Phycologia (1995) Volume 34 (6), 472–485

Molecular evolution of the *Alexandrium tamarense* 'species complex' (Dinophyceae): dispersal in the North American and West Pacific regions

C.A. SCHOLIN[1]*, G.M. HALLEGRAEFF[2] AND D.M. ANDERSON[3]

[1]*Monterey Bay Aquarium Research Institute, 160 Central Ave., Pacific Grove, CA 93950, USA*
[2]*Department of Plant Science, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia*
[3]*Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA*

C.A. SCHOLIN, G.M. HALLEGRAEFF AND D.M. ANDERSON. 1995. Molecular evolution of the *Alexandrium tamarense* 'species complex' (Dinophyceae), dispersal in the North American and West Pacific regions. *Phycologia* **34**: 472–485.

Hypotheses concerning the molecular evolution, population structure and dispersal of the toxic dinoflagellates *Alexandrium tamarense* (Lebour) Balech, *A. catenella* (Whedon et Kofoid) Balech and *A. fundyense* Balech (the '*tamarensis* species complex') are examined in light of previous reports that compared their small and large-subunit ribosomal RNA gene (SSU and LSU rDNA) sequences. Forty-eight cultures from North America, western Europe, Japan, Australia and Thailand were analysed by a restriction fragment length polymorphism (RFLP) assay of SSU rDNA, and 34 of those by sequencing a fragment of LSU rDNA. Results indicate that the *tamarensis* species complex comprises at least 5 genetically distinct evolutionary lineages ('ribotypes') whose phylogenetic relationships reflect geographic populations, not morphospecies. We believe this pattern reveals a monophyletic radiation from an ancestor that included or gave rise to multiple morphotypes. Accumulated mutations in descendants' SSU and LSU rDNA are suggested to reflect the prolonged geographic isolation and independent evolution of distinct populations. Novel SSU rDNA data are presented in support of this hypothesis. Given the proposed evolutionary framework and other historical considerations, we interpret the genetic diversity of Japanese *A. tamarense/catenella* as indicative of dispersed populations from genetically distinct sources. The possibility that *A. catenella* was introduced to Australia from an Asian source is also considered. In both cases, however, rDNA data alone are insufficient to distinguish whether this occurred thousands of years ago by natural immigrations or as a result of recent human activity (ballast water transport or relays of shellfish stocks). The uncertainty of dispersal timing stems from the relatively slow rate at which rDNA evolves and lack of fossil evidence. Ballast water samples show that viable toxigenic *Alexandrium* cysts have undergone human-assisted transoceanic transport, illustrating how a region could be 'seeded' with genetically distinct *A. tamarense* and *A. catenella* from a variety of regional populations.

INTRODUCTION

The geographic range of the toxic dinoflagellates *Alexandrium tamarense* (Lebour) Balech, *A. catenella* (Whedon et Kofoid) Balech and *A. fundyense* Balech (hereafter the '*tamarensis* complex') appears to be increasing on both regional and global scales (Anderson 1989; Hallegraeff & Bolch 1991, 1992). This is an alarming trend given that these organisms cause paralytic shellfish poisoning (PSP), a neurologic disorder with significant public health and economic impacts (Steidinger & Baden 1984). Several explanations have been put forward to explain this trend, including increased abundance of previously unnoticed endemic species, natural dispersal, human-assisted dispersal, or a combination of all the above (Anderson 1989; Smayda 1990; Hallegraeff & Bolch 1991, 1992). In order to distinguish between these hypotheses, endemic and introduced flora must be differentiated. Historical records of toxicity and species abundance in a region are useful in this regard, yet an absence of these indicators does not preclude the possibility that toxigenic *Alexandrium* are present in a given area. A further difficulty is that *A. tamarense/catenella/fundyense* resting cysts survive for 5–10 years in natural sediments (Keafer *et al.* 1992). Fossilization of these cysts is not known to occur, and therefore stratigraphy cannot be used to determine historical occurrences. Consequently, endemism and dispersal must be inferred from other data.

In an attempt to solve this problem, Scholin & Anderson (1993) proposed to elucidate the genetic relationships of different regional populations to provide a reference from which to view dispersal hypotheses. Before this approach could be applied, however, a long-standing debate as to the 'validity' of defining *A. tamarense*, *A. catenella* and *A. fundyense* as distinct species remained to be resolved. For example, *A. tamarense* and *A. catenella* isolated from Japan are distinguishable morphotypically, and may be separated into distinct groups based on isozyme electrophoretic patterns and immunogenicity – evidence that supports the morphospecies paradigm (Sako *et al.* 1990, 1993). In contrast, isolates of *A. tamarense*, *A. catenella* and *A. fundyense* from North America and elsewhere show no consistent correlation between morphological and biochemical characteristics – evidence that does not support the morphospecies concept (Cembella & Taylor 1986; Cembella *et al.* 1987, 1988; Hayhome *et al.* 1989; Anderson *et al.* 1994). Two schools of thought concerning the validity of species definitions thus emerged. Some researchers believe that detailed morphological features used to define *A. tamarense* (with a ventral pore on the first apical plate), *A. catenella* (without a ventral pore

*Corresponding author; fax: (408) 649-8587; Internet e-mail: scholin@mbari.org

on the first apical plate; chain forming, posteriorly compressed cells) and *A. fundyense* (identical to *A. catenella*, but occurring as isodiametrical, non-chain forming cells) are valid species-level criteria, whereas others view such morphological variations as 'strains' or 'varieties' of a single species (Balech 1985; Fukuyo 1985; Taylor 1985; Cembella & Taylor 1986; Steidinger & Moestrup 1990; Hallegraeff *et al.* 1991; Anderson *et al.* 1994). Each of these views infers a distinct evolutionary history, or phylogenetic tree: (1) that each morphospecies is ascribed by a single ancestral line whose terminus (or termini) consists exclusively of one morphotype; or (2) that each morphospecies is represented by one or more ancestral lines whose termini may contain multiple morphotypes.

Sequence analysis of small-subunit (SSU) and large-subunit (LSU) ribosomal RNA (rRNA) genes (rDNA) was undertaken to distinguish between these possibilities and to provide the necessary background for describing the population biogeography of the organisms. Collections of *A. tamarense*, *A. catenella* and *A. fundyense* from North America, western Europe, Japan, Australia, Thailand and the ballast water of several cargo vessels were compared on the basis of restriction fragment length polymorphisms (RFLPs) of SSU rDNA (Scholin & Anderson 1994) and sequences of LSU rDNA (Scholin *et al.* 1994a). The RFLP assay allowed the detection of a SSU pseudogene (the 'B gene;' Scholin *et al.* 1993), and the LSU sequences were used to construct a phylogenetic tree. Results indicate that the *tamarensis* complex comprises at least 5 genetically distinct evolutionary lineages or 'ribotypes.' Overall, ribotypes appear indicative of regional populations, not morphospecies, and were therefore named with reference to the geographic origins of the isolates compared: 'North American', 'western European', 'temperate Asian', 'Tasmanian' and 'tropical Asian'. The SSU B gene is thought to occur only in those organisms that belong to the North American ribotype (Fig. 1).

Recognition of phenotypic overlap between genetically distinct populations (e.g. North American and western European *A. tamarense*), as well as phenotypic plasticity within a genetically similar population (e.g. North American *A. tamarense/catenella/fundyense*), offers an explanation for the long-standing taxonomic controversy. Morphological features used to define *A. tamarense*, *A. catenella* and *A. fundyense* may indeed appear positively *or* negatively correlated with subcellular criteria; results depend on which regional population(s) are chosen for analysis, as well as the particular isolates compared (Fig. 1; see also Adachi *et al.* 1994; Anderson *et al.* 1994).

Definition of genetically distinct regional populations sets the stage for testing dispersal hypotheses, but two hurdles remain. First, an evolutionary model is needed to account for the confusing associations between morphospecies designations, ribotypes and geographic populations. Second, the population specificity of the B gene (and its utility as an indicator of dispersal) must be established in the context of this model, that is, the 'uniqueness' of the SSU B gene/North American LSU ribotype pair (Fig. 1) must be clarified since their strict association is considered tenuous. The latter difficulty arises from the fact that the RFLP assay used to define the B gene samples only a few B-specific nucleotides, leaving the possibility that 'B-like genes' occur in ribotypes other than the North American (Scholin & Anderson 1994; Scholin *et al.* 1994a).

In this report we address these problems by presenting an evolutionary model for the *tamarensis* complex and apply a simple method for screening different ribotypes for B-like genes. We then propose a conceptual framework from which to explore the differentiation of endemic and introduced *tamarensis* complex representatives. Lastly, dispersal hypotheses and their specific predictions are examined in light of this framework with particular emphasis on North American, Japanese and Australian regional populations.

MATERIALS AND METHODS

Cultured *Alexandrium* compared on the basis of SSU RFLP patterns and LSU rDNA sequences are presented in Table 1.

Solid-phase sequencing of SSU rDNA

SSU rDNA from representatives of the North American, western European, temperate Asian and Tasmanian ribotypes (Fig. 1, Table 1) were amplified using the polymerase chain reaction (PCR; Saiki *et al.* 1988) as described previously (Scholin & Anderson 1994), with the exception that the 3′ (reverse) primer was biotinylated. Purification of the biotinylated strand was achieved using streptavidin-coated magnetic beads ('Dyna Beads') following the recommendations of the manufacturer (Technical Handbook; Dynal AS, PO Box 158 Skøyen, N-0212 Oslo, Norway; cf. Hultman *et al.* 1989; Uhlen 1989; Scholin *et al.* 1994b). Primers complementary to *Dictyostelium* Brefeld SSU nucleotides 892–906, and 962–976 (Sogin & Gunderson 1987) were used to sequence (United States Biochemical Sequenase 2.0) a portion of the captured molecules (positions ~636 to ~1158; Scholin *et al.* 1993, 1994b). Products of the reactions were resolved on standard 6% polyacrylamide (19:1 acrylamide:bis-acrylamide), 8.3 M urea, 1× TBE gels (Ausubel *et al.* 1987) using a BioRad (Hercules, California, USA) Sequigencell apparatus. The top buffer chamber was filled with O.5× TBE and the bottom chamber filled with 1× TBE. Gels were pre-electrophoresed with a constant power setting until reaching ~50°C. Sequencing reactions were thawed on ice, heated to 80°C for 3 min and immediately returned to ice. Approximately 2.5 μl of each reaction were loaded per lane and run until the bromophenol blue dye had migrated roughly 1/3 the length of the gel. Electrophoresis was then briefly terminated while 1/2 volume of 3 M NaOAc (pH 5.0) was added to the bottom buffer chamber. Electrophoresis was resumed maintaining a surface plate temperature of 50–55°C, then terminated when the xylene cyanol dye front had migrated to within 10–12 cm from the bottom of the gel. Gels were fixed in 10% methanol/10% glacial acetic acid for 30 min, dried onto Whatman 3MM paper at 80°C with applied vacuum, and exposed to either XAR-5 or XRP-5 X-ray film (Kodak) from periods of overnight to 2 days.

Sequences from the western European, temperate Asian and Tasmanian representatives were compared to that from the eastern North American isolate from which the A and B SSU rDNAs were originally characterized (GtCA29; Scholin *et al.* 1993). Two western European representatives were chosen because one (Pgt 183) is non-toxic, while another

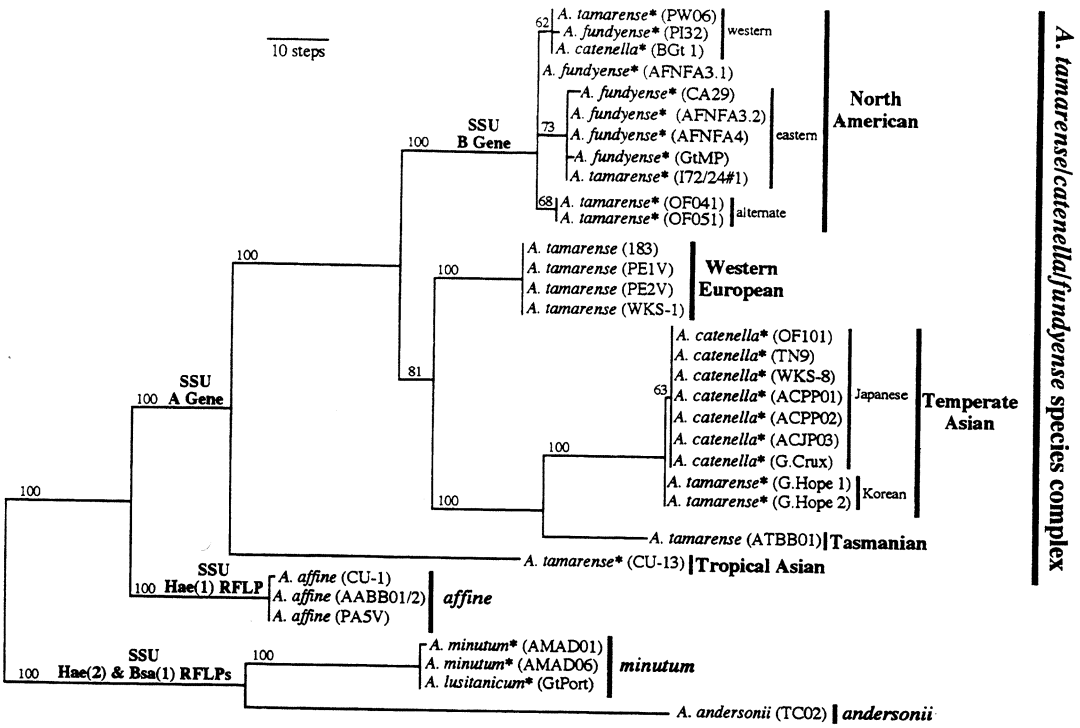


Fig. 1. Parsimony phylogenetic tree inferred from aligned *Alexandrium* LSU rDNA sequences (Scholin *et al.* 1994a) generated by PAUP 3.1.1 (Swofford 1993). Tree statistics are as follows: length = 403 steps; consistency index excluding uninformative characters = 0.802; rescaled consistency index = 0.790; retention index = 0.948. The tree was rooted using the outgroup method, with *A. minutum*, *A. lusitanicum* and *A. andersonii* defined as outgroup taxa [note: *A. andersonii* is spelled here using rules of the Botanical Code of Nomenclature; the original description employs the Zoological Code, being *A. andersoni* (Balech 1990)]. These isolates were chosen as the outgroup because their sequences share a common SSU rDNA restriction pattern that is distinct from *A. affine* and members of the *tamarensis* complex (Scholin & Anderson 1994), and because they are the most divergent taxa relative to representatives of the *tamarensis* complex (Scholin *et al.* 1994a). The *tamarensis* group remains monophyletic when *A. affine* is used as the outgroup root (not shown). Horizontal branch lengths reflect the relatedness of the sequences; scale bar represents a divergence of 10 steps (Swofford 1993). North American, western European, temperate Asian, Tasmanian, tropical Asian, *affine*, *minutum*, and *andersonii* are ribotype designations of terminal taxa; western, eastern and alternate, and Japanese and Korean are subribotypes of the North American and temperate Asian groups, respectively. The latter divisions are based on fine-scale SSU and LSU differences (Scholin & Anderson 1994; Scholin *et al.* 1994a). All eastern North American ribotype representatives contain at least 2 classes of LSU rDNA; both variants from AFNFA3 ('AFNFA3.1' and '.2') were included to illustrate this fact. Remaining eastern North American ribotype sequences were entered as the AFNFA3.2-like variant. *denotes toxic isolates. SSU rDNA RFLP characteristics for the cultures are also shown on the appropriate branches; note correspondence between those patterns and the LSU rDNA phylogeny (cf. Scholin & Anderson 1994). Numbers indicate the frequency that taxa to the right of the value were found to group together upon bootstrap analysis (250 iterations; Felsenstein 1985, Swofford 1993).

(PE1V) may make trace amounts of toxin (Table 1); the two isolates were included to determine if there was any strict association between the B gene and toxin production.

RESULTS AND DISCUSSION

Evolution of the *tamarensis* species complex: polyphyletic or monophyletic?

Gonyaulacoid dinoflagellates, a group that encompasses the genus *Alexandrium*, appear in the fossil record since at least the Cretaceous [~135 million years ago (Ma)]. A more precise estimate of the appearance of *A. tamarense*, *A. catenella* and *A. fundyense* is not possible because of the absence of fossil data (Taylor 1980). Nevertheless, it is widely accepted that over tens of millions of years, members of this species complex colonized many regions of the world (Taylor 1984, 1987). Since these organisms are not known to survive for long periods in the open ocean, transoceanic dispersal by means of ocean currents is highly improbable (Hallegraeff & Bolch 1992). However, disjunct populations could arise from coastal transport via near-shore currents, chance encounters with migratory waterbirds, or other episodic events (Anderson 1989; Franks & Anderson 1992; R. Scheltema, personal communication). Globally distributed populations of the *tamarensis* complex are thus predicted to have arisen as a result of dispersal and/or vicariance (cf. Brooks & McLennan 1991; Avise 1994). Millions of years of continental drift, changes in sea level, climate, etc., would result in reproductively isolated endemic populations. In turn, prolonged isolation and independent evolution of regional groups could be 'recorded' in the genomes of their descendants given the processes of genetic drift (i.e. neutral mutation) and selection (Ayala & Kiger 1980).

Present-day genetic affinities of globally distributed *A. tamarense/catenella/fundyense* depend on whether these species descended from independently evolved, distinct ancestral lines ('polyphyletic radiation,' Fig. 2a), or from a

Table 1. Isolation locales, species and strain designations, toxicity, and rDNA characteristics of *Alexandrium* cultures compared by RFLP analysis of SSU rDNA and sequence comparison of LSU rDNA

Geographic region	Isolation locale[a]	Species designation[b]	Culture source[c]	Strain[d]	Toxic?[e]	SSU rDNA B gene?[f]	LSU rDNA ribotype[g]
North America							
W. Coast							
	Port Benny, AK	*A. tamarense* (EB)	S. Hall	PW05	Yes	Yes	
	Port Benny, AK	*A. tamarense* (EB)	S. Hall	PW06	Yes	Yes	North American (western)*
	Porpoise Isl., AK	*A. fundyense* (EB)	S. Hall	PI32	Yes	Yes	North American (western)*
	AK	*A. fundyense* (EB)	S. Hall	IP02	Yes	Yes	
	Puget Sound, WA	*A. catenella* (EB)	S. Hall	ACQH01	Yes	Yes	
	Puget Sound, WA	*A. catenella* (EB)	S. Hall	ACQH02	Yes	Yes	
	Russian River, CA	*A. catenella* (EB)	D. Anderson	BGt1	Yes	Yes	North American (western)*
E. Coast							
	Gulf of St. Lawrence	*A. fundyense* (EB)	D. Anderson	ATSL01	Yes	Yes	
	Newfoundland	*A. fundyense* (EB)	D. Anderson	AFNFA3	Yes	Yes	North American (eastern)*
	Newfoundland	*A. fundyense* (EB)	D. Anderson	AFNFA4	Yes	Yes	North American (eastern)*
	Bay of Fundy	*A. fundyense* (EB)	A. White	Gony.#7	Yes	Yes	North American (eastern)
	Ipswich Bay, MA	*A. fundyense* (EB)	C. Martin	Gt429	Yes	Yes	North American (eastern)
	Cape Ann, MA	*A. fundyense* (EB)	D. Anderson	GtCA29	Yes	Yes	North American (eastern)*
	Orleans, MA	*A. fundyense* (EB)	D. Anderson	GtMP	Yes	Yes	North American (eastern)*
	Falmouth, MA	*A. tamarense* (EB)	D. Anderson	GtPP01	Yes	Yes	North American (eastern)
	Falmouth, MA	*A. tamarense* (EB)	D. Anderson	GtPP06	Yes	Yes	North American (eastern)
	Groton, CN	*A. tamarense* (EB)	D. Anderson	GtCN1	Yes	Yes	
	Groton, CN	*A. tamarense* (EB)	D. Anderson	GtCN16	Yes	Yes	North American (eastern)
	Babylon, NY	*A. tamarense* (EB)	D. Anderson	GtLI21	Yes	Yes	North American (eastern)
	Eastham, MA	*A. andersonii* (EB)**	D. Anderson	TC02	No	No	*andersonii**
Western Europe							
U.K.							
	Plymouth	*A. tamarense* (MT)	NEPCC	Pgt183	No	No	Western European*
Spain							
	Galicia	*A. tamarense* (EB)	I. Bravo	PE1V	No[δ]	No	Western European*
	Galicia	*A. tamarense* (EB)	I. Bravo	PE2V	No	No	Western European*
	Galicia	*A. affine* (IB)	I. Bravo	PA5V	No	No	*affine**
	Ria de Vigo	*A. lusitanicum* (IB)	I. Bravo	AL2V	Yes	No	
Portugal							
		A. lusitanicum (EB)	L. Provasoli	GtPort	Yes	No	*minutum**
France							
	Morlaix Bay	*A. minutum* (ED)	E. Erard-Le Denn	AM2	Yes	No	
	Morlaix Bay	*A. minutum* (ED)	E. Erard-Le Denn	AM3	Yes	No	
Japan							
North							
	Hachinobe Hbr.	*A. catenella* (YF)	NIES	Collection N520	Yes	No	
	Noda Bay	*A. tamarense* (YF)	M. Kodama	ND-1	Yes	Yes	
	Okkirai Bay	*A. tamarense* (YF)	M. Kodama	OK875-1	Yes	Yes	
	Ofunato Bay	*A. tamarense* (YF)	M. Kodama	OF875-8	nd	Yes	
	Ofunato Bay	*A. tamarense* (YF)	M. Kodama	OF84423D	Yes	Yes	
	Ofunato Bay	*A. tamarense* (YF)	Y. Sako	OF041	Yes	Yes	North American (alternate)*
	Ofunato Bay	*A. tamarense* (YF)	Y. Sako	OF051	Yes	Yes	North American (alternate)*
	Ofunato Bay	*A. catenella* (YF)	Y. Sako	OF101	Yes	No	Temperate Asian (Japanese)*
South							
	Tanabe Bay	*A. catenella* (YF)	Y. Sako	TN-9	Yes	No	Temperate Asian (Japanese)*
	Tanabe Bay	*A. tamarense* (YF)	M. Kodama	WKS-1	No	No	Western European*
	Tanabe Bay	*A. catenella* (YF)	M. Kodama	WKS-3	Yes	No	
	Tanabe Bay	*A. catenella* (YF)	M. Kodama	WKS-8	Yes	No	Temperate Asian (Japanese)*
	Harima Nada	*A. tamarense* (YF)	NIES Collection	N239	Yes	Yes	

Thailand							
	Gulf of Thailand	*A. affine* (YF)	M. Kodama	CU-1	No	No	*affine**
	Gulf of Thailand	*A. tamarense* (YF)	M. Kodama	CU-13	Yes	No	Tropical Asian*
Australia							
	Mainland						
	Port Phillip Bay, Vic.	*A. catenella* (EB, GH)	CSIRO Collection	ACPP01	Yes	No	Temperate Asian (Japanese)*
	Port Phillip Bay, Vic.	*A. catenella* (EB, GH)	CSIRO Collection	ACPP02	Yes	No	Temperate Asian (Japanese)*
	Port Phillip Bay, Vic.	*A. catenella* (EB, GH)	CSIRO Collection	ACPP03	Yes	No	Temperate Asian (Japanese)
	Port Phillip Bay, Vic.	*A. catenella* (EB, GH)	CSIRO Collection	ACPP09	Yes	No	Temperate Asian (Japanese)
	Port River, S.A.	*A. minutum* (EB, GH)	CSIRO Collection	AMAD01	Yes	No	*minutum**
	Port River, S.A.	*A. minutum* (EB, GH)	CSIRO Collection	AMAD06	Yes	No	*minutum**
Tasmania							
	Bell Bay	*A. tamarense* (GH)	CSIRO Collection	ATBB01	No[δ]	No	Tasmanian*
	Bell Bay	*A. affine* (GH)	CSIRO Collection	AABB01/2	No	No	*affine**
Ballast water							
	Muroran, Japan#	*A. tamarense* (GH)	CSIRO Collection	I72/21#2	Yes	Yes	North American (eastern)
	Muroran, Japan#	*A. tamarense* (GH)	CSIRO Collection	I72/22#2	Yes	Yes	
	Muroran, Japan#	*A. tamarense* (GH)	CSIRO Collection	I72/24#1	Yes	Yes	North American (eastern)*
	Kashima, Japan#	*A. catenella* (GH)	CSIRO Collection	ACJP03	Yes	No	Temperate Asian (Japanese)*
	Singapore##	*A. catenella* (GH)	CSIRO Collection	G. Crux	Yes	No	Temperate Asian (Japanese)*
	Samchonpo, S. Korea#	*A. catenella* (GH)	CSIRO Collection	G. Hope 1	Yes	No	Temperate Asian (Korean)*
	Samchonpo, S. Korea#	*A. catenella* (GH)	CSIRO Collection	G. Hope 2	Yes	No	Temperate Asian (Korean)*

[a] #=hailing port of vessel; ##=origin of ballast water uncertain (Hallegraeff & Bolch 1992).

[b] As determined by (or using the criteria of): E. Balech (EB), F.J.R. Taylor (MT), I. Bravo (IB), E. Erard-Le Denn (ED), Y. Fukuyo (YF), or G. Hallegraeff (GH); **=spelled using the Botanical Code of Nomenclature [the origninal description employs the Zoological Code, being *A. andersoni* (Balech 1990)].

[c] Individuals who supplied the culture; CSIRO=Commonwealth Scientific and Industrial Research Organisation (Division of Fisheries; Australia); NEPCC=North East Pacific Culture Collection (British Columbia); NIES=National Institute for Environmental Studies (Japan).

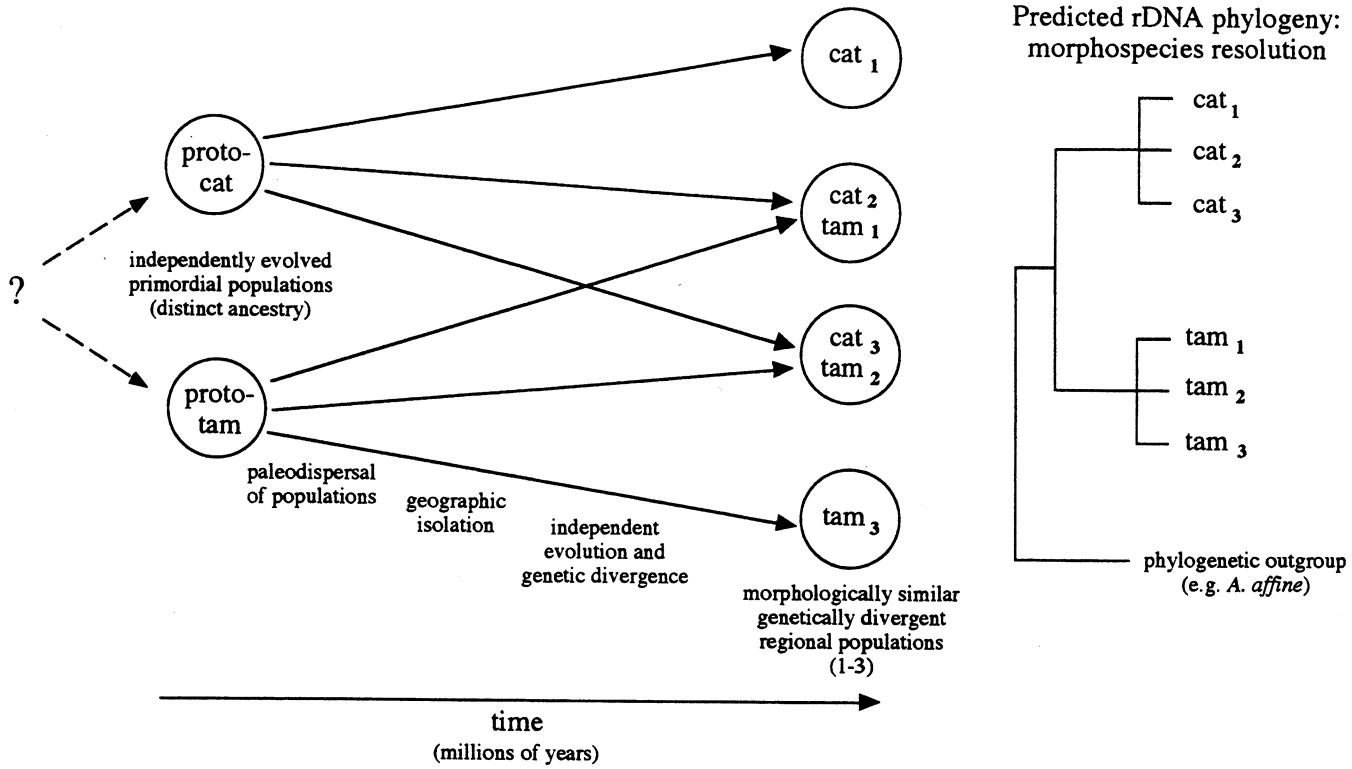
[d] Designations currently used in the D.M. Anderson culture collection, Woods Hole Oceanographic Institution, Woods Hole, MA, USA.

[e] Determined by mouse bioassay and/or HPLC analysis; nd=not determined; δ=may contain trace amounts of toxin (D. Kulis, personal communication).

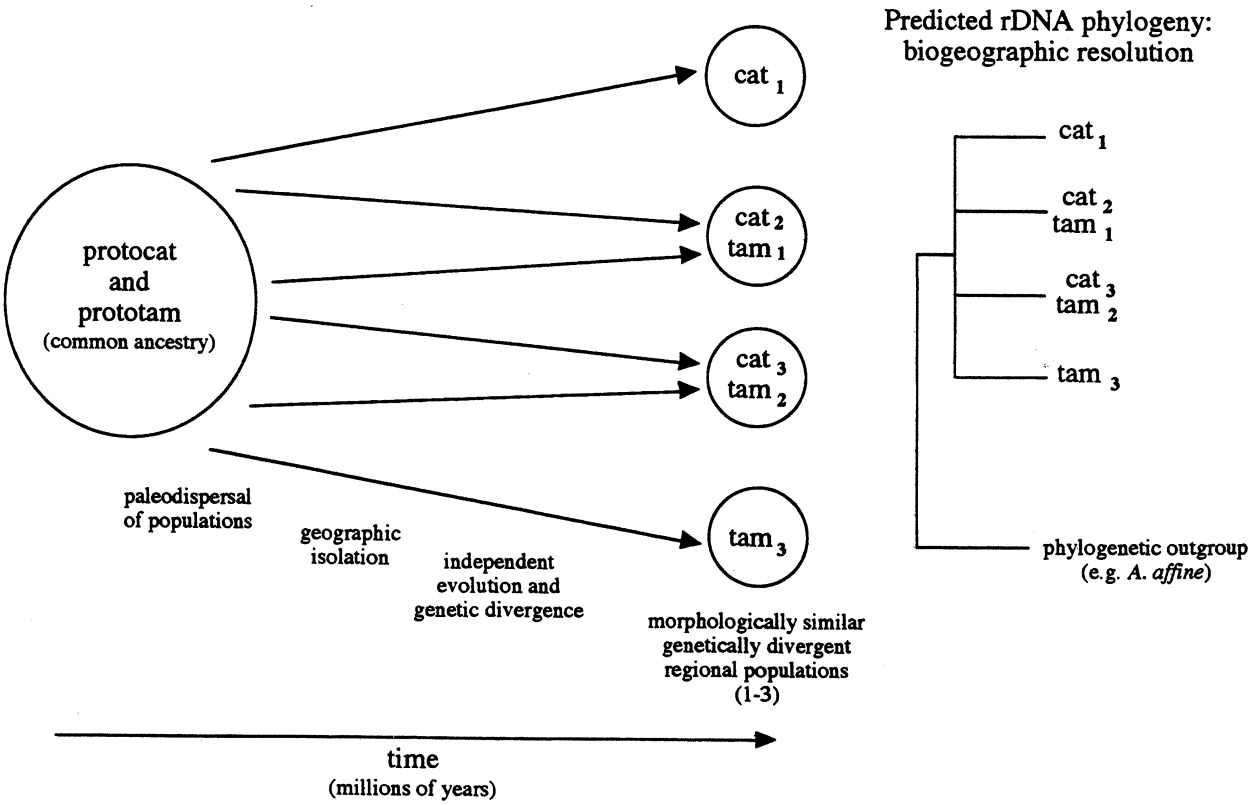
[f] As defined by SSU rDNA RFLP analysis (Scholin & Anderson 1994).

[g] Designation based on LSU rDNA sequence affinity with terminal groups defined in Fig. 1. Eastern, western and alternate, and Japanese and Korean are subribotype designations of the North American and Temperate Asian groups respectively. These designations are based on fine-scale SSU and LSU rDNA characteristics as noted by Scholin *et al.* 1994a. Isolates belonging to the eastern North American ribotype contain at least two distinct classes of LSU rDNA; these classes of sequence are shown by 'AFNFA3.1' and '.2' (those obtained from culture AFNFA3) in Fig. 1; *=cultures whose sequence were used to construct the phylogenetic tree shown in Fig. 1 (cf. Scholin *et al.* 1994a).

a. Polyphyletic Radiation



b. Monophyletic Radiation



more recent, common ancestor that included, or gave rise to, multiple morphotypic forms ('monophyletic radiation,' Fig. 2b). In both cases, globally distributed endemic populations could include one or more morphospecies, and each population may appear genetically divergent. However, if the progenitors of *A. tamarense*, *A. catenella*, and *A. fundyense* arose polyphyletically (Fig. 2a), then the different morphotypes should always be distinguishable at a subcellular level. Overall, their combined phylogenies should reflect the evolution of morphospecies – each morphotype ascribed by a single ancestral line whose terminus (or termini) consists exclusively of one morphospecies. Alternatively, if *A. tamarense*, *A. catenella* and *A. fundyense* arose monophyletically (Fig. 2b), then descendants of the same regional population should be most closely related, irrespective of their morphospecies designations. In the latter case, the phylogeny should reflect regional populations (strains or varieties) which may or may not share the same morphotype(s).

Sequence analysis of evolutionarily variable SSU and LSU rDNA sequences (variable domains) provides data useful in testing these predictions. Variable domains are subject to accelerated rates of nucleotide change and may ascribe species or even strain-specific genetic markers (e.g., Gobel *et al.* 1987; McCutchan *et al.* 1988; Lenaers *et al.* 1991). Such sequences are powerful tools for resolving evolutionary events that occurred in the geological past and are thus useful in testing the models presented in Fig. 2. The 5′ portion of LSU rDNA is valuable in this regard as it encompasses the so-called D1 and D2 domains, some of the most rapidly evolving portions of rRNA-encoding, eukaryotic DNA, interspersed among conserved sequence positions (Mitchot *et al.* 1984; Mitchot & Bachellerie 1987; Lenaers *et al.* 1989, 1991). The phylogeny shown in Fig. 1 is based on this portion of LSU rDNA, and is most consistent with the monophyletic radiation model (Fig. 2b). This conclusion is based on several facts: that representatives of the *tamarensis* complex are monophyletic with respect to other dinoflagellate genera (Lenaers *et al.* 1991), as well as the closely related congeners *A. affine* Fukuyo et Inoue, *A. minutum* Halim, *A. lusitanicum* Balech and *A. andersonii* Balech (Scholin *et al.* 1994); that distinct but co-occurring morphospecies can have similar (or identical) rDNA sequences (e.g. North American *A. tamarense/catenella/fundyense* versus temperate Asian *A. tamarense/catenella*); and that the overall tree topology is one of geography, not morphology (Fig. 1). Observations similar to the latter have been used to support dispersal and vicariance biogeography among a wide variety of organisms, including marine algae, barnacles, fishes, oysters, horseshoe crabs, terrapins, chickadees and humans (Lynch 1989; van den Hoek *et al.* 1990; Brooks & McLennan 1991; Avise 1992, Gill *et al.* 1993; Nei & Roychoudhury 1993; Avise 1994; van Oppen *et al.* 1994; Van Syoc 1994).

Validation of the B gene as a population-specific genetic marker

The biogeography of isolates found to carry the B gene (Table 1) also supports a monophyletic radiation of the *tamarensis* group. First, the B gene is found in all three morphotypes, consistent with the notion of a common ancestor. Second, the B gene is only associated with one ribotype, termed North American (so named because of the predominance of isolates from that region; Scholin *et al.* 1994a). This suggests that the B gene and only *one* of the LSU rDNA sequence types share a common evolutionary history (Fig. 1). According to our model, this particular combination thus ascribes only one of the many *tamarensis* complex lineages proposed to have arisen over the course of millions of years (i.e. delineates descendants of a unique regional population; Fig. 2b). Third, the B gene is found in all North American isolates examined to date, but only in a fraction of those from Japan and ship ballast water (Table 1). The latter observation is suggestive of a dispersal of B gene-containing *A. tamarense* between North America and Japan, and secondarily (as cysts in ballast water) from Japan to Australia (see below).

The strengths of these supporting observations rest on the supposition that the B sequence is unique to only one evolutionary lineage of *A. tamarense/catenella/fundyense* found throughout the world. To examine this idea more rigorously, we considered the relative age of the B gene and its population specificity in the context of two conflicting hypotheses:

(1) The B gene appeared 'late' in the evolutionary history of the *tamarensis* species complex (e.g. within the last several million years), and is a unique, population-specific marker with a restricted geographic distribution.
(2) The B gene appeared 'early' in the evolutionary history of the *tamarensis* species complex (e.g. tens of millions of years ago or more), and is widely distributed in populations inhabiting different regions of the world.

The first hypothesis predicts that the B gene is unique to the North American ribotype, while the second predicts that B-like genes occur in multiple LSU ribotypes and are pandemic among geographically isolated populations.

Distinguishing between these possibilities required sampling other portions of the SSU rDNA molecules not targeted in

←

Fig. 2a,b. Hypothetical models accounting for the evolution of the *A. tamarense/catenella/fundyense* species complex, and their respective phylogenetic predictions. For simplicity, the models consider only catenelloid and tamarensoid morphotypes. Primordial populations of *A. tamarense/catenella* ('protocat' and 'prototam,' respectively) are presumed to have dispersed to various regions of the world over millions of years and to have subsequently diverged, giving rise to morphologically similar, genetically distinct, regional populations (numbered 1–3). Present-day phylogenetic relationships of the morphotypes and their correspondence to geographic populations are hypothesized to depend on: (a) whether the organisms arose from distinct ancestral lines ('Polyphyletic Radiation'); or, (b) whether the organisms radiated from a more recent, common ancestor ('Monophyletic Radiation'). Hypothetical rDNA phylogenetic trees illustrate the expected outcomes for each model; in both cases, endemic, regional populations are predicted to be divergent. However, a polyphyletic radiation predicts that the overall tree topology resolves morphospecies, and that different morphotypes should always appear genetically divergent even in regions where they co-occur. In contrast, a monophyletic radiation predicts that the overall tree topology resolves distinct geographic populations (strains or varieties); descendants of the same regional population will appear most closely related, regardless of their morphospecies designation. These predictions assume no lateral gene transfer.

the original RFLP assay. To accomplish this task, we PCR-amplified SSU rDNA from isolates representing divergent LSU ribotypes and used each isolate's pool of PCR product as template in sequencing reactions. Figure 3 illustrates the type of results that were obtained. The region of the SSU molecule examined includes both evolutionarily variable and conserved sequences, encompasses multiple nucleotide differences in the SSU A and B genes, and is bracketed by restriction sites used in the A/B restriction tests (Sogin & Gunderson 1987; Scholin *et al.* 1993; Scholin & Anderson 1994). The A and B genes are clearly visible in rDNA from the North American *A. fundyense* as seen by the ambiguities that result from the presence of both genes (Fig. 3); a total of 7 transversions, 4 transitions and 3 single base-length differences were

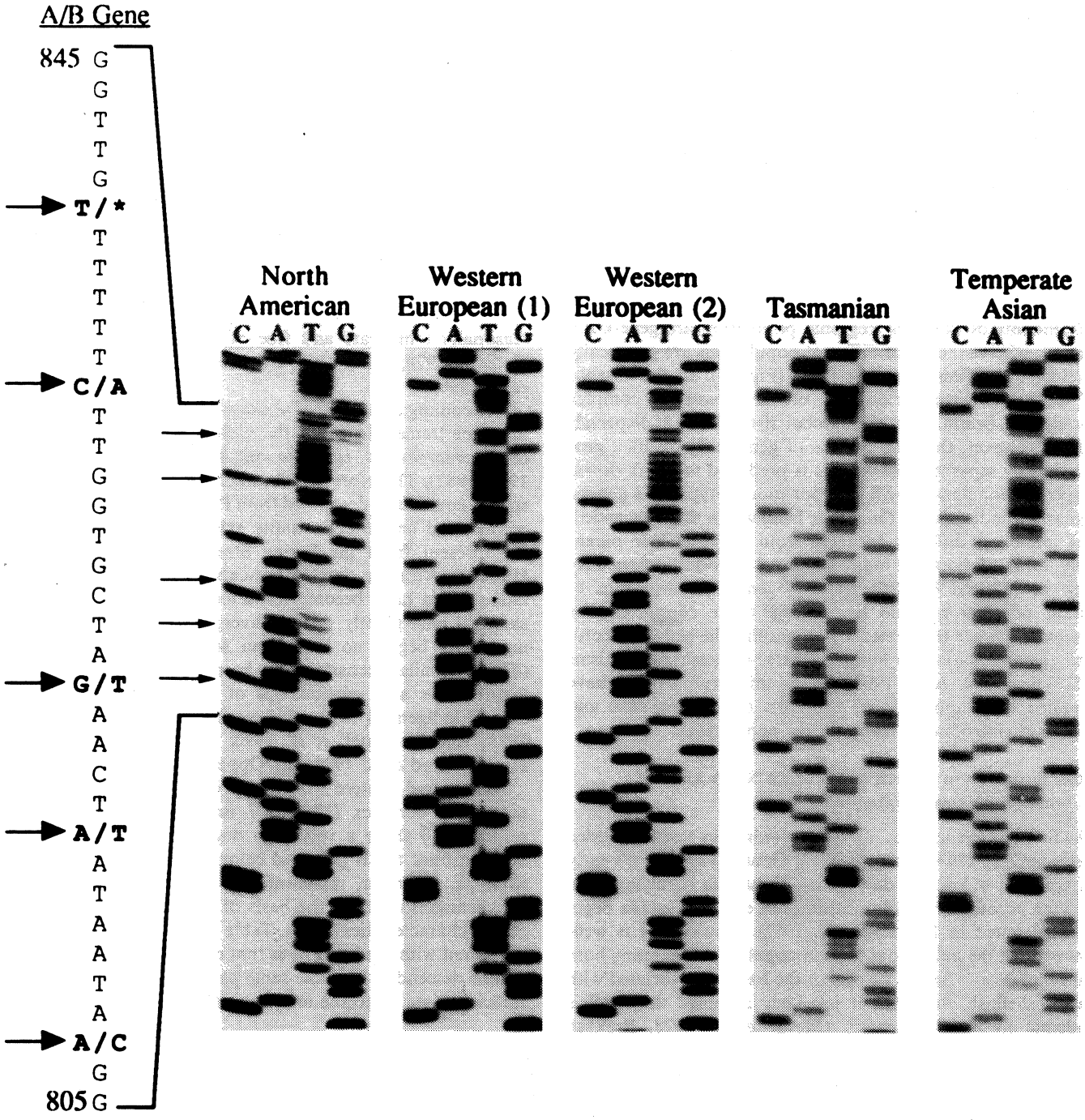


Fig. 3. Direct sequencing of PCR-amplified SSU rDNA from North American (*A. fundyense*; GtCA29), western European [*A. tamarense*; Pgt 183 (1) and PE1V (2)], Tasmanian (*A. tamarense*; ATBB01/2) and temperate Asian (*A. catenella*; ACPP01) LSU rDNA ribotype representatives using the magnetic bead technique. Two western European isolates were included because '1' is non-toxic, while '2' may produce trace amounts of toxin. Partial sequences of the A and B genes from GtCA29 (positions 805–845; Scholin *et al.* 1993) and their correspondence to that sequencing ladder are shown; arrows denote ambiguities and a single base length heterogeneity (T/*) expected if both genes are present. Multiple classes of SSU rDNA are seen in the North American strain, but are not observed in strains representative of other regional populations.

visualized using this procedure, all in agreement with previous determinations (Scholin *et al.* 1993). In contrast, all remaining isolates showed no evidence of these ambiguities and length heterogeneities, nor others indicative of multiple classes of SSU rDNA. When these results are combined with those of the RFLP tests (Scholin & Anderson 1994), a total of 17 positions that delineate the SSU A/B pair were sampled for each isolate compared.

Results of this study suggest that the B gene is not an 'ancient sequence' that has been differentially preserved in widely distributed populations. Rather, we favour the idea that it appeared 'late' in the evolutionary history of the *tamarensis* group [hypothesis (1)]. The B gene in our view is therefore indicative of a group of organisms that have descended from a unique regional population – a group that was geographically isolated and that evolved independently from other globally distributed *tamarensis* complex representatives.

Endemism or dispersal?

A prediction of the monophyletic radiation hypothesis is that descendants of the same regional population are genetically similar, whereas those of different populations are genetically distinct, regardless of morphotype (Fig. 2b). In the absence of dispersal, distinct ribotypes of the *tamarensis* complex should thus be limited in their global distribution. If dispersals occur, however, then the pattern of genetically distinct, geographically separate populations is predicted to break down: dispersed populations will harbour morphotypic and genetic signatures indicative of the region from which they descended, but inhabit a different location from that of their parent population. Patterns of indigenous and introduced flora may thus be inferred by defining phylogenetic relationships of extant populations, and by viewing those continuities or discontinuities in the context of geography, the historic record, and potential natural or human-assisted dispersal mechanisms (cf. Wiley 1980; Avise 1994). Using this approach, we have revealed what appear to be examples of both endemic and dispersed (introduced) *Alexandrium* populations.

Possible origins of eastern and western North American *A. tamarense/catenella/fundyense*

The first written account of PSP poisonings in North America dates back to 1793, during Captain George Vancouver's early exploration of present day British Columbia (Quayle 1969). Written accounts of PSP poisonings in eastern Canada begin in 1889 (cited in Prakash *et al.* 1971). However, it is well-known that the indigenous human population inhabiting both shores of North America knew of the hazards associated with eating shellfish, and thus it is widely accepted that PSP is endemic to North America (Meyer *et al.* 1928; Prakash *et al.* 1971).

PSP was not ascribed to a specific organism on the east coast until 1961 (Prakash *et al.* 1971) and on the west coast until 1965 (Quayle 1969). The causative organisms are now known as *A. tamarense*, *A. catenella* and *A. fundyense*. *Alexandrium catenella* is found exclusively on the west coast, and with one possible exception (strain PI32) *A. fundyense* is found only on the east coast. It should be noted that some taxonomists suggest that *A. fundyense* is a non-chain-forming variety of *A. catenella* (Hallegraeff *et al.* 1991; Y. Fukuyo, personal communication). *Alexandrium tamarense* occurs on both coasts.

Since the early Mesozoic (~250 Ma) the North American continent has been a barrier to, as well as a conduit for, the dispersal of marine organisms (Berggren & Hollister 1974; Marincovich *et al.* 1990; Thiede *et al.* 1990). During the late Cretaceous (~100 Ma), the Arctic Ocean is believed to have had connections to the Pacific and Tethys seas, but by the end of the Cretaceous became almost completely isolated from each (Marincovich *et al.* 1990, Thiede *et al.* 1990). A connection to the North Atlantic is believed to have opened sometime during the Paleogene (~40–50 Ma), providing the means for Atlantic fauna to enter the Arctic. The Pliocene opening of the Bering Strait (~3 Ma) then produced the most dramatic change in the composition of Arctic marine organisms: North Pacific species flooded the Arctic, largely displacing other organisms of Atlantic origin, while a limited number of Arctic-Atlantic species apparently entered the Pacific via the same seaway (Marincovich *et al.* 1990). These geologic and palaeoceanographic events took place when the Arctic climate was much milder than present – the region was seasonally temperate and free of an ice cap (Berggren & Hollister 1974; Clark 1990; Marincovich *et al.* 1990; Thiede *et al.* 1990).

The coming and going of seaways described above occurred in a time frame relevant to the evolution and palaeodispersal of *A. tamarense*, *A. catenella* and *A. fundyense* (Taylor 1980, 1984, 1987). Therefore North American populations of these species could have descended from Pacific, Tethyan or Atlantic realms, and ample opportunity existed for them to become omnipresent from the Bering Strait to the Labrador sea. However, with the onset of ice-cap formation such a population could have become restricted to eastern and western shores. Consequently, genetic divergence of these two groups may have begun no later than several million years ago (Fig. 4). Similar scenarios have been proposed to explain the distributions and relationships of other marine algae considered indigenous to North America (e.g. Estes & Steinberg 1988; van den Hoek *et al.* 1990; Peters & Breeman 1992; Hommersand *et al.* 1994; van Oppen *et al.* 1994).

Eastern and western North American representatives of the *tamarensis* complex (12 and 7 isolates examined to date respectively) share a very high degree of rDNA similarity: both harbour the B gene and belong to the North American ribotype. However, representatives of the two populations *are* distinguishable on the basis of fine-scale SSU and LSU rDNA characteristics (Fig. 1, Table 1). These observations are consistent with the hypothesis that eastern and western populations descended from the same parental stock, but also had sufficient time to accrue unique mutations.

Explanations for Japanese *A. tamarense/catenella* genetic heterogeneity

In contrast to North America, PSP toxicity was unknown in Japan until 1948 (Anraku 1984). Toxicity caused by *A. tamarense* and *A. catenella* was only confirmed in 1975 and 1976 respectively (Murano 1975; Hashimoto 1976). The recent appearance of PSP in Japan is noteworthy, given the high consumption of seafood and extensive farming of local

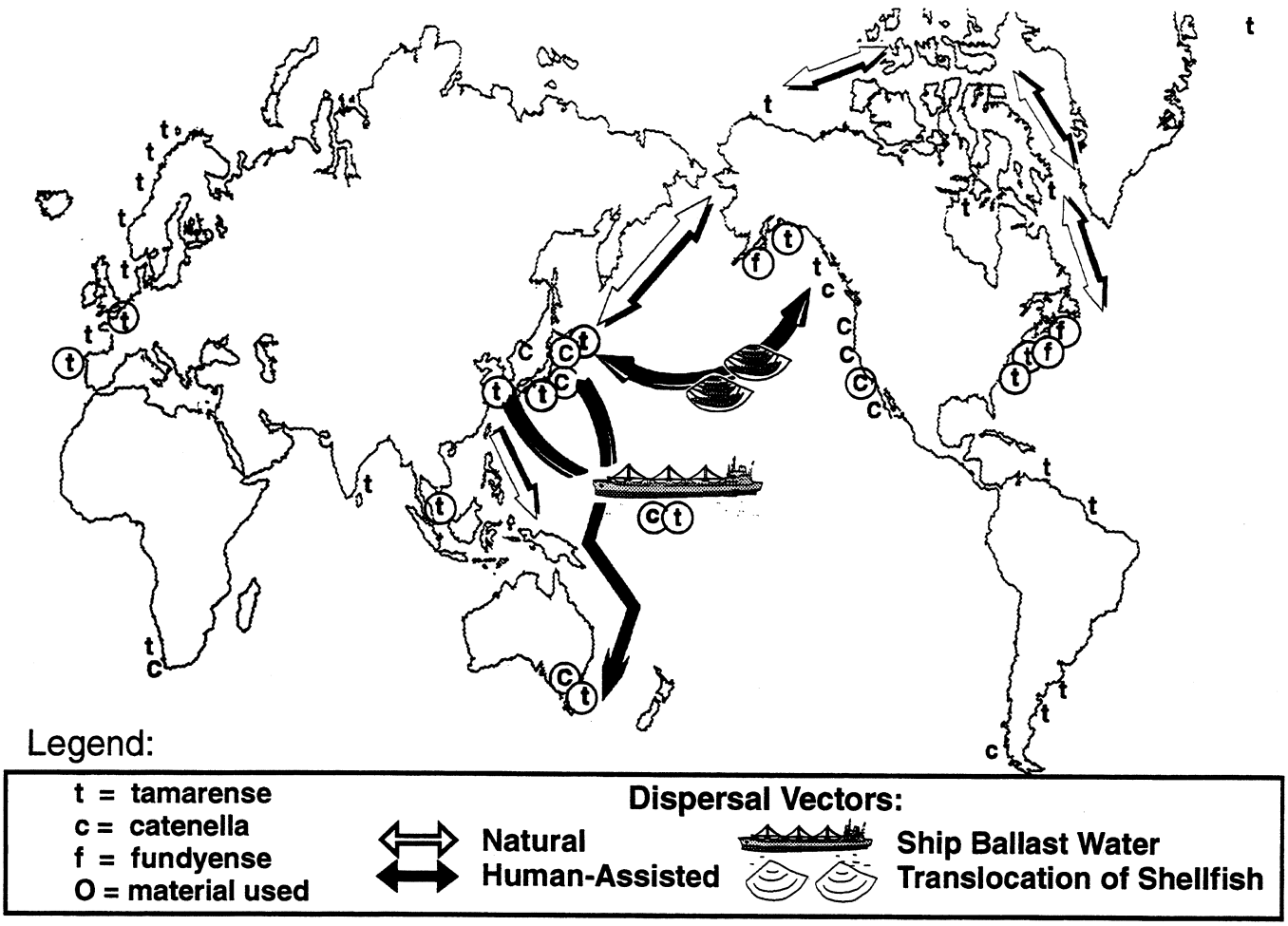


Fig. 4. Global map summarizing known distributions of *A. tamarense* (t), *A. catenella* (c), and *A. fundyense* (f), representatives of these populations brought into culture and used in this investigation (circled), hypothesized natural dispersal routes (hollow arrows), and possible human-assisted dispersal vectors (e.g., intercontinental transfers by ship ballast water and/or exchange of shellfish stocks; solid arrows).

waters in that country. An illustration by Oda (1935) of a *Diplopsalis* species probably refers to *A. tamarense* (G. Hallegraeff, personal observation). Work by Hirasaka (1923) published nearly two decades earlier indicates a possible earlier occurrence of *A. catenella*. These observations suggest that both *A. tamarense* and *A. catenella* may be among the 'hidden flora' whose growth has only recently been enhanced in Japanese coastal waters. However, the genetic heterogeneity among multiple isolates of these species examined thus far (Fig.1, Table 1) suggests that they also descended from different primordial populations – a pattern indicative of dispersal. The occurrence of the B gene-containing North American ribotype *A. tamarense* in Japanese waters is especially noteworthy in this regard.

Natural and human-mediated dispersals are both plausible mechanisms to explain this pattern. A prediction of the natural dispersal scenario is that extant populations of *A. tamarense* found between Japan and North America should share a high degree of genetic similarity. *Alexandrium tamarense* recently isolated from the Kamchatka Peninsula of Russia (between the Bering Strait and northern Japan) agree with this prediction, harbouring both the B gene and North American ribotype (unpublished data). Also of note is that some *A. tamarense* isolated from Ofunato Bay, Japan, are genetically similar to their counterparts from eastern and western North America, but *are* distinguishable as a unique cluster on the basis of fine-scale LSU rDNA sequence differences (the 'alternate North American' ribotype; Fig. 1, Table 1). As above, such fine-scale differences are consistent with a divergence of these groups in the recent *geological* past (e.g. within the last several million years).

In addition to natural dispersal, there is also good reason to believe that *A. tamarense* was introduced to Japan by human activity over the last 50 years. For example, the exchange of shellfish stocks between British Columbia and Japan (Taylor, personal communication), and increased shipping between Japan and other countries of the world are potential mechanisms whereby such transfers could occur (Fig. 4; Anderson 1989; Hallegraeff & Bolch 1992). Some *A. tamarense* found in Japan are *identical* to eastern North American and western European isolates with respect to their SSU and LSU rDNA characteristics, an observation consistent with introductions in modern times. As the population structure of *A. tamarense/catenella/fundyense* inhabiting North America, Asia and elsewhere becomes more rigorously defined and genetic variation within these populations docu-

mented, it may be possible to distinguish between natural and human-assisted dispersals. In either case, we view co-occurrence of multiple *tamarensis* complex ribotypes in Japanese coastal waters as the result of secondary contact of regional populations previously isolated for millions of years.

Potential dispersal of toxic *Alexandrium* species to Australia

The history of PSP toxicity in Australia has similarities to that of Japan. In Australia, incidences of PSP resulting from *A. catenella* and *A. minutum* were first confirmed in 1986 (Hallegraeff *et al.* 1988). Prior to that time, there is only a single account of suspected PSP toxicity (Le Messurier 1935), but the causative species was never identified. A taxonomic survey of Australian dinoflagellates published by Wood (1954) includes a chain-forming species, *Gonyaulax conjuncta* Wood, which most likely is a mis-identified *A. catenella* (G. Hallegraeff, personal observation). However, the recent appearance of conspicuous blooms of toxigenic dinoflagellates adjacent to major shipping ports is noteworthy (Hallegraeff *et al.* 1991; Hallegraeff & Bolch 1992). Once again, one is confronted with difficulties distinguishing between recent dispersal (in this case by ships' ballast water) and the possibility that a 'hidden flora' has become a more visible part of the phytoplankton community. Sequence analysis of rDNA from Australian *A. tamarense* and *A. catenella* is one way to address this question: endemic populations may have a unique genetic signature relative to others in the world, while an introduced population would have a genetic signature indicative of the population from which it dispersed.

The non-toxic *A. tamarense* isolated from Tasmania is unique, being the sole representative of the Tasmanian ribotype (Fig. 1, Table 1). In that sense, it conforms to our theoretical notion of a hidden flora. In contrast, rDNA sequences of Australian and Japanese *A. catenella* are very similar. The heterogeneity within and between these two regional populations is less than that observed in the North American cluster, suggesting that Japanese and Australian *A. catenella* have recently descended from the same parental stock. Natural dispersal of *Alexandrium* from Asia to Australia cannot be ruled out. For example, reductions of sea level and equatorial sea surface temperatures during the last Pleistocene glacial maximum (*c.* 18 000 years ago) may have provided a means by which this could have occurred (Potts 1983; Fleminger 1985; van Oppen *et al.* 1994). As above, this natural dispersal predicts a genetic continuity of *A. tamarense/catenella* populations along the expected route (Fig. 4). *Alexandrium catenella* recently isolated from the coast of China agree with this prediction, as they belong to the temperate Asian ribotype (unpublished data).

Alternatively, Hallegraeff & Bolch (1991, 1992) conclusively demonstrated that viable *A. tamarense/catenella* resting cysts have been discharged from the ballast tanks of cargo vessels into Australian ports. Some vessels are known to have carried these cysts from documented blooms in Japan and Korea (Table 1). The occurrence of temperate Asian ribotype *A. catenella* cysts in ballast water from southern Japan are significant, since they are identical to those isolated from established populations of this species in both Japan and Australia. The occurrence of eastern North American ribotype *A. tamarense* in the ballast water of a ship that originated in Japan is also of particular interest. This vessel had never been to North America, yet it contained *A. tamarense* cysts whose rDNA is indistinguishable from that obtained from other eastern North American ribotype representatives (Fig. 1, Table 1). We suspect this ribotype was introduced to Japan from a North Amercian population in modern times, and has subsequently been transported to Australia as cysts in ship ballast water. To date, free-swimming cells of North American ribotype *A. tamarense* are unknown in Australia.

Ballast water samples show that viable toxigenic *Alexandrium* cysts are dispersing as a direct result of human activity, and serve to illustrate how a region can be 'seeded' with genetically distinct *A. tamarense* and *A. catenella* from a variety of regional populations (Fig. 4, Table 1). If the introduced cysts ultimately give rise to blooms in Australia, then resulting 'founder' populations should reflect the morphological, biochemical and genetic signatures of their parents; that is, the regional populations from which they originated (e.g. Geller 1994). On-going dispersal of toxic *Alexandrium* cysts to Australia may serve as a contemporary example of what might have occurred in Japan.

CONCLUSIONS

Use of the species designations, *A. tamarense*, *A. catenella* and *A. fundyense*, is an indispensable means of conveying a sense of what these organisms look like. For that reason alone we submit that morphological-based descriptions should never be replaced by a classification scheme based purely on molecular criteria. However, in some cases recognition of these species' subcellular characteristics (toxin composition, genetic or immunogenic profiles, etc.) is necessary when morphological criteria fail to provide adequate resolution of the organisms in question (see also Anderson *et al.* 1994). This communication exemplifies the latter, because *both* morphological and subcellular characteristics are relevant to reconstructing the organisms' evolution and dispersal.

We believe that the long-standing disagreements over *A. tamarense*, *A. catenella* and *A. fundyense* morphospecies designations and their confusing relationship to subcellular characteristics and geographic populations are rooted in the organisms' natural history – a period of time in which the earth has seen dramatic climatic changes, alterations of sea level, the opening and closing of seaways, and an increase in intercontinental shipping. It seems undeniable that such processes have affected the primordial biogeography of populations in the ancient and recent geological past, with further changes resulting from human activity only within the last few centuries. The monophyletic dispersal hypothesis (Fig. 2b) offers a genetic reference from which to view dispersal pathways in the light of problems based largely on morphotaxonomy. However, rDNA data alone may be insufficient to distinguish whether dispersals occurred thousands of years ago by natural immigrations, or are a result of recent human activity (ballast water transport or relays of shellfish stocks). The uncertainty of dispersal timing stems from the relatively slow rate at which rDNA evolves and a lack of fossil evidence. Focusing on portions of the genome known to accrue mutations at a higher frequency than SSU or LSU rDNA may improve estimates of dispersal timing. Also of tremen-

dous value are numerous case studies concerning the evolution and global dispersal of other marine organisms whose biogeography is similar to that of the *tamarensis* complex (e.g., Berggren *et al.* 1985; Newton 1988; Bot *et al.* 1990; Cambridge *et al.* 1990; Prud'Homme van Reine & van den Hoek 1990; van den Hoek & Breeman 1990; Lindberg 1991; Peters & Breeman 1992; Breeman & Pakker 1994; van Oppen *et al.* 1994; Wiencke *et al.* 1994).

As the genetic relationships of additional, globally distributed populations are characterized, further inference of ancient and recent dispersals may be possible. Of particular interest are regions affected at present by toxic *A. tamarense* or *A. catenella* blooms that have no long-term history of such events (e.g. Australia, South America), regions where the debate over a change in a species abundance versus human-assisted introduction is perhaps greatest. The conceptual framework outlined here may be of use in delineating 'endemic' and 'introduced' species in these areas with appropriate reference to genetic data, the historic record, and consideration of any recent, localized alterations of the coastal environment (e.g. aquaculture, eutrophication, ballast water discharge).

ACKNOWLEDGEMENTS

The authors thank I. Bravo, S. Hall, M. Kodama, L. Provasoli, Y. Sako and A. White for providing cultures used in this investigation. D. Kulis, S. Blackburn and C. Bolch contributed to culturing efforts and toxicity determinations, as well as light and electron microscopy. C. Stubbs is thanked for excellent clerical assistance. This work was supported by grants from the National Science Foundation No. OCE 8911226 (DMA), the National Sea Grant College Progam Office grant No. NA46RG0470 [WHOI Sea Grant project R/B 121 (DMA)], the Woods Hole Oceanographic Institution Ocean Ventures Fund (CAS), and the David and Lucile Packard Foundation through funds allocated by the Monterey Bay Aquarium Research Institute (CAS). The Australian ballast water studies were supported by grant 89/39 from the Fishing Industry Research Development Council (GMH). Contribution number 8917 from the Woods Hole Oceanographic Institution.

REFERENCES

ADACHI M., SAKO Y. & ISHIDA Y. Restriction fragment length polymorphism of ribosomal DNA internal transcribed spacer and 5.8S regions in Japanese *Alexandrium* species (Dinophyceae). *Journal of Phycology* **30**: 857–863.

ANDERSON D.M. 1989. Toxic algal blooms and red tides: a global perspective. In: *Red Tides: Biology, Environmental Science and Toxicology* (Ed. by T. Okaichi, D.M Anderson & T. Nemoto), pp. 11–20. Elsevier, New York.

ANDERSON D.M., KULIS D.M., DOUCETTE G.J., GALLAGHER J.C. & BALECH E. 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. *Marine Biology, Berlin* **120**: 467–478.

ANRAKU M. 1984. Shellfish poisoning in Japanese waters. In: *Toxic Red Tides and Shellfish Toxicity in Southeast Asia* (Ed. by A.W. White, M. Anraku & K.-K. Hooi), pp. 105–109. Southeast Asian Fisheries Development Center, Singapore, and the International Development Research Centre, Ottawa, Canada.

AUSUBEL F.M., BRENT R., KINGSTON R.E., MOORE D.D., SIEDMAN J.G., SMITH J.A. & STRUHL K. (EDS). 1987. *Current Protocols in Molecular Biology*, Vols. 1 and 2. Wiley Interscience, New York.

AVISE J.C. 1992. Molecular population structure and the biogeographic history of a regional fauna: mtDNA analyses of marine, coastal, and freshwater species in the southeastern United States. *Oikos* **63**: 62–76.

AVISE J.C. 1994. *Molecular Markers, Natural History and Evolution.* Chapman and Hall, New York. 511 pp.

AYALA F.J. & KIGER J.A. 1980. *Modern Genetics.* Benjamin/Cummings, California. 844 pp.

BALECH E. 1990. Four new dinoflagellates. *Helgoländer Meeresuntersuchungen* **44**: 387–396.

BALECH E. 1985. The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. In: *Toxic Dinoflagellates* (Ed. by D.M. Anderson, A.W. White & D.G. Baden), pp. 33–38. Elsevier, New York.

BALECH E. & TANGEN K. 1985. Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae): *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb. nov. *Sarsia* **70**: 333–343.

BERGGREN W.A. & HOLLISTER C.D. 1974. Paleogeography, palaeobiogeography and the history of circulation in the Atlantic Ocean. In: *Studies in Paleoceanography* (Ed. by W.W. Hay), pp. 126–185. Society of Economic Paleontologists and Minerologists special publication No. 20.

BERGGREN W.A., KENT D.V. & VAN COUVERING J.A. 1985. The Neogene: Part 2. Neogene geochronology and chronostratigraphy. In: *The Chronology of the Geological Record* (Ed. by N.J. Snelling), pp. 211–260. Blackwell, Oxford, Boston.

BOT P.V.M., STAM W.T. & VAN DEN HOEK C. 1990. Genotypic relations between geographic isolates of *Cladophora.laetevirens* and *C. vagabunda. Botanica Marina* **33**: 441–446.

BREEMAN A.M & PAKKER H. 1994. Temperature ecotypes in seaweeds: adaptive significance and biogeographic implications. *Botanica Marina* **37**: 171–180.

BROOKS D.R. & MCLENNAN D.A. 1991. *Phylogeny, Ecology and Behavior. A Research Program in Comparative Biology.* University of Chicago Press, Chicago, London. 434 pp.

CAMBRIDGE M.L., BREEMAN A.M. & VAN DEN HOEK C. 1990. Temperature limits at the distribution boundaries of four tropical to temperate species of *Cladophora* (Cladophorales: Chlorophyta) in the North Atlantic Ocean. *Aquatic Botany* **38**: 135–151.

CEMBELLA A.D., TAYLOR F.J.R. & THERRIAULT J.-C. 1988. Cladistic analysis of electrophoretic variants within the toxic dinoflagellate genus *Protogonyaulax. Botanica Marina* **31**: 39–51.

CEMBELLA A.D., SULLIVAN J.J., BOYER G.L., TAYLOR F.J.R. & ANDERSEN R.J. 1987. Variation in paralytic shellfish toxin composition within the *Protogonyaulax tamarensis/catenella* species complex: red tide dinoflagellates. *Biochemical Systematics and Ecology* **15**: 171–186.

CEMBELLA A.D. & TAYLOR F.J.R. 1986. Electrophoretic variability within the *Protogonyaulax tamarensis/catenella* species complex: pyridine linked dehydrogenases. *Biochemical Systematics and Ecology* **14**: 311–323.

CLARK D.L. 1990. Arctic Ocean ice cover; geologic history and climatic significance. In: *The Geology of North America Vol. L. The Arctic Ocean Region* (Ed. by A. Grantz, L. Johnson & J.F. Sweeny), pp. 53–62. Geological Society of North America, Boulder, CO.

CULOTTA E. 1992. Red menace in the world's oceans. *Science, Washington, DC* **257**: 1476–1477.

ESTES J.A. & STEINBERG P.D. 1988. Predation, herbivory, and kelp evolution. *Paleobiology* **14**: 19–36.

FELSENTEIN J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **38**: 16–24.

FLEMINGER A. 1985. The Pleistocene equatorial barrier between the Indian and Pacific Oceans and a likely cause for Wallace's line. In: *Pelagic Biogeography. Proceedings of an International Confer-*

ence. The Netherlands (Ed. by A.C. Pierot-Bults, S. van der Spoel, B.J. Zahuranec & R.K. Johnson,), pp. 84–97. Unesco Technical Papers in Marine Science 49.

FRANKS P.J.S. & ANDERSON D.M. 1992. Along shore transport of a toxic phytoplankton bloom in a buoyancy current: *Alexandrium tamarense* in the Gulf of Maine. *Marine Biology, Berlin* **12**: 153–164.

FUKUYO Y. 1985. Morphology of *Protogonyaulax tamarensis* (Lebour) Taylor and *Protogonyaulax catenella* (Whedon and Kofoid) Taylor from Japanese coatal waters. *Bulletin of Marine Science* **37**: 529–537.

GELLER J.B. 1994. Marine biological invasions as models of dispersal: tracking secondary spread and introgressive gene flow. California Cooperative Oceanic Fisheries Investigations **35**: 68–72.

GILL F.B, MOSTROM A.M. & MACK A.L. 1993. Speciation patterns in North American chickadees: I. patterns of mtDNA genetic divergence. *Evolution* **47**: 195–212.

GOBEL U.B., GEISER A. & STANBRIDGE E.J. 1987. Oligonucleotide probes complementary to variable regions of ribosomal RNA discriminate between *Mycoplasma* species. *Journal of General Microbiology* **133**: 1969–1974.

GUADET J., JULIEN J., LAFAY J.F. & BRYGOO Y. 1989. Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison. *Molecular Biological Evolution* **6**: 227–242.

HALLEGRAEFF G.M. & BOLCH C.J. 1991. Transport of diatom and dinoflagellate cysts via ship's ballast water. *Marine Pollution Bulletin* **22**: 27–30.

HALLEGRAEFF G.M., BOLCH C.J., BLACKBURN S.I. & OSHIMA Y. 1991. Species of the toxigenic dinoflagellate genus *Alexandrium* in southeastern Australian waters. *Botanica Marina* **34**: 575–587.

HALLEGRAEFF G.M. & BOLCH C.J. 1992. Transport of diatom and dinoflagellate resting spores via ship's ballast water: implications for plankton biogeography and aquaculture. *Journal of Plankton Research* **14**: 1067–1084.

HALLEGRAEFF G.M., STEFFENSEN D.A. & WETHERBEE R. 1988. Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. *Journal of Plankton Research* **10**: 533–541.

HASHIMOTO Y., NOGUCHI T. & ADAICHI R. 1976. Occurrence of toxic bivalves in association with the bloom of *Gonyaulax spp. in Owase Bay. Nippon Suisan Gakkaishi* **42**: 671–676.

HIRAKASA K. 1923. Second note on red tide. *Zoological Magazine (Tokyo)* **35**: 84–95.

HOMMERSAND M.H., FREDERICQ S. & FRESHWATER D.W. 1994. Phylogenetic systematics and biogeography of the Gigartinaceae (Gigartinales, Rhodophyta) based on sequence analysis of *rbc*L. *Botanica Marina* **37**: 193–203.

HULTMAN T., STAHL S., HORNES E. & UHLEN M. 1989. Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *Nucleic Acids Research* **17**: 4937–4946.

KEAFER B.A., BUESSELER K.O. & ANDERSON D.M. 1992. Burial of living dinoflagellate cysts in estuarine and nearshore sediments. *Marine Micropaleontology* **20**: 147–161.

LE MESSURIER D.J. 1935. A survey of mussels on a portion of the Australian coast. *Medical Journal of Australia* **1**: 490–492.

LENAERS G., SCHOLIN C.A., BHAUD Y., SAINT-HILAIRE D. & HERZOG M. 1991. A molecular phylogeny of dinoflagellate protists (Pyrrhophyta) inferred from the sequence of the 24S rRNA divergent domains D1 and D8. *Journal of Molecular Evolution* **32**: 53–63.

LENAERS G., MAROTEAUX L., MICHOT B. & HERZOG M. 1989. Dinoflagellates in evolution. A molecular phylogenetic analysis of large subunit ribosomal RNA. *Journal of Molecular Evolution* **29**: 40–51.

LENAERS G., NIELSEN H., ENGBERG J. & HERZOG M. 1988. The secondary structure of large-subunit rRNA divergent domains, a marker for protist evolution. *BioSystems* **21**: 215–222.

LINDBERG D.R. 1991. Marine biotic interchange between the northern and southern hemispheres. *Paleobiology* **17**: 308–324.

LYNCH J.D. 1989. The gauge of speciation: on the frequencies of modes of speciations. In: *Speciation and its Consequences* (Ed. by D. Otte & J.A. Endler), pp. 527–553. Sinauer Associates, Sunderland, MA.

MCCUTCHAN T.F., DE LA CRUZ V.F., LAL A.A., GUNDERSON J.H., ELWOOD H.J. & SOGIN M.L. 1988. Primary sequences of two small subunit ribosomal RNA genes from *Plasmodium falciparum. Molecular and Biochemical Parasitology* **28**: 63–68.

MARINCOVICH L. JR, BROUWERS E.M., HOPKINS D.M. & MCKENNA M.C. 1990. Late Mesozoic and Cenozoic paleogeographic and paleoclimatic history of the Arctic Ocean Basin, based on shallow-water marine faunas and terrestrial vertebartes. In: *The Geology of North America Vol. L. The Arctic Ocean Region* (Ed. by A. Grantz, L. Johnson & J.F. Sweeny), pp. 403–426. Geological Society of America, Boulder, CO.

MEYER K.F., SOMMER H. & SCHOENHOLZ P. 1928. Mussel poisoning. *Journal of Preventative Medicine* **2**: 365–394.

MITCHOT B., HASSOUNA N. & BACHELLERIE J.P. 1984. Secondary structure of mouse 28S rRNA and general model for the folding of the large RNA in eukaryotes. *Nucleic Acids Research* **12**: 4259–4279.

MITCHOT B. & BACHELLERIE J.P. 1987. Comparisons of large subunit rRNAs reveal some eukaryote-specific elements of secondary structure. *Biochimie* **69**: 11–23.

MURANO 1975. Paralytic shellfish poisoning in Ofunato Bay and a suspected species of plankton. *Bulletin of the Plankton Society of Japan* **22**: 33–38.

NEWTON C.R. 1988. Significance of 'Tethyan' fossils in the American cordillera. *Science, Wahsington, DC.* **242**: 385–391.

ODA M. 1935. The red tide of *Gymnodinium mikimotoi* Miyake et Kominami n. sp. (ms) and the influence of copper sulfate on the red tide. *Zoological Magazine of Japan* **47**: 35–48.

PETERS A.F. & BREEMAN A.M. 1992. Temperature responses of disjunct temperate brown algae indicate long-distance dispersal of microthalli across the tropics. *Journal of Phycology* **28**: 428–438.

POTTS D.L. 1983. Evolutionary disequilibrium among Indo-Pacific Corals. *Bulletin of Marine Science* **33**: 619–632.

PRAKASH A., MEDCOF J.C. & TENNANT A.D. 1971. Paralytic shellfish poisoning in eastern Canada. *Bulletin of the Fisheries Research Board of Canada* **177**: 1–87.

PRUD'HOMME VAN REINE W.F. & VAN DEN HOEK C. 1990. Biogeography of Macronesian Seaweeds. *Courier Forschungsinstitut Senckenberg* **129**: 55–73.

QUAYLE D.B. 1969. Paralytic shellfish poisoning in British Columbia. *Bulletin of the Fisheries Research Board of Canada* **168**: 1–68.

SAIKI R.K., GELFAND D.H., STOFFEL S., SCHARF S.J., HIGUCHI R., HORN G.T., MULLIS K.B. & ERLICH H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science, Wahsington, DC.* **239**: 487–491.

SAKO Y., ADACHI M. & ISHIDA Y. 1993. Preparation and characterization of monoclonal antibodies to *Alexandrium* species. In: *Toxic Phytoplanton Blooms in the Sea* (Ed. by T.J. Smayda & Y. Shimizu), pp. 87–93. Elsevier, New York.

SAKO Y., KIM C.H., NINOMIYA H., ADACHI M. & ISHIDA Y. 1990. Isozyme and cross analysis of mating populations in the *Alexandrium catenella/tamarense* species complex. In: *Toxic Marine Phytoplankton* (Ed. by E. Granéli, B. Sundström, L. Edler & D.M. Anderson), pp. 320–323. Elsevier, New York.

SCHOLIN C.A. & ANDERSON D.M. 1993. Population analysis of toxic and non-toxic *Alexandrium* species using ribosomal RNA signature sequences. In: *Toxic Phytoplanton Blooms in the Sea* (Ed. by T.J. Smayda & Y. Shimizu), pp. 95–102. Elsevier, New York.

SCHOLIN C.A. & ANDERSON D.M. 1994. Identification of species and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *Journal of Phycology* **30**: 744–754.

SCHOLIN C.A., ANDERSON D.M. & SOGIN M. 1993. The existence of two distinct small-subunit rRNA genes in the toxic dinoflagellate *Alexandrium fundyense* (Dinophyceae). *Journal of Phycology* **29**: 209–216.

SCHOLIN C.A., HERZOG M., SOGIN M.L. & ANDERSON D.M. 1994a.

Identification of group and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *Journal of Phycology* **30**: 999–1011.

SCHOLIN C.A., VILLAC M.C., BUCK K.R., KRUPP J.M., POWERS D.A., FRYXELL G.A. & CHAVEZ F.P. 1994b. Ribosomal DNA sequences discriminate among toxic and non-toxic *Pseudonitzschia* species. *Natural Toxins* **2**: 152–165.

SMAYDA T.J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: *Toxic Marine Phytoplankton* (Ed. by E. Granéli, B. Sundström, L. Edler & D.M. Anderson), pp. 29–40. Elsevier, New York.

SOGIN M.L. & GUNDERSON J.H. 1987. Structural diversity of eukaryotic small subunit ribosomal RNAs: evolutionary implications. *Endocytobiology III. Annals of the New York Academy of Science* **503**: 125–139.

STEIDINGER K.A. & BADEN D.G. 1984. Toxic marine dinoflagellates. In: *Dinoflagellates* (Ed. by D.L. Spector), pp. 201–261. Academic Press, San Diego.

STEIDINGER K.A. & MOESTRUP Ø. 1990. The taxonomy of *Gonyaulax*, *Pyrodinium*, *Alexandrium*, *Gessnerium*, *Protogonyaulax* and *Goniodoma*. In: *Toxic Marine Phytoplankton* (Ed. by E. Granéli, B. Sundström, L. Edler & D.M. Anderson), pp. 522–523. Elsevier, New York.

SWOFFORD D.L. 1993. PAUP: Phylogenetic analysis using parsimony, version 3.1.1. Computer program and documentation distributed by the Illinois Natural History Survey, Champaign, IL.

TAYLOR F.J.R. 1987. General and marine ecosystems. In: *The Biology of Dinoflagellates* (Ed. by F.J.R. Taylor), pp. 399–502. Blackwell, Oxford.

TAYLOR F.J.R. 1985. The taxonomy and relationships of red tide dinoflagellates. In: *Toxic Dinoflagellates* (Ed. by D.M. Anderson, A.W. White & D.G. Baden), pp. 11–26. Elsevier, New York.

TAYLOR F.J.R. 1984. Toxic dinoflagellates: taxonomic and biogeographic aspects with emphasis on *Protogonyaulax*. In: *Seafood Toxins* (Ed. by E.P. Ragelis), pp. 77–97. American Chemical Society Symposium Series No. 262, Washington, DC.

TAYLOR F.J.R. 1980. On dinoflagellate evolution. *Biosystems* **13**: 65–108.

THIEDE J., CLARK D.L. & HERMAN Y. 1990. Late Mesozoic and Cenozoic paleoceanography of the northern polar oceans. In: *The Geology of North America Vol. L. The Arctic Ocean Region* (Ed. by A. Grantz, L. Johnson & J.F. Sweeny), pp. 427–458. Geological Society of America, Boulder, CO.

UHLEN M. 1989. Magnetic separation of DNA. *Nature, London* **340**: 733–734.

VAN DEN HOEK C. & BREEMAN A.M. 1990. Seaweed biogeography on the North Atlantic: where are we now? In: *Evolutionary Biogeography of the Marine Algae of the North Atlantic* (Ed. by D.J. Garbary & G.R. South), pp. 55–86. Nato ASI Series Vol. G 22. Springer-Verlag, Berlin, New York.

VAN DEN HOEK C., BREEMAN A.M. & STAM W.T. 1990. The geographic distribution of seaweed species in relation to temperature: past and present. In: *Expected Effects of Climate Change on Marine Coastal Ecosystems* (Ed. by J.J. Beukema, W.J. Wolf & J.J.W.M. Brouns), pp. 55–67. Kluwer Academic, Boston, Dordrecht.

VAN OPPEN M.J.H., DIEKMANN O.E., WIENCKE C., STAM W.T. & OLSEN J.L. 1994. Tracking dispersal routes: phylogeography of the Arctic-Antarctic disjunct seaweed *Acrosiphonia arcta* (Chlorophyta). *Journal of Phycology* **30**: 67–80.

VAN SYOC R.J. 1994. Genetic divergence between subpopulations of the eastern Pacific goose barnacle *Pollicipes elegans*: mitochondrial cytochrome c subunit 1 nucleotide sequences. *Molecular Marine Biology and Biotechnology* **3**: 338–346.

WILEY E.O. 1980. Phylogenetic systematics and vicariance biogeography. *Systematic Botany* **5**: 194–220.

Accepted 18 April 1995