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Variation among congeneric dinoflagellates from the northeastern United States and Canada

I. Enzyme electrophoresis

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Abstract

Relationships among dinoflagellates of the genus *Alexandrium* (= *Protogonyaulax*) from Long Island, New England, USA, and northeastern Canada were investigated between 1983 and 1987 using enzyme electrophoresis. A relative lack of heterogeneity among toxic isolates was observed despite large geographic separations of source populations. This observation is in marked contrast to observations on toxic members of this genus from the west coast of Canada and the United States. These different enzyme variability patterns support the proposal that dispersal of toxic strains in the east from a common source has occurred recently, presumably from established populations in northern Maine, USA, or Canada. Non-toxic strains from the study area were only distantly related to toxic isolates, consistent with their different morphology and separate species designations. One non-toxic isolate from a different region, that is conspecific with a number of the toxic strains, was more similar to these isolates than to other non-toxic clones.

Introduction

The presence of toxic marine dinoflagellates of the genus *Alexandrium* (= *Protogonyaulax*) in the northeast United States and Canada is a serious economic and public health concern, as well as a unique and important problem in population biology. These organisms produce potent neurotoxins which accumulate in filter-feeding shellfish, resulting in episodes of paralytic shellfish poisoning or PSP. The PSP problem has a long history in eastern Canada, dating back hundreds of years (Prakash 1975), but toxicity was first reported in southern Maine, New Hampshire, and Massachusetts, USA in 1972 (Bicknell and Walsh 1975, Hurst 1975), and in Connecticut and Long Island in 1982 (M. Schute, personal communication) and 1986 (E. Carpenter, personal communication), respectively. An important event in this sequence is the 1972 New England red tide (LoCicero 1975) which resulted in shellfish quarantines in Maine, New Hampshire, and Massachusetts. The subsequent recurrence of PSP in these waters in ensuing years has been interpreted as a clear indication of the dispersal and colonization of these organisms within the region (Dale 1977, Anderson and Morel 1979, Anderson et al. 1982).

The relatively recent detection of toxic *Alexandrium* species in the waters of Connecticut and Long Island, USA is more difficult to explain, since no toxicity was detected in 1972, and there has been no subsequent bloom event to which dispersal can be attributed. One clear dispersal mechanism is the dormant cyst stage in the *Alexandrium* life history (Dale 1977, Anderson and Wall 1978). These resting cells are produced in the late stages of blooms, falling to the sediments where they germinate in subsequent years to initiate new blooms. Cysts form when the typical asexual reproduction of the dinoflagellate changes to sexual, with gametes being formed which fuse to produce swimming zygotes and eventually cysts (Turpin et al. 1978, Anderson and Morel 1979).

As studies of the *Alexandrium* populations in this large region were completed, differences between northern and southern populations became apparent. The first differences noted were related to habitat; the southern populations were shown to be isolated within certain estuaries and embayments, whereas populations to the north appeared to be far more widespread, extending well out into nearshore coastal waters (Anderson and Wall 1978, Anderson et al. 1982). A survey of the intrinsic toxicity of a collection of *Alexandrium* isolates from the region between Nova Scotia and Long Island by Maranda et al. (1985) demonstrated a surprising trend of decreasing toxicity from north to south. Isolates from northern waters were as much as 50 times as toxic as those from the south, and two strains from the center of the region were non-toxic.

Within the general framework of the dispersal of species into a region in the past 15 yr, and the apparent heterogeneity of the resulting populations, a research program was established to compare *Alexandrium* isolates from the region

using three independent methods: (a) traditional taxonomy based on thecal morphology; (b) toxin composition, (i.e., the suite of saxitoxin and its derivatives produced by each isolate); and (c) enzyme electrophoresis. This paper describes the electrophoresis results.

In the last 10 to 15 yr, isozyme electrophoresis has resulted in a better understanding of phytoplankton population variation. Studies of diatom populations have shown both genetic heterogeneity (Murphy and Guillard 1976, Gallagher 1980) and genetic homogeneity (Soudek and Robinson 1983). Genetic variation among isolates of the chlorophyte *Pandorina morum* (Muell.) Bory (Fulton 1977) and of the dinoflagellate genera *Symbiodinium* (Schoenberg and Trench 1980) and *Heterocapsa* Stein (Watson and Loeblich 1983) has been demonstrated by isozyme electrophoresis. In addition, geographically separated isolates of the heterotrophic dinoflagellate *Crypthecodinium cohnii* Chatton en Grasse seemed to show isozyme patterns that correlated with breeding behavior (Beam and Himes 1987). Finally, investigations of dinoflagellate population variability in both freshwater isolates of *Peridinium volzii* Lemmerman (Hayhome et al. 1987) and marine isolates of the *Alexandrium tamarense/catenella* species complex (Cembella and Taylor 1985, Cembella and Taylor 1986, Cembella et al. 1988) have shown a high degree of intra- and interpopulation genetic polymorphism for both isozyme profiles and nuclear DNA quantities. In this population study we compared a group of isolates that shared certain characteristics (a common general morphology of motile or encysted stages, or an ability to produce PSP toxins) by determining their isozyme banding patterns for nine different systems.

Materials and methods

Twenty-three clones of *Alexandrium* taken from waters (in 1980) ranging from Canada in the north to Long Island in the south were placed into culture. Most of the strains examined in this study originated from "tamarensis-type" cysts collected during a large regional survey (Anderson et al. 1982). Cultures established from individual germinated cysts were then re-isolated to establish true clonal cultures (a cyst is a single cell, but the products of germination might include the two mating types that fused as gametes). Two additional cultures (*Gonyaulax* #2, 7) were obtained from A. W. White who isolated them from the Bay of Fundy, New Brunswick, Canada.

The taxonomy of the armoured, saxitoxin-producing dinoflagellates remains a contentious and confusing issue (Taylor 1985) that is especially problematic with respect to this paper. *Gonyaulax* is generally considered to be an incorrect genus for the "tamarensis complex" (Steidinger 1971, Taylor 1975). The two alternatives that are in the most favor are *Alexandrium* Halim and *Protogonyaulax* Taylor. In this and subsequent papers, we provisionally will use the oldest genus name (*Alexandrium*) until studies on the morphology of the type species are completed.

A related problem is the species designation. Morphological characteristics commonly used to separate species within the "tamarensis complex", (i.e., ability to form long chains, general shape) have changed in laboratory cultures (Cembella and Taylor 1985, Taylor 1985), and biochemical techniques have not yielded a clear separation (Cembella and Taylor 1986, Cembella et al. 1987). Continued use of different species names may not be justified. However, on the basis of morphology, cultures examined in this study were assigned to three *Alexandrium* species (*fundyense, tamarense,* and one new species, here referred to as *Alexandrium* sp., that will shortly be described by E. Balech, unpublished data). Isolate designations, origins, and toxicity values are summarized in Table 1. Subsequently, three additional isolates from other regions were added for purposes of taxonomic comparison. The first additional strain, designated isolate x, was *Protogonyaulax affinis* (= *Alexandrium fukuyoi*) Inoue and Fukuyo. This strain is characterized by a sub-rectangular apical pore complex, a round cyst, and by the ability to form long chains (Fukuyo et al. 1985). Strain x was isolated from the coast of Spain and provided to us by S. Fraga. The second additional strain, designed isolate y, was *Alexandrium tamarense* Lebour, which is a non-toxic clone of the type-species originally isolated from the Tamar Estuary, Plymouth, UK. This strain was kindly provided by F. J. R. Taylor from the University of British Columbia culture collection, where its designation is NEPCC 183 (*Protogonyaulax tamarensis*). The third additional isolate, designated as isolate z, was *Gymnodinium catenatum* Graham isolated by I. Bravo as a single swimming cell from Ria de Vigo, Spain (strain GCIV). Although this species is unarmoured, it produces toxins similar to those produced by toxic *Alexandrium* species (D. M. Anderson, unpublished data) and has a unique microreticulate cyst (Anderson et al. 1988). All of the above isolates may be obtained as cultures from D. M. Anderson.

For electrophoretic analysis, isolates were grown to late exponential phase under identical conditions at the Woods Hole Oceanographic Institution in 2 or 3 l batch cultures in K/2 medium (Keller and Guillard 1985) at 20 °C under a 14 h light : dark regime (ca 250 μE m^{-2} s^{-1} from cool white lamps). Cultures were harvested during the midpoint of the light cycle to yield a pellet of ca 8×10^6 cells. In harvesting, cells were concentrated on 15 μm nitex mesh, rinsed into a sterile 50 ml centrifuge tube with 50 volumes of cold 500 m*M* Tris-HCl, pH 8, and centrifuged at 4 °C for 5 min at 1750 $\times g$. The supernatant was aspirated and the pellet washed in 50 volumes of cold 500 m*M* Tris-HCl, pH 8. After centrifugation (4 °C, 5 min, 1750 $\times g$), the supernatant was aspirated again, the washed cell pellet was quickly frozen to −70 °C, and packed in dry ice for shipment by overnight express mail to Omaha for electrophoretic analysis. To avoid bias during scoring of electrophoresis results, isolates were labeled according to a coded identification system which was not disclosed to investigators in Omaha until all electrophoresis and similarity coefficient calculations were completed.

The frozen pellets were thawed rapidly on the day of electrophoresis and resuspended in cold sonication solution (25 m*M* Tris-HCl, pH 8, 0.006% aprotinin, 0.1%

Table 1. *Alexandrium* spp. Designation, origin, and toxicity of isolates from Canada and the northeastern United States. NB: New Brunswick; ME: Maine; NH: New Hampshire; MA: Massachusetts: CT: Connecticut; NY: New York. Toxicity in μg saxitoxin equivalent $\cdot 10^6$ cells

Designation	Isolate code	Origin	Toxicity
a-*fundyense*	*Gonyaulax* #2	Bay of Fundy, NB (45N)	20.93
b-*fundyense*	*Gonyaulax* #7	Bay of Fundy, NB (45N)	12.67
c-*fundyense*	GtME05	Deer Isle,ME (44N)	23.97
d-*fundyense*	GtME20	Monhegan Island, ME (43°45′N)	17.16
e-*fundyense*	GtCA01	Gulf of Maine, NH (43N)	14.63
f-*fundyense*	GtCA29	Gulf of Maine, NH (43N)	16.74
g-*fundyense*	Gt429	Ipswich Bay, MA (42°40′N)	19.36
h-*tamarense*	GtSP1	Salt Pond, Eastham, MA (41°45′N)	3.95
i-*sp.*	GtTC02	Town Cove, Eastham, MA (41°45′N)	0.00
j-*sp.*	GtTC03	Town Cove, Eastham, MA (41°45′N)	0.00
k-*fundyense*	GtMR01	Mitchell River, Orleans, MA (41°45′N)	6.28
l-*tamarense*	GtMP02	Mill Pond, Orleans, MA (41°45′N)	10.38
m-*fundyense*	GtMMP101	Mill Pond, Orleans, MA (41°45′N)	2.88
n-*fundyense*	GTMMP103	Mill Pond, Orleans, MA (41°45′N)	4.64
o-*tamarense*	GtPP02	Perch Pond, Falmouth, MA (41°30′N)	9.30
p-*tamarense*	GtPP10	Perch Pond, Falmouth, MA (41°30′N)	2.66
q-*fundyense*	GtCN02	Palmer Cove, Groton, CT (41°20′N)	28.87
r-*tamarense*	GtCN10	Palmer Cove, Groton, CT (41°20′N)	3.73
s-*tamarense*	GtCN16	Mumford Cove, Groton, CT (41°20′N)	3.01
t-*fundyense*	GtCN15	Mumford Cove, Groton, CT (41°20′N)	5.03
u-*tamarense*	GtLI21	Mud Creek, Moriches Bay. NY (40°45′N)	1.99
v-*tamarense*	GtLI22	Mud Creek, Babylon, NY (40°40′N)	3.38
w-*fundyense*	GTLI12	Mud Creek, Babylon, NY (40°40′N)	0.75

2-mercaptoethanol) with 5×10^5 cells ml^{-1} of sonication solution. Cells were disrupted by sonication (max. power output of intermediate tip of Virsonic model 16-850) with one-fourth volume of 2 mm diam. glass beads at 4 °C by alternating intervals of sonication with intervals of cooling. Cell disruption was monitored microscopically and was usually 90% in ca 2 min. The sonicates were centrifuged (20 000 $\times g$, 30 min, 4 °C) to pellet debris. The supernatants containing the soluble proteins ranged in color from light pink to a deep red-orange due to the presence of peridinin (B. Boczar, personal communication). They were passed through pre-chilled XAD-2 minicolumns (Sigma) to remove substances that interfered with electrophoretic separation of enzymes. Sucrose was added to each sample to the level of 10% and protein levels were determined (Bradford 1976). Approximately 80 to 100 μg of protein for each sample was loaded onto gels.

Vertical slab polyacrylamide gel electrophoresis of enzymes was performed on the samples according to Hayhome and Pfiester (1983). Methods for visualizing enzymes (with minor modifications of pH and substitution of MTT Tetrazolium for NitroBlue Tetrazolium) were those of Nerad and Daggett (1979) and Soltis et al. (1983). Selected isolates initially were screened for 25 different enzyme activities. Of these, nine systems were selected for further analysis on the basis of their diversity of functions and reproducibility. The nine enzyme systems surveyed were NAD-dependent malate dehydrogenase (NAD-MDH), malic enzyme (ME), glucose-6-phosphate dehydrogenase (GPD), glutamate dehydrogenase (GDH), phosphoglucomutase (PGM), aldolase (ALD), superoxide dismutase (SOD), alanine dehydrogenase (AlaDH) and NADP-dependent malate dehydrogenase (NADP-MDH). Gel banding patterns were scored after 1 to 2 h incubation and again after an overnight incubation. Each gel was scored independently by two investigators to reduce bias. Enzyme banding positions were determined to the nearest mm. For gel to gel comparisons, the banding pattern of clone SP1 (h) was used as an internal standard for each system and the migration distances of all bands were converted to relative mobilities (R_f) by selecting one prominent, reproducible band from the SP1 (h) banding pattern and using its migration distance as a common denominator. Each clone was analyzed electrophoretically at least twice or until reproducible banding patterns were obtained for all systems. Consistent and reproducible results for isolates were observed throughout the four year period of culturing and electrophoretic analysis needed for this study. Reproducibility of banding pattern R_f values was within 1%. This value falls within the range of previously reported percentages of reproducibility (Soudek and Robinson 1983). Accordingly, bands with R_f values within 1% of each other were considered to be homologous.

For data analysis, all enzyme banding patterns were converted to binary presence-absence data using the similarity coefficient of Jaccard (Sneath and Sokal 1973), S_J; where $S_J = [a/(a+u)] \times 100$, where a is the number of matches and u is the number of mismatches, when isolate banding patterns are compared pairwise. These similarity coefficients were then used for unweighted pair-group arithmetic average (UPGMA) cluster analysis (Ferguson 1980) to reveal the relative electrophoretic similarities of isolates. This phenetic method of data analysis was chosen over phylogenetic methods because the simple genetic patterns needed for phylogenetic analysis should not be inferred from gel banding pat-

Rf	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	F
NAD-MDH																											
5				x																							1
23	x		x	x	x	x	x	x		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	23
27									x																		1
36													x														1
41	x		x																					x			3
45	x																				x	x					3
50				x											x							x		x			4
64			x																								1
68																							x			x	2
73			x																								1
75									x															x		x	3
77	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	23
86	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	23
91	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	25
96	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x			x	22
100	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		23
109				x											x												2
116											x	x														x	3
127							x					x									x			x		x	4
182			x										x														2
232													x														1
246									x			x							x								3
ME																											
23	x		x	x			x	x		x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	20
34		x																				x		x			3
45	x	x		x	x		x	x			x	x	x	x	x	x		x		x	x	x	x		x	x	19
63																								x	x	x	3
68			x	x		x	x	x			x	x			x	x	x		x	x				x		x	14
77	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x			x	23
86	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x			x	22
91	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x			22
96	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		23
100	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x			x	23
109																									x		1
120																										x	1
125														x													1
130														x													1
136													x								x						2
141				x					x																		2
146									x																		1
227														x													1
250									x			x	x						x								4
GPD																											
100	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	25
150	x					x	x	x		x	x	x			x	x	x	x			x	x		x			14
220	x	x	x	x	x		x	x			x	x	x	x	x	x		x	x	x	x	x	x		x		20
250												x													x		2
350																										x	1
480																										x	1
800			x																								1
900									x																		1
1080									x			x							x								3
GDH																											
50	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x				22
100																								x	x		2
160					x			x													x	x	x				5
200	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	23
1000														x													1
1060									x			x															2

Fig. 1. *Alexandrium* spp. Diagrammatic representation of enzyme banding patterns for NAD-dependent malate dehydrogenase (NAD-MDH), malic enzyme (ME), glucose-6-phosphate dehydrogenase (GPD), and glutamate dehydrogenase (GDH). Lower case letters across the top designate each isolate. An x indicates the presence of a band of a particular R_f. F stands for the overall frequency of each band of a given R_f

terns for organisms such as dinoflagellates for which so little is known of genetics and breeding systems.

Cultures for toxin analysis were grown and harvested under the same conditions as the cells for electrophoresis with these exceptions: each 1 l batch culture was concentrated to a pellet of ca 4×10^6 cells. One ml of 50 m*M* acetic acid was added to the pellet, and the sample stored at −20 °C. Prior to HPLC analysis, the sample was thawed and frozen three times, centrifuged (1 750 $\times g$, 3 min, 23 °C), and 400 μl of the supernatant was 45 μm filtered into 1.5 ml autoanalyzer vials. Extracts were analyzed by HPLC (Sullivan and Wekell 1988) and the concentration of individual toxins converted to saxitoxin equivalents using conversion factors given by Hall and Reichardt (1984).

Results

Electrophoretic enzyme banding patterns for all isolates and all enzyme systems are summarized in Figs. 1 and 2. A total of 118 bands with different electrophoretic mobilities were observed. There were 22 different NAD-MDH enzymes, 19 ME, 9 GPD, 6 GDH (Fig. 1), 14 PGM, 12 ALD, 13 SOD, 9 AlaDH, and 14 NADP-MDH enzymes (Fig. 2). All toxic isolates from Canadian and northeastern US waters (a to h and k to w) had an average total band number of 46 (range, 37 to 57), while the two non-toxic isolates from this region (i and j) had a total of 17 and 18 bands, respectively. The three isolates from other regions had an average total band number of 38 (range, 36 to 41).

Fig. 3 shows the mean similarity coefficients that result when all enzyme banding patterns are converted to binary presence-absence data and the number of matches and non-matches between isolate banding patterns are compared pairwise for all isolates. The closer to 100 each mean similarity coefficient is, the less electrophoretically different and more related each pair of isolates is considered to be. For example, isolates h and r with a mean similarity coefficient of 95 are the two isolates that are the least different electrophoretically. When similarity coefficients for only those isolates having the same geographical origin are examined, we see the array of values summarized in Table 2. Except for

R_f	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	F
PGM																											
100	x	x	x	x	x	x	x	x		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	24
140			x			x	x	x			x	x	x		x			x			x	x	x				12
150			x	x		x	x	x			x	x	x		x			x			x	x	x				13
160																								x			1
180																								x			1
190	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		23
200	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		22
210	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		21
260																								x			1
420																										x	1
580																								x			1
620																									x		1
1000														x													1
1160									x			x	x							x							4
ALD																											
50	x	x	x	x	x	x	x	x		x	x	x	x		x		x	x	x	x	x	x	x				20
75							x								x						x	x		x			5
95	x	x	x	x	x		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		22
100	x	x	x	x	x		x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		21
105	x	x	x	x	x		x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		22
130					x									x							x	x		x			5
150																										x	1
165																										x	1
175																										x	1
220																									x	x	2
525									x			x	x							x							4
540													x														1
SOD																											
80											x				x												2
88									x	x	x																3
94											x																1
100	x		x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		21
104	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		22
116	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		23
120	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		23
130									x	x														x		x	4
144										x												x					2
160	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x				21
168	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		23
180	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		23
192																								x			1
AlaDH																											
50	x		x	x		x	x	x		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	22
75							x	x			x	x			x			x				x		x			8
100	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		22
130				x		x	x						x						x	x	x			x	x		9
210																								x		x	2
230																									x		1
450									x																		1
500													x														1
550									x			x				x			x								4
NADP-MDH																											
34																					x	x					2
45	x	x		x	x	x	x	x			x	x	x	x	x	x	x	x			x	x	x	x	x	x	21
59									x															x		x	3
68			x	x			x	x			x	x			x	x	x	x	x	x				x		x	14
77	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	23
86	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x			22
91	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	23
96	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x				21
100	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		23
118																										x	1
141				x																				x			2
205									x																		1
232													x														1
246				x					x			x															3
SUM	43	37	47	49	40	39	49	47	18	17	50	57	53	42	50	42	40	45	47	42	50	52	43	41	36	37	

Fig. 2. *Alexandrium* spp. Diagrammatic representation of enzyme banding patterns for phosphoglucomutase (PGM), aldolase (ALD), superoxide dismutase (SOD), alanine dehydrogenase (AlaDH), and NADP-dependent malate dehydrogenase (NADP-MDH). Lower case letters across the top designate each isolate.An x indicates the presence of a band of a particular R_f. F stands for the overall frequency of each band of a given R_f. The sum across the bottom is the total number of enzyme bands in each isolate for all enzyme systems in Fig. 1 and 2

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z
a		82	77	77	81	74	82	83	0	39	83	69	74	65	82	89	85	88	76	89	81	79	87	36	58	28
b			71	73	89	66	72	73	0	27	73	60	70	70	71	81	75	77	74	84	68	68	81	28	54	23
c				82	69	73	83	79	0	32	79	68	80	55	79	78	73	80	79	85	76	68	84	33	48	26
d					71	70	81	77	2	31	77	67	76	56	79	78	72	79	79	84	74	67	81	36	59	27
e						66	71	79	0	27	70	60	69	71	69	80	74	76	73	83	77	75	80	32	53	24
f							79	74	0	37	73	64	69	53	73	76	80	77	69	71	75	67	73	41	48	26
g								90	0	33	89	76	75	62	92	85	78	92	77	82	84	86	81	43	56	28
h									0	34	91	76	71	59	90	86	80	95	73	84	84	87	83	44	52	25
i										7	1	15	4	0	0	0	0	0	12	0	0	0	0	4	0	7
j											36	26	27	20	33	34	42	36	34	33	31	32	34	34	34	18
k												76	70	60	92	85	79	84	73	83	76	83	82	38	52	30
l													67	50	73	73	67	79	79	68	66	68	68	34	48	26
m														56	71	70	65	78	81	78	76	66	82	31	55	23
n															57	64	62	60	58	64	59	57	63	21	50	16
o																83	78	91	72	81	81	84	82	39	52	28
p																	88	88	82	90	75	73	81	37	57	29
q																		82	74	85	71	68	77	41	56	29
r																			76	86	81	84	88	39	56	28
s																				86	69	63	79	33	54	26
t																					74	71	92	33	60	30
u																						89	78	41	53	25
v																							76	42	47	24
w																								30	57	28
x																									24	32
y																										20
z																										

Fig. 3. *Alexandrium* spp. Mean similarity coefficients for all isolates

Table 2. *Alexandrium* spp. Similarity coefficients for Canadian and northeastern United States isolates by geographic origin for isolates from the same locale. NB: New Brunswick; NH: New Hampshire; MA: Massachusetts; CT: Connecticut; NY: New York

Locale and isolate designation	Enzyme systems									
	NAD-MDH	ME	GPD	GDH	PGM	ALD	SOD	Ala-DH	NADP-MDH	Mean
Bay of Fundy, NB a, b	63	75	67	100	100	100	86	50	100	82
Gulf of Maine, NH e, f	100	71	33	67	67	20	100	33	100	66
Town Cove, MA i, j	0	0	0	0	0	0	66	0	0	7
Mill Pond, MA l, m, n	54	54	37	61	59	59	100	28	67	58
Perch Pond, MA o, p	75	88	100	100	67	80	88	50	100	83
Groton, CT q, r, s, t	93	71	84	100	74	78	100	59	100	84
Babylon, NY v, w	67	88	67	67	100	67	88	67	71	76

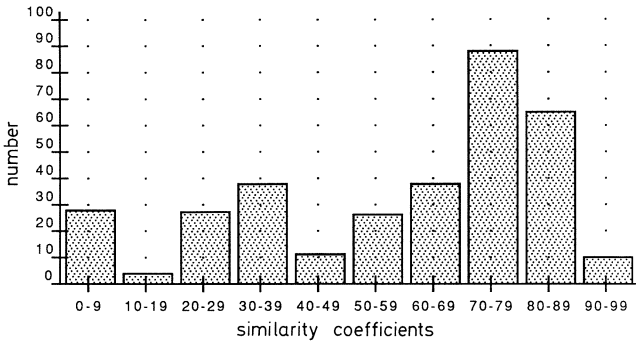

Fig. 4. *Alexandrium* spp. Frequency distribution for mean similarity coefficients of isolate pairs

the Town Cove, Massachusetts, isolates of *Alexandrium* sp., which were quite dissimilar, mean similarity coefficients for other locales from which multiple isolates were analyzed ranged from 58 to 84. The similarity coefficients from Mill Pond, Massachusetts, were the lowest and those from Palmer/Mumford Cove, Groton, Connecticut, the highest. Palmer and Mumford Cove isolates are considered to have a common geographical origin since these two contiguous embayments share common waters. Furthermore, strains from these two different locales were a mix of *A. tamarense* (l, r and s) and *A. fundyense* (m, n, q and t).

Another way in which these data can be viewed is simply by plotting the frequencies of the different mean S_J values (Fig. 4). This figure shows a discontinuous distribution with what appears to be three clusters of values. Those values grouped below 30 are attributable to the two *Alexandrium* sp. strains (i and j), those between 30 and 49 to the isolates from Spain (x and z), and those values above 50 are attributable to the *A. tamarense* and *A. fundyense* isolates.

Finally, the mean similarity coefficients in Fig. 3 were subjected to UPGMA clustering analysis to produce the dendrogram in Fig. 5. This dendrogram shows that all *Alexandrium tamarense* and *A. fundyense* isolates except n and y form a cluster of high similarity (70%). Isolates n and y join this cluster at a somewhat lesser similarity value of 50%. The degree of relatedness among those isolates from Canada and northeastern USA frequently has little to do with geographical origin. For example, h and r are the two most closely related isolates, but h was isolated from Salt Pond, Massachusetts, and r from Palmer Cove, Connecticut. Although isolates l and n, both from Mill Pond, are the last northeastern USA strains to join this cluster, isolate n does so at a level of similarity not much greater than that of isolate y which is the non-toxic *A. tamarense* type from the UK. Even though strains x and z have different morphologies and one is toxic (z) while the other is non-toxic (x), they are both more closely related to the *A. tamarense/fundyense* cluster than are the two non-toxic *Alexandrium* sp. isolates from Town Cove, Massachusetts (i and j).

Discussion

The data obtained in this study can be interpreted to have significance in two areas: taxonomy and ecology. Results indicate support for some aspects of the morphology-based taxonomy and lack of support for certain other aspects. The relative lack of enzymatic heterogeneity among toxic isolates of the east coast *Alexandrium* species, when compared to the high degree of enzymatic heterogeneity observed for the toxic west coast *Alexandrium* species (Cembella and Taylor 1986), suggests a common origin for the east coast isolates and supports the dispersal hypothesis (Dale 1977, Anderson and Morel 1979).

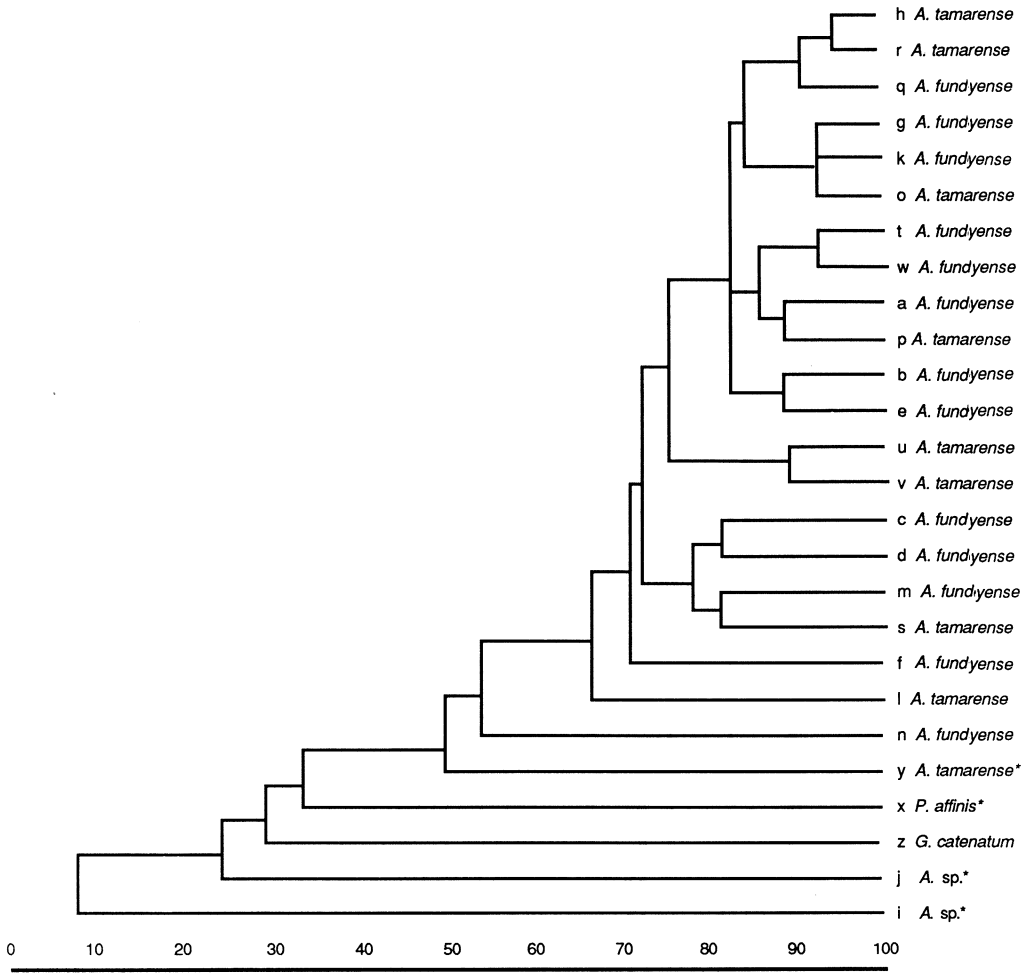

Fig. 5. *Alexandrium* spp. Dendrogram from UPGMA clustering analysis of mean similarity coefficients. Lower case letters and species names on the right designate each isolate (*A.: Alexandrium; G.: Gymnodinium; P.: Protogonyaulax*). The presence of an asterisk after a species name indicates non-toxic isolates. The scale indicates the amount of electrophoretic heterogeneity with 100 being the value for a complete lack of heterogeneity

Taxonomic significance

Electrophoretic data obtained in this study provide clear support for some aspects of the morphology-based taxonomy. The distinct electrophoretic differences observed between clones i and j and the other east coast isolates (a to h, k to w) in Fig. 5 supports Balech's (unpublished) designation of these two clones as a separate species here designated as *Alexandrium* sp. The relative electrophoretic similarity of the non-toxic *A. tamarense* type species clone from the UK (isolate y) with *A. tamarense* clones from the east coast study area (h, l, o, p, r, s, u, and v) also shows concordance with a taxonomic system based on morphology. In addition, the morphology-based taxonomy is supported by the relatedness of isolate x (*Protogonyaulax affinis = Alexandrium fukuyo*) to the cluster of *A. fundyense/tamarense* isolates in Fig. 5 since this isolate (x) is more similar morphologically to members of the cluster (Fukuyo et al. 1985) than it is to the remaining three isolates (i, j, and z). The relatedness of the *Gymnodinium catenatum* isolate (z) to the *A. fundyense/tamarense* cluster is interesting, and adds additional information to the debate on the nature of the relationship of this isolate to members of the cluster (Anderson et al. 1988).

The electrophoretic data from this study, however, do not support the morphology-based species designations of *Alexandrium fundyense* and *A. tamarense*. At the very least, when other studies with dinoflagellates are taken into account (Hayhome and Pfiester 1983, Cembella and Taylor 1986, Hayhome et al. 1987), the electrophoretic data argue for a close relationship between the two species and perhaps support the concept of an east coast *A. fundyense/tamarense* species complex analogous to the west coast *Protogonyaulax tamarensis/catenella* species complex described by Cembella and Taylor (1985, 1986).

Ecological significance

Toxic Canadian (east coast) and New England *Alexandrium* isolates are much more enzymatically homogeneous than toxic strains of the same genus from the west coast (Cembella and Taylor 1986). East coast toxic isolates all form one

large related cluster regardless of geographic origin (Fig. 5) with a mean similarity coefficient of 71 while that for all the west coast isolates is 22.

Another major difference is that toxic west coast isolates of this genus from a single embayment (English Bay, British Columbia, Canada) are more closely related than isolates from geographically-different locations along the coast (Cembella and Taylor 1986). This is not the case for the toxic east coast *Alexandrium* species, where strains from the same location are no more closely related than those from different regions. For example, isolates from the Bay of Fundy, (a and b) have mean similarity coefficients of 89 when matched with strains from Massachusetts (p) or New Hampshire (e) respectively, but have a coefficient of 82 when matched with each other (Fig. 3). On the other hand, four isolates from Palmer/Mumford Cove (Connecticut, USA) have an average similarity of 85. Essentially, similarity coefficients are high throughout the collection of east coast toxic strains.

Another interesting point of comparison with the study done by Cembella and Taylor (1986) is that all the east coast isolates in the *Alexandrium fundyense/tamarense* cluster form the cluster at a similarity coefficient of 54 while those west coast isolates comprising the English Bay population, the most homogeneous group in the west coast study, form a cluster at a similarity coefficient of about 30. Hence, the toxic east coast isolates from Canada to Long Island appear to be at least as similar electrophoretically as members of a localized population on the west coast.

Since protein polymorphisms, such as changes in isozyme banding patterns, usually are considered to be due to genetic drift of selectively neutral mutations (Kimura 1983), we believe that the relative lack of electrophoretic enzyme variation in toxic east coast isolates supports the dispersal hypothesis (Dale 1977, Anderson and Morel 1979). According to this hypothesis, a massive coastal red tide in 1972 introduced toxic populations to previously unaffected areas. The 1972 red tide occurred after the passage of Hurricane Carrie, and, thus, may have originated from benthic cyst accumulations from Canadian and northern Maine waters where red tides had been common phenomena for years (Anderson et al. 1982). The degree of enzymatic homogeneity among toxic isolates ranging from Canada to New York could thus be explained by the deposition of dormant cysts from motile populations originating in the north to southern areas where cysts subsequently inoculated overlying waters with motile cells to cause recurrent annual blooms (Anderson and Wall 1978, Anderson and Morel 1979). This inference applies to Connecticut and Long Island as well as to northern regions, although the timing of the dispersal remains problematic. The 1972 red tide caused toxicity only as far south as Massachusetts, so it is not clear how much further south the species had been transported during that event. The recent occurrence of PSP in Connecticut (Anderson et al. 1982, M. Schute personal communication) and Long Island (E. Carpenter, personal communication), following a history free from such concerns, has led to the speculation that further dispersal has occurred subsequent to the massive 1972 bloom. Our results cannot resolve the timing of the introduction of the species to the southernmost states, but they do indicate that the spreading events were recent and from a common origin. This could have occurred in stages rather than with one major event. If one accepts the enzymatic similarity of toxic isolates with different geographic origins as support for the dispersal hypothesis, variation observed in toxin content (Alam et al. 1979, Maranda et al. 1985) and composition (D. M. Anderson, unpublished data) may be accounted for by at least two alternative hypotheses. Since toxin composition appears to be a genetically determined polymorphism (Cembella et al. 1987), this trait might be subject to selection and toxin variability the consequence of recent genetic divergence after local populations were established by colonization from the north. On the other hand, given the relatively small number of isolates analyzed, toxin variability could be the result of genetic drift as cysts from somewhat heterogeneous northern source populations were transported down the coast. Hopefully, analysis of yet unpublished toxin composition data currently underway will shed some light on this question.

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Literature cited

Alam, M. I., Hsu, C. P., Shimizu, Y. (1979). Comparison of toxins in three isolates of *Gonyaulax tamarensis* (Dinophyceae). J. Phycol. 15: 106–110

Anderson, D. M., Jacobson, D. M., Bravo, I., Wrenn, J. H. (1988). The unique microreticulate cyst of the naked dinoflagellate *Gymnodinium catenatum*. J. Phycol. 24: 255–262

Anderson, D. M., Kulis, D. M., Orphanos, J. A., Ceurvels, A. R. (1982). Distribution of the toxic red tide dinoflagellate *Gonyaulax tamarensis* in the southern New England region. Estuar. cstl Shelf Sci. 14: 447–458

Anderson, D. M., Morel, F. M. M. (1979). The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. Estuar. cstl mar. Sci. 8: 279–293

Anderson, D. M., Wall, D. (1978). The potential importance of benthic cysts of *Gonyaulax tamarensis* and *Gonyaulax excavata* in initiating toxic dinoflagellate blooms. J. Phycol. 14: 224–234

Beam, C. A., Himes, M. (1987). Electrophoretic characterization of members of the *Crypthecodinium cohnii* (Dinophyceae) species complex. J. Protozool. 34: 204–217

Bicknell, W. J., Walsh, D. C. (1975). The first "red tide" in recorded Massachusetts history. Managing an acute and unexpected public health emergency. In: LoCicero V. R. (ed.) Toxic dinoflagellate blooms. Proc. Int. Conf. (1st) Mass. Sci. and Tech. Found., Wakefield, Massachusetts, p. 447–458

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248–254

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. Biochem. Syst. Ecol. 15: 171–186

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/North Holland, New Yor , p. 55–60

Cembella, A. D., Taylor, F. J. R. (1986). Electrophoretic variability within the *Protogonyaulax tamarensis/catenella* species complex: pyridine linked dehydrogenases. Biochem. Syst. Ecol. 14: 311–321

Cembella, A. D., Taylor, F. J. R., Therriault, J. C. (1988). Cladistic analysis of electrophoretic variants within the toxic dinoflagellate genus *Protogonyaulax*. Botanica mar. 31: 39–51

Dale, B. (1977). Cysts of the toxic red tide dinoflagellate *Gonyaulax excavata* Balech from Oslofjorden, Norway. Sarsia 63: 29–34

Ferguson, A. (1980). Biochemical systematics and evolution. John Wiley and Sons, Inc., New York

Fukuyo, Y., Yoshida, K., Inoue, H. (1985). *Protogonyaulax* in Japanese coastal waters. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 27–32

Fulton, A. B. (1977). Isozyme band variation within the algal species *Pandorina morum*. Biochem. Syst. Ecol. 5: 261–264

Gallagher, J. C. (1980). Population genetics of *Skeletonema costatum* (Bacillariophyceae) in Narragansett Bay. J. Phycol. 16: 464–474

Hall, S., Reichardt, P. B. (1984). Cryptic paralytic shellfish toxins. In: Ragelis, E. P. (ed.) Seafood toxins. American Chemical Society, Washington, p. 813–823

Hayhome, B. A., Pfiester, L. A. (1983). Electrophoretic analysis of soluble enzymes in five freshwater dinoflagellate species. Am. J. Bot. 70: 1165–1172

Hayhome, B. A., Whitten, D. J., Harkins, K. R., Pfiester, L. A. (1987). Intraspecific variation in the dinoflagellate *Peridinium volzii*. J. Phycol. 23: 573–580

Hurst, J. W. (1975). History of paralytic shellfish poisoning on the Maine coast. In: LoCicero V. R. (ed.) Toxic dinoflagellate blooms. Proc. Int. Conf. (1st), Mass. Sci. and Tech. Found., Wakefield, Massachusetts, p. 525–528

Keller, M. D., Guillard, R. R. L. (1985). Factors significant to marine dinoflagellate culture. In: Anderson D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 113–116

Kimura, M. (1983). The neutral theory of molecular evolution. In: Nei, M., Koehn, R. (eds.) Evolution of genes and proteins. Sinauer Associated, Sunderland, Massachusetts, p. 208–233

LoCicero, V. R. (1975). Toxic dinoflagellate blooms. Mass. Sci. Technol. Found., Wakefield, Massachusetts

Maranda, L., Anderson, D. M., Shimizu, Y. (1985). Toxicity of *Gonyaulax tamarensis* clones from eastern North American waters. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 349–350

Murphy, L. S., Guillard, R. R. L. (1976). Biochemical taxonomy of marine phytoplankton by electrophoresis of enzymes. I. The centric diatoms *Thalassiosira pseudonana* and *T. fluviatilis*. J. Phycol. 12: 9–13

Nerad, T. A., Daggett, P. M. (1979). Starch gel electrophoresis: an effective method for separation of pathogenic and nonpathogenic *Naegleria* strains. J. Protozool. 26: 613–615

Prakash, A. (1975). Land drainage as a factor in "red tide" development. Envir. Letters 9: 121–128

Schoenberg, D. A., Trench, R. K. (1980). Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and its specificity in its symbiosis with marine invertebrates. I. Isoenzyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum*. Proc. R. Soc. (Ser. B) 207: 405–427

Sneath, P. H. H., Sokal R. R. (1973). Numerical taxonomy. W. H. Freeman, San Francisco

Soltis, D. E., Haufler, C. H., Darrow, D. C., Gastony, G. J. (1983). Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern J. 73: 9–27

Soudek, D., Robinson, G. G. C. (1983). Electrophoretic analysis of the species and population structure of the diatom *Asterionella formosa*. Can. J. Bot. 61: 418–433

Steidinger, K. A. (1971). *Gonyaulax balechii* sp. nov. (Dinophyceae) with a discussion of the *Gonyaulax* and *Heteraulacus*. Phycologia 10: 183–187

Sullivan, J. J., Wekell, M. M. (1988). The application of HPLC in a PSP monitoring program. In: Kramer E. E., Liston J. (eds.) Seafood Quality Determinations. Seagrant College Program, Anchorage, Alaska

Taylor, F. J. R. (1975). Taxonomic difficulties in red tide and paralytic shellfish poison studies: the "*tamarensis* complex" of *Gonyaulax*. Envir. Letters 9: 103–119

Taylor, F. J. R. (1985). The taxonomy and relationships of red tide dinoflagellates. In: Anderson D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 11–26

Turpin, D. H., Dobell, P. E. R., Taylor, F. J. R. (1978). Sexuality and cyst formation in Pacific strains of the toxic dinoflagellate *Gonyaulax tamarensis*. J. Phycol. 14: 235–238

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

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