estuary, Tasmania	S. Blackburn	+	+	+
DE06	8 January 1987	Derwent Estuary, Tasmania	S. Blackburn	+		+
DE08	15 June 1987	Derwent Estuary, Tasmania	S. Blackburn	+	+	+
DE09	15 June 1987	Derwent Estuary, Tasmania	S. Blackburn	+		+
DE9301	15 May 1993	Derwent Estuary, Tasmania	H. Ling	+		+
DE9302	15 May 1993	Derwent Estuary, Tasmania	H. Ling	+		+
DE9303	15 May 1993	Derwent Estuary, Tasmania	H. Ling	+		+
DE9304	15 May 1993	Derwent Estuary, Tasmania	H. Ling	+		+
DE9305	15 May 1993	Derwent Estuary, Tasmania	H. Ling	+		+
HA01	29 June 1990	Hastings Bay, Tasmania	C. Bolch/S. Blackburn	ND		+
HA02	29 June 1990	Hastings Bay, Tasmania	C. Bolch/S. Blackburn	ND	+	+
HU02	6 June 1986	Deep Bay, Huon Estuary, Tasmania	S. Blackburn	+	+	+
HU07	15 June 1987	Deep Bay, Huon Estuary, Tasmania	S. Blackburn	+		+
HU08	15 June 1987	Deep Bay, Huon Estuary, Tasmania	S. Blackburn	+		+
HU09	15 June 1987	Deep Bay, Huon Estuary, Tasmania	S. Blackburn	+	+	
HU11	20 June 1988	Deep Bay, Huon Estuary, Tasmania	S. Blackburn	+		+
HU15	5 April 1990	Purcell's Bay, Huon Estuary, Tasmania	S. Blackburn	+		+
HU16	7 April 1990	Purcell's Bay, Huon Estuary, Tasmania	S. Blackburn	+		+
HU20	4 April 1990	Killala Bay, Huon Estuary, Tasmania	S. Blackburn	+		+
PTL01-4	17 April 1996	Boston Bay, Port Lincoln, South Australia	C. Bolch	+		+
PTL02	17 April 1996	Boston Bay, Port Lincoln, South Australia	C. Bolch	+		+
TRA06	5 March 1993	Spring Bay, Triabunna, Tasmania	M. Ellegaarde	+		+
TRA14	5 March 1993	Spring Bay, Triabunna, Tasmania	M. Ellegaarde	+		+
Japan, Spain, and Portugal						
JP01	4 September 1985	Harimanada, Japan	S. Yoshimatsu	ND	+	+
JP03	1995	Harimanada, Japan	S. Yoshimatsu/C. Bolch	+		+
JP10	1986	Senzaki Bay, Japan	T. Ikeda	+	+	+
SNZ01	1990	Senzaki Bay, Japan	Y. Oshima	+		+
SP01	5 November 1985	Ria de Vigo, Spain	I. Bravo			+
SP03	5 November 1985	Ria de Vigo, Spain	I. Bravo/S. Blackburn	+		+
SP04	5 November 1985	Ria de Vigo, Spain	I. Bravo/S. Blackburn	+		+
SP09	5 November 1985	Ria de Vigo, Spain	I. Bravo/S. Blackburn	+	+	
PT01	1986	Figueira da Foz, Portugal	E. S. Silva/S. Franca	+	+	
PT02	August 1989	Aguda, Portugal	S. Franca	+	+	+
PT03	August 1989	Aguda, Portugal	M. Sampayo/S. Franca	+	+	+
Other specis (Australia)						
Gyrodinium uncatenum CS289	1988	Bathurst Harbour, Tasmania	S. Blackburn	+		+
Gyrodinium impudicum C5-3	15 June 1996	Cowans Creek, Hawkesbury, Estuary, New South Wales	C. Bolch	+		+

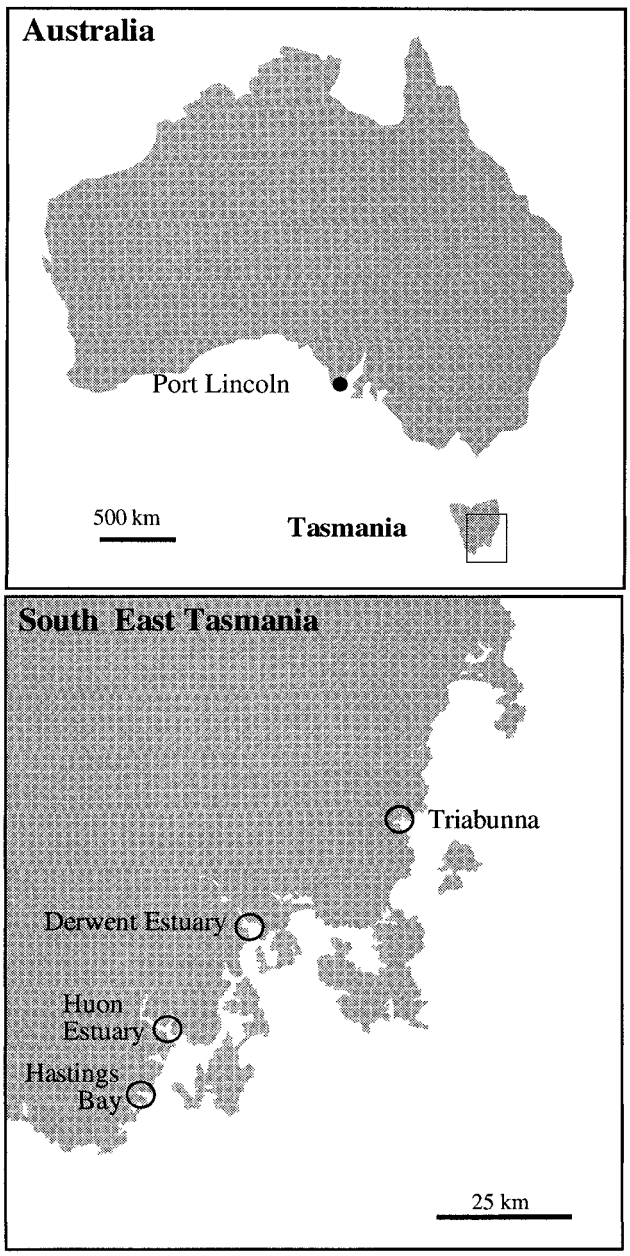

FIG. 1. Geographic origin of Australian strains of *G. catenatum* used in this study.

carried out to assess the reproducibility of RAPD patterns generated. The PCR amplifications were carried out in 20-μL volumes using thin-walled 200-μL reaction tubes (Quantum Specialised Plastics, Brisbane, Australia). Reactions contained 25 ng of genomic DNA, 0.5 U of *Taq* DNA polymerase (Advanced Biotechnologies, Surrey, United Kingdom), 3.0 mM $MgCl_2$, 200 μM of each dNTP (Promega, Madison, WI), 3 μL of bovine serum albumin (1 mg·mL^{-1}, Boehringer-Mannheim), and 0.5 μM of primer using PCR buffer IV (Advanced Biotechnologies, 200 mM $[NH_4]_2SO_4$, 750 mM Tris-HCl, pH 9.0, 0.1% Tween). Amplifications were performed in a Perkin-Elmer/Cetus GeneAmp 9600 thermocycler using the fastest possible transition times as follows: 2 min of 94° C denaturation followed by 40 cycles of 94° C for 10 s, 40° C for 50 s, and 72° C for 2 min and a final extension step of 8 min. The entire 20-μL volume was loaded and electrophoresed for 1200 v h through 1.5% agarose (Molecular Biology Grade, Promega, Madison, WI) dissolved in 1 × TBE buffer containing 0.2 μg·mL^{-1} of ethidium bromide. Gels were illuminated with UV light and photographed with Polaroid 665 positive/negative instant film.

Data analysis. All strains were scored for the presence (scored as 1) or absence (scored as 0) of comigrating RAPD bands to produce a binary matrix of bands for each primer. All visible bands were scored between 2000 bp and 250 bp. Band frequencies among the strains examined were calculated (Table 2), and those present among *G. catenatum* strains at a frequency (F) <0.10 (effectively present in three *G. catenatum* strains or less) were considered unreliable markers and excluded from the analyses (see Table 2). Primer matrices were combined, and a pairwise euclidean distance matrix was calculated using the SYSTAT® Version 5.1 statistical software package.

The distance matrix was subjected to multidimensional scaling (MDS) analysis (Kruskal 1964) in two dimensions using SYSTAT®, with the two outgroup species included. The MDS analysis was repeated in three dimensions, with *G. catenatum* strains only, to obtain an even dispersion of *G. catenatum* strains and to avoid clustering distortions induced by the outgroups; this provided a ''better fit'' to the distance matrix as estimated from the ''Kruskal stress'' of the final configuration (Kruskal 1964). The averages and standard deviations of pairwise euclidean RAPD distances within and between species and populations were calculated and tabulated (Table 3).

To examine the subdivision and patterns of genetic variance within and between the sample populations, analysis of molecular variance (AMOVA) was carried out with AMOVA Version 1.55 (Excoffier et al. 1992) according to the procedure for RAPD data described by Huff et al. (1993). The test statistic Φ_{ST} is analogous to Wright's F_{ST} (Wright 1951). Strains were grouped according to their isolation location and then into three regional groups: Australia, Japan, and Spain/Portugal. Australian strains were subdivided into Huon/Hastings (southern-southeastern Tasmania), Derwent (northern-southeastern Tasmania), Triabunna (eastern Tasmania), and Port Lincoln (mainland Australia) populations (Table 4). Pairwise distances (measured by Φ_{ST}) among regions (Japan, Spain/Portugal, and Australia) and among individual ungrouped population samples were calculated. Population differentiation significance levels were obtained by nonparametric permutation using 9999 iterations (max. allowable using AMOVA Ver. 1.55; Excoffier et al. 1992).

RESULTS

Allozyme diversity. Allozyme variation was found to be exceedingly low among the 12 *G. catenatum* strains examined. Of the 11 enzymes that produced well-resolved and consistent banding patterns, only one reproducible polymorphism was noted. The Portuguese strain PT01 possessed a slower migrating GPI allele from all other strains.

RAPD genetic diversity. The 31 strains of *G. catenatum* spanned four Japanese blooms from two sites, one Portuguese bloom, one Spanish bloom, one Port Lincoln bloom, and eight Tasmanian blooms from four collection sites between January 1986 and May 1996. Therefore, these strains represent a highly diverse sample.

The 11 primers used in this study amplified a total of 375 scorable bands. Primers produced, on average, 34 distinct bands per primer, ranging between 3 and 15 bands per strain. The RAPD-PCR reactions were generally found to be highly reproducible, showing essentially the same banding pattern in repeated amplifications. Some primers showed variation in faint-scored bands, resulting in an average

TABLE 2. RAPD Band frequencies of strains examined. * Bonds excluded from AMOVA analysis.

Primer	Total bands (N_t)	Polymorphic bands (N_p)				Bands unique to outgroups
		F > 0.95	0.95 < F > 0.50	0.10 > F < 0.50	0.10 < F > 0.0*	
OPB-20	41	0	7	15	14	5
OPG-02	23	2	9	7	5	0
OPG-04	37	5	7	11	9	5
OPG-06	36	0	4	18	14	0
OPG-15	36	0	15	14	7	0
OPG-19	33	3	5	15	7	3
OPI-04	34	0	10	12	10	2
OPI-09	34	1	6	19	7	1
OPJ-04	34	3	10	11	8	2
OPQ-03	33	0	12	13	7	1
OPQ-10	34	2	8	13	8	3
Total	375	16	93	148	96	22

euclidean distance of $D = 0.108$ between replicate amplifications. The diversity of RAPD banding patterns was high, with more than 95% of bands considered polymorphic at $F < 0.95$ (Table 2). The primers used were able to distinguish all strains examined, each with a distinct RAPD genotype. Five strains isolated from a single bloom in the Derwent River during 1993 (DE9301, 9302, 9303, 9304, and 9305) exhibited pairwise euclidean distances in excess of 0.484. Outgroup species accounted for 5.8% of unique bands and rose to 7.8% after low-frequency ($F < 0.10$) bands were removed from the data matrix.

Average euclidean distances between *G. catenatum* and the two outgroup species were higher than that found among *G. catenatum* strains (Table 3). Average distances between *G. catenatum* from different global regions were marginally greater than those found between strains within regions and within the species as a whole, with the exception of the interpopulation distance between Japan and Spain/Portugal. The Spain/Portugal and Japanese populations were closer to each other ($D = 0.5327$) than to the Australian population ($D \geq 0.5932$). Strains from the Australian region were slightly more similar to Spain/Portugal than to Japan and exhibited a higher average distance between strains. Average distances between strains from within Australian populations were less than those between strains from different Australian populations.

Analysis of molecular variance. The results of the AMOVA analysis are presented in Table 4. The nested AMOVA analysis showed that most variation was found within populations (87%). Genetic variation attributable to between region differences accounted for 8% of the variation compared to 5% between populations within the regions (Table 4).

For the pairwise comparison of population differentiation (measured by Φ_{ST}), a *P*-value less than 0.01 was considered statistically significant. When AMOVA was carried out with all populations considered separately, including separation of the Derwent 1987 and 1993 collections, the Spanish and Portu-

TABLE 3. Average euclidean distances between *Gymnodinium catenatum* and the two outgroup species, and among strains of *G. catenatum*. N = number of pairwise comparisons.

Species/Population	N	Euclidian distance	
		Average	SD
Between species			
G. catenatum–G. impudicum	29	0.6951	0.0234
G. catenatum–G. uncatenum	29	0.6886	0.0245
G. uncatenum–G. impudicum	1	0.6450	—
Within *Gymnodinium catenatum*	465	0.5817	0.0435
Between regions			
Japan–Australia	88	0.5952	0.0312
Spain/Portugal—Australia	110	0.5932	0.0302
Japan–Spain/Portugal	20	0.5327	0.0526
Within regional populations			
Australia	231	0.5840	0.0336
Japan	6	0.4587	0.0699
Spain/Portugal	10	0.4572	0.0593
Between Australian populations			
Huon/Hastings–Derwent	81	0.5902	0.0362
Within Australian populations			
Derwent	36	0.5686	0.0461
Huon/Hastings	36	0.5695	0.0259

TABLE 4. Analysis of molecular variance between and within populations and regions. Regions and populations defined in text.

Variance component	Variance value	Proportion of total variance (%)
Nested AMOVA analysis		
Between regions	0.0238	7.8
Between populations, within regions	0.0156	5.1
Within populations	0.2669	87.1
Analysis among populations		
Between populations	0.0292	9.9
Within populations	0.2669	90.1
Analysis among regions		
Between regions	0.0312	10.2
Within regions	0.2762	89.8

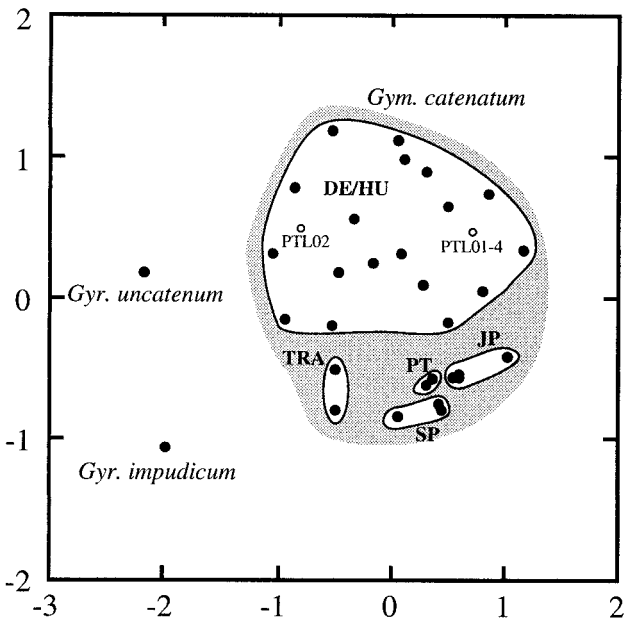

FIG. 2. Two-dimensional MDS plot of RAPD-PCR data for *G. catenatum* strains (shaded area) and *G. uncatenum* and *G. impudicum* (outgroup species). *G. catenatum* clusters (bounded by solid lines): Derwent Estuary, Huon Estuary, and Hastings Bay (DE/HU); Triabunna (TRA); Japan (JP); Spain (SP); and Portugal (PT). Kruskal stress = 0.249.

guese strains were significantly different from each other (Φ_{ST} = 0.1507, $P < 0.001$). However, these two population samples were not significantly different from the Japanese population (Φ_{ST} = 0.1548, Φ_{ST} = 0.1996; $P \geq 0.026$). Within Australian population samples, the Derwent 1987 and 1993 samples were significantly different from each other (Φ_{ST} = 0.0845, $P < 0.001$), and each was significantly different from the Huon Estuary population (Φ_{ST} = 0.0562, P = 0.0034; Φ_{ST} = 0.0560, $P < 0.001$, respectively). The Triabunna and Port Lincoln samples were significantly different from each other (Φ_{ST} = 0.0959, $P < 0.001$) but not from either the Huon or the Derwent sample ($P \geq 0.0479$). When all Derwent strains were grouped as a single population sample, they remained significantly different from the Huon sample (Φ_{ST} = 0.0359, P = 0.0018).

When genetic differentiation was considered at a regional level, Australian strains were significantly different from both Japanese (Φ_{ST} = 0.0962, $P <$ 0.001) and Spanish/Portuguese (Φ_{ST} = 0.0997, $P <$ 0.001) strains; however, the difference between the Japan and Spain/Portugal regions was not significant (Φ_{ST} = 0.1404, P = 0.0122).

Multidimensional scaling analysis. The two-dimensional MDS analysis, with the two outgroup species included, clearly resolved both the comparison species (*Gyrodinium impudicum* and *Gyrodinium uncatenum*) from *Gymnodinium catenatum* strains on the horizontal axis (Fig. 2). The final Kruskal stress of the plot was 0.249, which is considered a ''fair'' fit to the distance matrix according to the criteria of Kruskal (1964). Within the cluster, the Japanese, Spanish, and Portuguese strains were clearly separated on the vertical axis below the Australian strains. The two Triabunna (eastern Tasmania, TRA) strains were also displaced on vertical axis but clearly separated from the non-Australian strains. The two Port Lincoln (SA) strains fell within the Tasmanian cluster.

Three-dimensional MDS analysis, with *G. catenatum* strains only, provided a superior correspondence of the MDS analysis (Kruskal stress = 0.193) to the euclidean distance matrix (Fig. 3a, b). Non-Australian strains were clearly separated in dimensions 1 and 2 (Fig. 3a) and further resolved into Spanish, Portuguese, and Japanese clusters in dimension 3 (Fig. 3b). Within the Australian strains, Triabunna strains were again clearly displaced in dimensions 1 and 2. Port Lincoln (PTL) strains did not cluster closely together but fell within the Tasmanian cluster of strains. Tasmanian strains formed two discrete, nonoverlapping clusters in dimensions 1 and 2 (Fig. 3a), one cluster composed of the Derwent Estuary strains and the other of the Huon Estuary and Hastings Bay strains. Dimension 3 resolved two clusters within the Derwent group: a cluster of strains isolated in 1987 that was clearly separated from a cluster of five strains isolated in 1993 (Fig. 3b).

DISCUSSION

Interspecific RAPD variation. One of the traditional drawbacks of using RAPD markers in population genetics is that they represent dominant markers; that is, heterozygotes are masked by the dominant amplified (present) allele (Williams et al. 1993). Although this presents difficulties in diploid organisms, in haploid plankton dinoflagellates, such as *G. catenatum,* heterozygotes do not exist. In haploid systems, RAPD loci are thought to be biallelic (present or absent) loci, and genetic diversity can be assessed directly from the presence or absence of RAPD bands (Lynch and Milligan 1994).

The present study is one of the first to examine genetic variability within a dinoflagellate species using RAPD markers despite their clear suitability in haploid microalgae. The higher genetic similarities within species and populations compared to that between species indicate that RAPD can clearly resolve different taxa. The finding that *G. catenatum* was marginally more similar to the non-chain-forming *Gyrodinium uncatenum* than to the morphologically similar *Gyrodinium impudicum* confirms the results of a previous study of D9 and D10 domain DNA sequences of the LSU rDNA of *G. catenatum* and *G. impudicum* that indicated extensive divergence of these two species despite their morphological similarity (Costas et al. 1995, Zardoya et al. 1995). Although RAPD analysis clearly resolved the three species in this study, assessment of the phylogenetic relationships in this group using RAPD should be in-

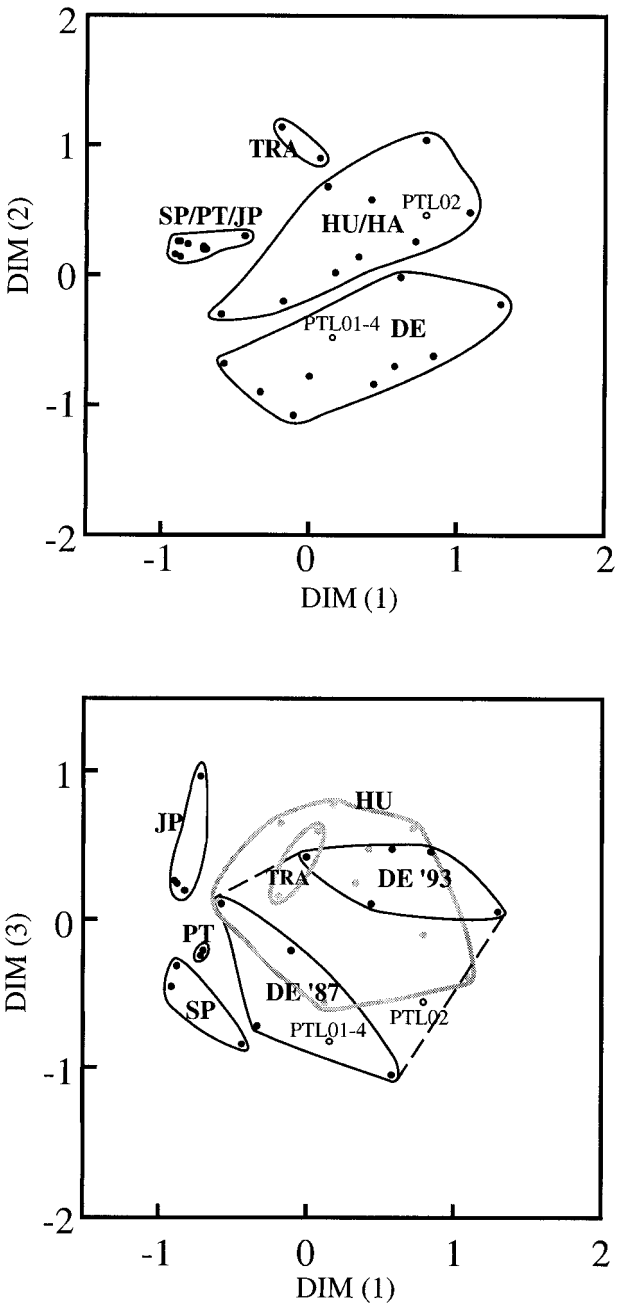

FIG. 3. Three-dimensional MDS analysis of RAPD data from *G. catenatum* strains, not including the two outgroup species. Kruskal stress = 0.193. (a) Plot of first and second dimension of the three-dimensional MDS analysis of *G. catenatum* strains. Region/population clusters (bounded by solid line): Huon Estuary and Hastings Bay (HU/HA), Derwent Estuary 1987 (DE'87) and 1993 (DE'93), Triabunna (TRA), and Japan, Spain, and Portugal (JP/SP/PT). Port Lincoln, South Australia, strains (PTL) marked by strain number. (b) Plot of the first and third dimension of the three-dimensional MDS analysis of *G. catenatum* strains. *G. catenatum* clusters bounded by solid or shaded lines: Huon Estuary and Hastings Bay (HU/HA, shaded), Derwent Estuary 1987 (DE'87) and 1993 (DE'93), Triabunna (TRA, shaded), Japan (JP), Spain (SP), and Portugal (PT). Port Lincoln, South Australia, strains (PTL) marked by strain number.

terpreted with some caution. The gymnodinoid dinoflagellates represent a morphologically and phylogenetically diverse collection of protistan lineages (Saunders et al. 1998), increasing the likelihood of scoring nonhomologous comigrating RAPD bands and perhaps resulting in poorly resolved higher-order relationships among taxa (Medlin et al. 1996). However, in the absence of variation using other common approaches (e.g. allozymes or rDNA sequence variation), RAPD provides useful genetic markers for resolving closely related species and species complexes.

Intraspecific variation in G. catenatum. The level of RAPD diversity found between *G. catenatum* strains, as measured by average simple matching (SM coefficient; Gower 1972) rather than euclidean distance, is within the ranges found for many outcrossing higher-plant species. For example, average SM coefficients between *G. catenatum* strains (population averages = 0.718–0.800; see Bolch et al. 1998) were larger than those found between two races of the common bean, *Phaseolus vulgaris* (average = 0.374; Johns et al. 1997) and fell within the same ranges of RAPD distances found between half-siblings of the forest tree *Eucalyptus globulus* (average = 0.802) and those of unrelated individuals of *E. globulus* (average = 0.745) (Nesbitt et al. 1997).

From the AMOVA analysis, most of the RAPD variation in *G. catenatum* is found within populations rather than between populations or regions. No comparative data are available for other phytoplankton species; however, the level of RAPD variation has been examined extensively in several outcrossing and partial inbreeding higher-plant populations. For example, populations of Buffalo grass (*Buchloë dactyloides*) exhibit from 70% to 81% of variation within regions (Huff et al. 1993), and *Eucalyptus globulus* populations in southern Australia exhibit from 74% to 95% (average = 80%) of RAPD variation within populations (Nesbitt et al. 1995). Plants with higher levels of inbreeding generally display much lower levels of within-population variation (e.g. *Hordeum spontaneum,* 43% within-population variation; Dawson et al. 1993). The proportion of RAPD variation within populations of *G. catenatum* (87%; Table 4) suggests that this species is essentially an outbreeding species with a low level of self-crossing or biparental (related) mating. This is supported by interbreeding analysis using clonal strains, which indicates that the mating system of *G. catenatum* is a complex, multigroup, outbreeding system (Blackburn et al., unpubl.).

The higher level of RAPD diversity within Australian strains of *G. catenatum* strains compared to both the Spanish/Portuguese and the Japanese strains (Table 3) might be partially attributed to the considerably higher number of Australian strains compared with the other sample populations. However, a previous RAPD-PCR study of eight *G. catenatum* strains from the same regions also found a higher

diversity of RAPD banding patterns among four Tasmanian strains compared to more conservative patterns for the four Japanese, Spanish, and Portuguese strains (Adachi et al. 1997). These data and the present analysis strongly suggest that the Japanese and Spanish/Portuguese populations are more similar to each other (marginally differentiated, $P = 0.0122$) than either population is to Australian strains ($P < 0.001$).

Global population variation and dispersal hypotheses. Earlier genetic studies of several dinoflagellates using allozyme electrophoresis have shown considerable genetic heterogeneity among strains of dinoflagellate morphospecies (e.g. *Alexandrium* spp., Cembella and Taylor 1985, Cembella et al. 1988, Hayhome et al. 1989, Sako et al. 1990; *Heterocapsa* spp., Watson and Loeblich 1983; *Peridinium volzii,* Hayhome et al. 1987; *Gambierdiscus toxicus,* Chinain et al. 1997). Similarly, variation has now been shown in the sequences of rDNA genes within species of dinoflagellates. For example, the *Alexandrium catenella/tamarense* complex exhibits extensive rDNA gene and ITS sequence divergence between different global populations that is thought to represent population divergence times approaching millions of years (Scholin et al. 1995, Adachi et al. 1996). Genetic variation in rDNA genes has also been reported both within and between Atlantic and Pacific Ocean strains of *Gambierdiscus toxicus* (Babinchak et al. 1996), indicating that genetic isolation and differentiation of dinoflagellate populations on a global scale might be a common feature of many dinoflagellate species.

In contrast, Ellegaard and Oshima (1998) showed no allozyme variation between strains of *G. catenatum* from Spain and Australia. The present work also confirms the low level of allozyme polymorphism among *G. catenatum* strains from Tasmania, Japan, Spain, and Portugal. Similarly, ribosomal RNA gene (rDNA) sequence variation among *G. catenatum* strains is also limited or nonexistent. Single-base inserts, deletions, or substitutions are reported in the 5.8S rDNA and rDNA-ITS region (13 variable sites in 575 bp, 0.25%) among Japanese, Spanish, Portuguese, and Australian strains, but the authors considered this insignificant (Adachi et al. 1997). These sequence variations could not be detected by sequence analysis or be confirmed by restriction enzyme analysis of ITS fragments using enzymes that should have detected the reported polymorphisms (data not shown). The significance of this lack of rDNA and allozyme variation between *G. catenatum* strains is difficult to explain. One explanation is that many dinoflagellate species might display little allozyme or rDNA variation, so that the level of variation within other species examined so far might represent unresolved species or species complexes (e.g. *Alexandrium catenella/tamarense;* Scholin et al. 1994, 1995). Alternately, the distinct global populations of *G. catenatum* might not have had sufficient time to accumulate detectable mutations at the rDNA locus, supporting the hypothesis of recent dispersal of *G. catenatum* to many areas from a genetically homogeneous single-source population. This interpretation is consistent with the absence of fossilized cysts in Tasmania and the appearance of *G. catenatum* cyst walls around 1973 in cores from the Huon Estuary (McMinn et al. 1997).

Introduced populations often undergo significant losses of genetic diversity during population bottlenecks during introduction and establishment; this is often called ''the founder effect'' (e.g. the Pacific seastar *Asterias amurensis* to the southeast of Tasmania; Ward and Andrew 1995). Assuming that *G. catenatum* was introduced to Tasmanian waters, Tasmanian *G. catenatum* strains have no detectable loss of diversity as a result of introduction. Several factors decrease the likelihood of a loss of diversity in *G. catenatum.* First, the most likely introduction vector, bulk carrier ballast water, has already been demonstrated to occasionally harbor extremely high numbers (up to 300 million in one tank) of viable dinoflagellate cysts (Hallegraeff and Bolch 1992), thus the high probability of a large inoculum population. Second, *G. catenatum* demonstrates a wide temperature tolerance, forming blooms in periods when surface water temperatures range from 12° to 20° C. In southern Tasmanian waters, surface water temperatures are within this range much of the year, dropping significantly below 12° C only during a few winter months (Hallegraeff et al. 1989, 1995). Cysts discharged into favorable growth conditions could undergo rapid population expansion, suffer no severe genetic bottleneck, and maintain a high level of genetic diversity during establishment.

Comparative studies of strains from the same populations examined here indicate that strains from each of the four populations are sexually compatible to the point of cyst formation (Oshima et al. 1993b, Ellegaard and Oshima 1998). However, progeny of some Spain/Tasmania crosses exhibit low long-term viability compared to other intra- and interpopulation progeny (Blackburn et al., unpubl.). Comparison of PSP toxin profiles between strains from the same three populations found fixed differences in the mol% ratios of saxitoxin (STX) fractions, with Tasmanian strains being unique in their ability to produce deoxy-decarbamoyl-STX compounds (Oshima et al. 1993a). The RAPD analysis presented here supports and confirms inferences drawn from the toxin and interbreeding studies: Australian strains appear distinct from both Japanese and Spanish and Portuguese strains; Spanish, Portuguese, and Japanese strains appear more closely related to one another than to Australian strains.

The separate regional clustering from the RAPD analysis and other supporting data do not disprove the hypothesis of recent introduction. However, it does question the proposed Japanese or Spanish origin of Tasmanian *G. catenatum* populations. All

three regions are extremely similar, as evidenced by the lack of fixed RAPD band differences. The biogeographical significance of the discrete nature of the regions is difficult to ascertain; however, several scenarios could explain the pattern of genetic relationships. Australian strains might have originated from an as-yet-unsampled overseas region, a hypothesis that can be rigorously tested only by including representatives of a number of other regions, such as those in the Gulf of California, Argentina and Uruguay, Korea, China, or tropical populations from western India or the Philippines. Alternately, Tasmanian populations might have undergone genetic drift after introduction or experienced selective pressure imposed by a new environment, accounting for the divergence from the possible donor populations examined here. For example, the wood-chip port of Triabunna is the proposed point of introduction of *G. catenatum* to Tasmania. The two strains from Triabunna are displaced from other Australian strains in the MDS analyses in a similar direction to the non-Australian strains, and it is tempting to interpret this as evidence for a possible introduction followed by drift or selection. The port of Triabunna continues to receive ballast water from ports known to harbor *G. catenatum.* Therefore, the displacement of Triabunna strains might be the result of repeated seeding of the Triabunna population with overseas *G. catenatum* genetic material that is integrated via recombination during sexual reproduction. This process could continue to ''push'' the process of genetic drift toward those of the donor populations. Analysis of additional strains from Triabunna is planned to aid the interpretation of relationships between this population and potential overseas donor populations.

Until 1993, the Australian distribution of *G. catenatum* was restricted to southeastern Tasmania (Bolch and Hallegraeff 1990). However, plankton cells and cysts have been recorded in May 1993 from southern Victorian waters (Sonneman and Hill 1997) and, more recently, from Port Lincoln, South Australia, in April 1996 (Bolch et al. 1998). The position of the two Port Lincoln strains within the Tasmanian cluster is consistent with the hypothesis of a recent dispersal from Tasmania to mainland Australia. Direct, regular shipping links exist between the Port of Hobart (Derwent Estuary) and Port Lincoln, providing a direct transport vector for the dispersal of *G. catenatum,* although it is difficult to establish which area might have been the original source. Analysis of resting cysts in dated sediment cores from Port Lincoln and of RAPD and toxin profiles of additional strains from mainland Australian and other global populations is in progress to fully resolve these issues.

Genetic variation and bloom populations. The present study demonstrates that *G. catenatum* is not a globally distributed clone but possesses a high level of genetic variation at RAPD loci. Like most planktonic dinoflagellates, the primary mode of reproduction of haploid vegetative cells of *G. catenatum* in the water column is thought to be asexual, leading to populations consisting of a series of clones. A sexual life cycle has been described for *G. catenatum* that appears to be triggered mainly by nitrate and/or phosphate limitation in laboratory culture, resulting in a zygotic resting cyst (Blackburn et al. 1989). Despite the apparent dominance of asexual reproduction, this study found all strains examined to be genetically distinct, even among those isolated from the same field sample at the peak of a prolonged bloom (strains DE9301, 02, 03, 04, and 05). Using RAPD-PCR, similarly low levels of clonality have been found among strains of the offshore-bloom-forming coccolithophorid *Emiliania huxleyi* (Lohmann) Hay et Mohler isolated from single bottle casts. This diversity was interpreted either as a high mutation rate of RAPD loci in *E. huxleyi* or, more plausibly, as evidence of sexual recombination within the population (Medlin et al. 1996).

Although the observed diversity in a mainly asexual reproducing organism seems counterintuitive, it suggests that *G. catenatum* blooms arise from a genetically diverse inoculum of overwintering vegetative cells or from a genetically diverse population of benthic resting cysts. If no strong environmental selection exists for a particular genotype during a bloom, or if a high level of habitat diversity exists, a high level of genetic diversity could be retained during bloom development, albeit at increasing cell concentrations. Alternately, cyst germination and sexual reproduction could be progressing at a low level throughout prolonged blooms, continually seeding new genotypes into the population. In both cases, the population would be represented by a vast series of clones; however, the probability of sampling the same clone twice might remain relatively low.

The observed spatial clustering of isolates into estuarine groups (Fig. 3) is surprising given the small distance between the Derwent and Huon Estuaries ($<$50 km). Blooms of *G. catenatum* are sometimes widespread, giving the appearance of a continuous distribution throughout the estuaries and protected coastal areas between Hastings Bay and the Derwent Estuary (see Fig. 1; Hallegraeff et al. 1989, 1995). The distinct clustering of isolates, collected over a period of several years, between these two geographically proximate areas suggests that genetic exchange between areas is limited. Blooms might arise within the estuaries from benthic cysts, undergo cyst formation, and resettle in the same area, effectively isolating estuarine blooms from those in neighboring estuaries. *Gymnodinium catenatum* cells sampled outside the Derwent Estuary, in Storm Bay, always appear unhealthy and do not survive well in coastal Tasmanian waters (Hallegraeff et al. 1989). Vegetative cells washed out of an estuary might rarely survive long enough to be transported in significant

numbers to neighboring estuaries and undergo sexual recombination and cyst formation.

Evidence for the nonclonal nature of dinoflagellate blooms and for geographical relationships between strains has been previously reported in the freshwater dinoflagellate *Peridinium volzii* (Hayhome et al. 1987) using allozyme analyses. Whereas long-term reproductive isolation might be easily envisaged between freshwater lakes, population structure in a coastal species at small scales seems less probable. However, morphological, mating compatibility, and toxin composition data have been used to conclude that the *Alexandrium fundyense/tamarense* complex populations along the northeastern coast of the United States and Canada are not a widespread, homogeneous population. The data suggested that, especially in the most southern locations sampled, they represented localized populations with little mixing of genotypes (Anderson et al. 1994). Additional studies with isolates from other Tasmanian sites are required to verify the genetic structure of Tasmanian populations; however, the current evidence indicates that localized subpopulations, with limited genetic exchange between them, might be a common feature of coastal dinoflagellate populations.

The clustering of strains from an estuary by bloom year suggests temporal shifts in bloom genotypes over longer time scales (years). It is interesting to note that the 1993 bloom was preceded by three nonbloom years in southeastern Tasmania (Hallegraeff et al. 1995); this might have isolated this major bloom from the more or less annual blooms during the late 1980s. This temporal differentiation might be due to stochastic changes in the mix of genotypes available to seed localized estuarine blooms or, more likely, result from changing environmental conditions, leading to the selection of particular genotypic subsets from the population. For example, different *G. catenatum* strains isolated from Tasmanian waters are known to have significantly different selenium requirements for optimal growth (Doblin 1998). Similar spatial and temporal patterns of RAPD diversity were found between strains isolated from North Atlantic bloom samples of *E. huxleyi* (Medlin et al. 1996). Isolates from the same bottle cast tended to group together, and isolates collected at different times (1 month apart) from the same area clustered separately, suggesting that the genotype mix had shifted at the collection site, possibly because of physical cell transport.

In conclusion, the present data from RAPD-PCR analysis suggest that *G. catenatum* blooms are not simply large masses of genetic clones arising from asexual reproduction in the water column. Rather, they appear to be genetically heterogeneous, outbreeding populations that might show distinct population structure at surprisingly small scales. Potentially introduced Australian populations are significantly different from strains from Japan, Spain, and Portugal despite the limited number of non-Australian strains examined. Representative strains from additional global populations are required to clarify the most probable source. The data also suggest that mainland Australian populations in Port Lincoln, South Australia, could have arisen by translocation of Tasmanian strains. Additional studies with *G. catenatum* aim to examine population clustering among Australian bloom areas in more detail and to examine additional strains from a range of other global populations. Such studies can lead to important insights into the dispersal, population genetics, and biology of *G. catenatum* and other coastal dinoflagellate populations.

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Adachi, M., Sako, Y. & Ishida, Y. 1994. Restriction fragment length polymorphism of ribosomal DNA internal transcribed spacer and 5.8S regions in Japanese *Alexandrium* species (Dinophyceae). *J. Phycol.* 30:857–63.

——— 1996. Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. *J. Phycol.* 32:424–32.

——— 1997. Analysis of *Gymnodinium catenatum* (Dinophyceae) using sequences of the 5.8S rDNA-ITS regions and random amplified polymorphic DNA. *Fish. Sci.* 63:701–7.

Anderson, D. M., Kulis, D. M., Doucette, G. J., Gallagher, J. C. & Balech, E. 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from northeastern United States and Canada. *Mar. Biol.* 120:467–78.

Babinchak, J. A., Doucette, G. J. & Ball, R. M. 1996. Partial characterization of the LSU rRNA gene from the ciguatoxic dinoflagellate *Gambierdiscus toxicus*. *In* Yasumoto, T., Oshima, Y. & Fukuyo, Y. [Eds.] *Harmful and Toxic Algal Blooms,* UNESCO, Paris, pp. 459–62.

Balech, E. 1964. El plancton de Mar del Plata durante el período 1961–1962. *Bol. Inst. Biol. Mar. Univ. Nac. B. Aires, Mar del Plata.* 4:1–49.

Blackburn, S. I., Hallegraeff, G. M. & Bolch, C. J. S. 1989. Vegetative reproduction and sexual life cycle of the toxic dinoflagellate *Gymnodinium catenatum* from Tasmania, Australia. *J. Phycol.* 25:577–90.

Bolch, C. J., Blackburn, S. I., Hallegraeff, G. M. & Vaillancourt, R. E. 1998. Genetic variation among different global populations of *Gymnodinium catenatum* revealed by RAPD-PCR. *In* Reguera, B., Blanco, J., Fernandez, M. L. & Wyatt, T. [Eds.] *Harmful Microalgae.* Xunta de Galicia and UNESCO, Paris, pp. 283–6.

Bolch, C. J., Elliott, N. E. & Ward, R. D. 1993. Enzyme variation in south-eastern Australian samples of the blue-eye or deep-sea trevalla (*Hyperoglyphe antarctica*). *Aust. J. Mar. Freshw. Res.* 44:687–97.

Bolch, C. J. S. & Hallegraeff, G. M. 1990. Dinoflagellate cysts in recent marine sediments from Tasmania, Australia. *Bot. Mar.* 33:173–92.

Cembella, A. D. & Taylor, F. J. R. 1985. Biochemical variability within the *Protogonyaulax tamarensis/catenella* species complex.

In Anderson, D. M., White, A. W. & Baden, D. G.[Eds.] *Toxic Dinoflagellates.* Elsevier, New York, pp. 55–60.
Cembella, A. D., Taylor, F. J. R. & Therriault, J.-C. 1988. Cladistic analysis of electrophoretic variants within the toxic dinoflagellate genus *Protogonyaulax. Bot. Mar.* 31:39–51.
Chinain, M., Germain, M., Sako, Y., Pauillac, S. & Legrand, A.-M. 1997. Intraspecific variation in the dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). I. isozyme analysis. *J. Phycol.* 33:36–43.
Costas, E., Zardoya, R., Bautista, J., Garrido, A., Rojo, C. & Lopez-Rodas, V. 1995. Morphospecies vs. genospecies in toxic marine dinoflagellates: an analysis of *Gymnodinium catenatum/ Gyrodinium impudicum* and *Alexandrium minutum/A. lusitanicum* using antibodies, lectins, and gene sequences. *J. Phycol.* 31:801–7.
Dawson, I. K., Chalmers, K. J., Waugh, R. & Powell, W. 1993. Detection and analysis of genetic variation in *Hordeum spontaneum* populations form Israel using RAPD markers. *Mol. Ecol.* 2:151–9.
Doblin, M. 1998. Importance of selenium and humic substances from land runoff in the development of *Gymnodinium catenatum* toxic dinoflagellate blooms. Ph.D. thesis, University of Tasmania, Tasmania, Australia, 138 pp.
Ellegaard, M. & Oshima, Y. 1998. *Gymnodinium nolleri* Ellegaard et Moestrup sp. ined. (Dinophyceae) from Danish waters, a new species producing *Gymnodinium catenatum*-like cysts: molecular and toxicological comparisons with Australian and Spanish strains of *Gymnodinium catenatum. Phycologia* 37:369–78.
Estrada, M., Sanchez, F. J. & Fraga, S. 1984. *Gymnodinium catenatum* Graham en las rias gallegas. (NO Espana). *Investig. Pesq.* 48:31–40. (in Spanish)
Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial restriction data. *Genetics* 131:479–91.
Fang, G., Hammar, S. & Grumet, R. 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Biotechniques* 13:52–6.
Franca, S. & Almeida, J. F. 1989. Paralytic shellfish poisons in bivalve molluscs on the Portugese coast caused by a bloom of the dinoflagellate *Gymnodinium catenatum. In* Okaichi, T., Anderson, D. M. & Nemoto, T.[Eds.] *Red Tides: Biology, Envionmental Science and Toxicology.* Elsevier, New York, pp. 93–6.
Gallagher, J. C. 1980. Population genetics of *Skeletonema costatum* (Bacillariophyceae) in Narranganset Bay. *J. Phycol.* 16:464–74.
Gower, J. C. 1972. Measures of taxonomic distance and their analysis. *In* Weiner, J. S. & Huizinga, J. [Eds.] *The Assessment of Population Affinities in Man.* Clarendon Press, Oxford, pp. 1–24.
Graham, H. W. 1943. *Gymnodinium catenatum,* a new dinoflagellate from the Gulf of California. *Trans. Am. Microsc. Soc.* 62:259–61.
Hada, Y. 1967. Protozoan plankton of the Inland Sea Setonaikai. I. the Mastigophora. *Bull. Suzugamine Women's Coll. Nat. Sci.* 13:1–26.
Hallegraeff, G. M. 1993. Review of harmful algal blooms and their apparent global increase. *Phycologia* 32:79–99.
Hallegraeff, G. M. & Bolch, C. J. 1992. Transport of dinoflagellate cysts in ship's ballast water: implications for plankton biogeography and aquaculture. *J. Plankton Res.* 14:1067–84.
Hallegraeff, G. M. & Fraga, S. 1998. Bloom dynamics of the toxic dinoflagellate *Gymnodinium catenatum,* with emphasis on Tasmanian and Spanish coastal waters. *In* Anderson, D. M., Cembella, A. D. & G. M. Hallegraeff. [Eds.] *Physiological Ecology of Harmful Algal Blooms.* Springer-Verlag, Heidelberg, pp. 59–80.
Hallegraeff, G. M., McCausland, M. A. & Brown, R. K. 1995. Early warning of toxic dinoflagellate blooms of *Gymnodinium catenatum* in southern Tasmanian waters. *J. Plankton Res.* 17: 1163–76.
Hallegraeff, G. M., Stanley, S. O., Bolch, C. J. & Blackburn, S. I. 1989. *Gymnodinium catenatum* blooms and shellfish toxicity in Southern Tasmania, Australia. *In* Okaichi, T., Anderson, D. M. & Nemoto, T. [Eds.] *Red Tides: Biology, Envionmental Science and Toxicology.* Elsevier, New York, pp. 77–80.
Hallegraeff, G. M. & Sumner, C. E. 1986. Toxic plankton blooms affect shellfish farms. *Aust. Fish.* 45:15–8.
Hayhome, B. A., Anderson, D. M., Kulis, D. M. & Whitten, D. J. 1989. Variation among congeneric dinoflagellates from northeastern United States and Canada. *Mar. Biol.* 101:427–35.
Hayhome, B. A., Whitten, J. D., Harkins, K. R. & Pfiester, L. A. 1987. Intraspecific variation in the dinoflagellate *Peridinium volzii. J. Phycol.* 23:573–80.
Huff, D. R., Peakall, R. & Smouse, P. E. 1993. RAPD variation within and among natural populations of outcrossing buffalo grass [*Buchloë dactyloides* (Nutt.) Engelm.]. *Theor. Appl. Genet.* 86:927–34.
Ikeda, R., Matsuno, S., Sato, S., Ogata, T., Kodama, M., Fukuyo, Y. & Takayama, H. 1989. First report of paralytic shellfish poisoning caused by *Gymnodinium catenatum* Graham (Dinophyceae) in Japan. *In* Okaichi, T., Anderson, D. M. & Nemoto, T.[Eds.] *Red Tides: Biology, Envionmental Science and Toxicology.* Elsevier, New York, pp. 411–4.
Johns, M. A., Skroch, P. W., Nienhuis, J., Hinrichsen, P., Bascur, G. & Muñoz-Schick, C. 1997. Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. *Crop Sci.* 37:605–13.
Kruskal, J. B. 1964. Multidimensional scaling by optimising goodness of fit to a non-metric hypothesis. *Psychometrika* 29:1–27.
La Barbera-Sanchez, A., Hall, S. & Ferraz-Reyes, E. 1993. *Alexandrium* sp., *Gymnodinium catenatum* and PSP in Venezuela. *In* Smayda, T. J. & Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea.* Elsevier, New York. pp. 281–5.
Lynch, M. & Milligan, B. G. 1994. Analysis of population genetic structure with RAPD markers. *Mol. Ecol.* 3:91–9.
McMinn, A., Hallegraeff, G. M., Thomson, P., Jenkinson, V. & Heijnis, H. 1997. Cyst and radionucleotide evidence for the recent introduction of the toxic dinoflagellate *Gymnodinium catenatum* into Tasmanian waters. *Mar. Ecol. Prog. Ser.* 161: 165–72.
Medlin, L. K., Barker, G. L. A., Campbell, L., Green, J. C., Hayes, P. K., Marie, D., Wreiden, S. & Vaulot, D. 1996. Genetic characterisation of *Emiliania huxleyi* (Haptophyta). *J. Mar. Syst.* 9: 13–31.
Mee, L. D., Espinosa, M. & Diaz, G. 1986. Paralytic shellfish poisoning with a *Gymnodinium catenatum* red-tide on the Pacific coast of Mexico. *Mar. Environ. Res.* 19:77–92.
Nesbitt, K. A., Potts, B. M., Vaillancourt, R. E. & Reid, J. B. 1997. Fingerprinting and pedigree analysis in *Eucalyptus globulus* using RAPDs. *Silvae Genet.* 46:6–11.
Nesbitt, K. A., Potts, B. M., Vaillancourt, R. E., West A. K. & Reid, J. B. 1995. Partitioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus* (Myrtaceae). *Heredity* 74:628–37.
Oshima, Y., Blackburn, S. I. & Hallegraeff, G. M. 1993a. Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. *Mar. Biol.* 116:471–6.
Oshima, Y. Itakura, H., Lee, K-C., Yasumoto, T., Blackburn, S. I. & Hallegraeff, G. M. 1993b. Toxin production by the dinoflagellate *Gymnodinium catenatum. In* Smayda, T. J. & Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea.* Elsevier, New York, pp. 907–12.
Sako, Y., Chang-Hoon, K., Ninomiya, H., Adachi, M. & Ishida, Y. 1990. Isozyme and cross analysis of mating populations in the *Alexandrium catenella/tamarense* species complex. *In* Graneli, E., Edler, L. & Sundstrom, B. [Eds.] *Toxic Marine Phytoplankton.* Elsevier, New York, pp. 320–3.
Saunders, G. W., Hill, D. R. A., Sexton, J. P. & Andersen, R. A. 1998. Small-subunit ribosomal RNA sequences from selected dinoflagellates: testing classical evolutionary hypotheses in the age of molecular systematics. *Plant Syst. Evol.* 11(Suppl.): 237–59.
Scholin, C. A. & Anderson, D. M. 1994. Identification of group-

and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *J. Phycol.* 30:744–54.

Scholin, C. A., Hallegraeff, G. M. & Anderson, D. M. 1995. Molecular evolution of the *Alexandrium* tamarense ''species complex'' (Dinophyceae): dispersal in the North American and West Pacific regions. *Phycologia* 34:472–85.

Scholin, C. A., Herzog, M., Sogin, M. & Anderson, D. M. 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* 30:999–1011.

Sonneman, J. A. & Hill, D. R. A. 1997. A taxonomic survey of cyst-producing dinoflagellates from the coastal waters of Victoria, Australia. *Bot. Mar.*40:149–77.

Ward, R. D. & Andrew, J. 1995. Population genetics of the northern Pacific seastar *Asterias amurensis* (Echinodermata: Asteriidae): allozyme differentiation among Japanese, Russian, and recently introduced Tasmanian populations. *Mar. Biol.* 124: 99–109.

Watson, D. A. & Loeblich III, A. R. 1983. The application of electrophoresis to the systematics of the marine dinoflagellate genus *Heterocapsa*. *Biochem. Syst. Ecol.* 11:67–71.

Williams, J. G. K., Hanafey, M. K., Rafalski, J. A. & Tingey, S. V. 1993. Genetic analysis using random amplified polymorphic DNA markers. *Methods Enzymol.* 218:704–41.

Wright, S. 1951. The genetic structure of populations. *Ann. Eugen.* 15:323–54.

Zardoya, R., Costas, E., Lopez-Rodas, V., Garrido-Pertierra, A. & Bautista, J. M. 1995. Revised dinoflagellate phylogeny inferred from molecular analysis of large-subunit ribosomal RNA gene sequences. *J. Mol. Evol.* 41:637–45.