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Genetic structure of asexually reproducing *Enteromorpha linza* (Ulvales: Chlorophyta) in Long Island Sound

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Abstract

Thirteen asexually reproducing clones of *Enteromorpha linza* were found in samples collected from March to September 1982 in Long Island Sound, USA, based on variation at five enzyme loci. Significant differences in the relative frequency of each clone were observed among samples from 16 localities. There was a tendency for localities at the eastern end of the Sound to form a group genetically differentiated from localities at the western end, but in general there was no strong relationship between geographic distance and genetic distance. Areas separated by only a few hundred meters were genetically differentiated, despite the presence of a dispersing spore stage in this species. Samples from areas of low salinity were genetically similar but distinct from adjacent high salinity areas. One clone restricted to high salinity localities and one associated with low salinity localities were tested for growth under high and low salinity conditions in the laboratory. The clone from the high salinity habitat grew more slowly under low salinity conditions. However, there was no evidence for a detrimental effect of high salinity on the low salinity associated clone. Additional environmental factors, as well as biological factors such as limited recruitment and competitive interactions among clones, may also be important determinants of genetic differentiation among populations of *E. linza*. Temporal shifts in clone frequency were observed within some localities and may be due to seasonal-dependent regeneration from microscopic holdfasts for different clones or a seasonal recruitment of new clones from other areas. The pattern of differentiation among populations of *E. linza* in Long Island Sound appears to be maintained by factors operating on a microgeographic scale.

Introduction

The life-cycle of many species of marine invertebrates consists of a sessile or sedentary adult stage and a larval stage which may or may not be planktonic (Thorson, 1950). Some species have an extended planktonic larval stage with the potential for extensive gene flow among geographically separated populations and would be expected to show much less genetic differentiation among populations, compared to species with a reduced or no planktonic stage (Berger, 1973; Gooch, 1975). This does not appear to be the case. Using information from protein polymorphisms, several species of marine invertebrates with planktonic larval stages show marked differentiation over short distances (Burton, 1983). These observations provide a basis for re-evaluating the dispersal potential of a species and examining environmental factors which may be associated with population differentiation (Koehn *et al.*, 1980; Burton, 1983).

Much less is known about the genetic structure of populations of marine benthic algae. A few reports of ecotypic variation indicate that populations of some species may be genetically differentiated (Innes, 1984). In addition, a limited number of studies have used electrophoretically detectable enzyme variation to assess differentiation among populations of marine benthic algae (reviewed by Innes, 1984; Cheney, 1986). Miura *et al.* (1979) found significant differentiation among populations of *Porphyra yezoensis* using information from six polymorphic loci. No differentiation was found among ten populations of *Codium fragile* for four polymorphic loci (Malinowski, 1974). All individuals were found to be the same four-locus genotype and it was concluded that these populations consisted of a single asexually reproducing genotype.

The importance of gene flow between genetically differentiated populations of marine algae is not known. Although most species of benthic algae release spores into the water column, several studies have documented a

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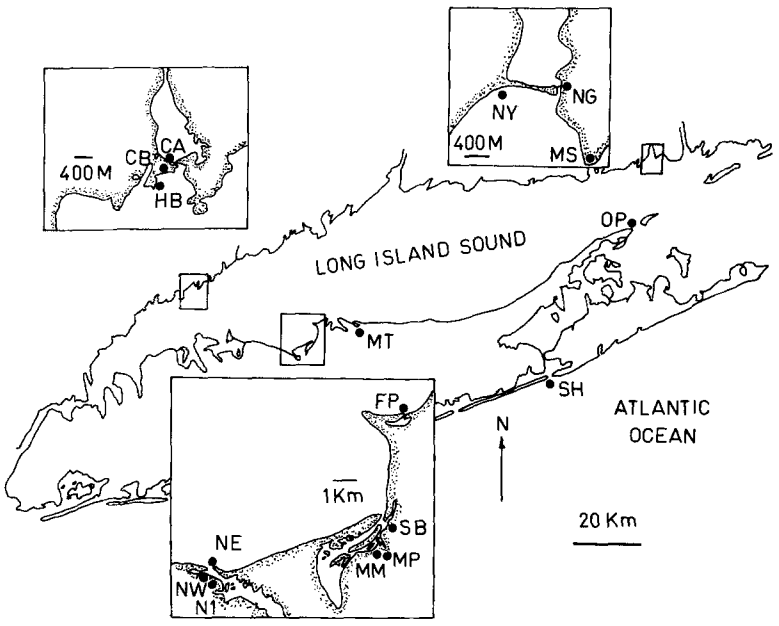

Fig. 1. *Enteromorpha linza.* Map of the 16 localities sampled: Stony Brook, NY (SB); Flax Pond, NY (FP); Mill Pond, NY (MP); Mill Pond Mouth, NY (MM); Nissequogue East, NY (NE); Nissequogue West, NY (NW); Nissequogue West-1, NY (N1); Mount Sinai, NY (MT); Shinnecock, NY (SH); Orient Pt., NY (OP); Horseshoe Beach, CT (HB); Cove Island Below, CT (CB); Cove Island Above, CT (CA); Millstone, CT (MS); Niantic Bridge, CT (NG); Niantic Bay, CT (NY)

restricted potential for dispersal. For example, dispersal of spores from the brown alga *Postelsia palmaeformis* has been shown to be restricted to within a three-meter radius of the sporophyte (Dayton, 1973). Restricted spore dispersal has also been found in the kelp *Macrocystis* sp. (Anderson and North, 1966).

In contrast to these species, spore dispersal in species of *Enteromorpha* appears to be much greater. *Enteromorpha* spp. spores are positively phototactic when first released and may remain in the upper portion of the water column for as long as several days (Jones and Babb, 1968). This should enhance dispersal by surface currents and may explain the occurrence of *Enteromorpha* spp. spores at offshore sampling sites (Amsler and Searles, 1980; Zechman and Mathieson, 1985). The capacity for extensive dispersal of large numbers of spores and the ability to survive in a variety of coastal habitats both contribute to the high colonizing potential of *Enteromorpha* species.

Enteromorpha linza occurs in a variety of estuarine environments and represents one of the most common species of *Enteromorpha* found in Long Island Sound (Schneider *et al.*, 1979). Genetic information from five polymorphic enzyme loci revealed that individuals from this area appeared to produce spores only by asexual reproduction (Innes and Yarish, 1984). Samples from four localities identified 13 different enzyme phenotypes, which are assumed to represent genetically distinct clones. These clones are probably diploid since most were heterozygous for at least one locus and there was no evidence for the occurrence of haploid genotypes as part of a sexual alternation of haploid and diploid generations. This study examines the genetic structure of *E. linza* in Long Island Sound to determine if the scale of differentiation among localities is consistent with the extensive dispersal of asexually produced spores. Several localities covering a range of salinity conditions were sampled to assess the distribution of each clone and to measure the level of genetic differentiation among these localities.

Materials and methods

Individuals of *Enteromorpha linza* were collected from the edge of the water at low tide from 15 localities in Long Island Sound and one locality on the Atlantic shore of Long Island (Fig. 1). Single individuals were easily identified and collected at least several centimeters apart, usually on separate rocks. *E. linza* does not spread vegetatively over the substrate, however, several fronds may proliferate from the same basal disc or holdfast (de Silva, 1969). Most localities were sampled at least twice in 1982 and each sample consisted of 23 or more ($\bar{x}=41$) individuals. Salinity measurements were taken at each site using a Goldberg refractometer (American Optical, Buffalo, NY). Individuals were analyzed electrophoretically as outlined previously (Innes and Yarish, 1984). Variation was found at five presumed gene loci using four enzymes: glutamate oxaloacetate transaminase (GOT-1, GOT-2), tetrazolium oxidase (TO), amylase (AMY) and phosphoglucomutase (PGM). Enzyme phenotypes conformed to the homozygous and heterozygous patterns expected for different alleles (electromorphs) associated with each locus (Innes and Yarish, 1984). This variation was used to determine the five-locus phenotype of each individual. The observation of large deviations from Hardy-Weinberg equilibrium at individual loci, non-random association among loci and mode of reproduction in the laboratory suggest that only asexual reproduction occurs at the localities sampled (Innes and Yarish, 1984). Each five-locus phenotype was therefore assumed to represent a genetically distinct clone with the understanding that additional loci may split some of these designated clones into additional clones.

Clone diversity

The number of clones and a measure of clonal diversity, G_O, were calculated for each locality. Clonal diversity was calculated as $1/\sum_{i=1}^{k} g_i^2$, where g_i is the relative frequency of the i^{th} clone in a sample of k clones (Black and Johnson, 1979). A measure of clonal evenness within each locality was calculated as G_O/k, which has a maximum value of 1.00 when the k clones are in equal frequency.

Population differentiation

Differentiation in clone frequency among localities was tested separately for samples collected in the spring and late summer. Differentiation in clone frequency among localities was also tested by unweighted pooling of samples within each locality. This tested for the presence of population differentiation and was followed by a more detailed analysis of within- and between-locality variation. Heterogeneity in clone frequency among localities was tested using a row (locality) × column (clone) G-test on the number of each clone found at each locality (Sokal and Rohlf, 1981). Clones with expected values less than 1.0 were pooled together to form a new clone class for the G-test. Clones were also individually tested for heterogeneity in frequency among the localities.

The relationship between genetic distance and geographic distance among all pairs of localities (pooled samples within localities) was tested using Spearman's rank correlation. Genetic distance was calculated as the complement of a similarity coefficient, $1-I_H$ (Hedrick, 1971, 1975), based on the relative frequency of each clone among all pairs of localities. Populations of asexually reproducing organisms are often not in Hardy-Weinberg equilibrium, and Hedrick's measure of similarity (based on genotype or in this case clone frequency) provides a better measure of similarity than coefficients based on gene frequencies. For example, two populations with the same gene frequency may have different genotype frequencies and a similarity measure based on gene frequencies would not detect genotypic differentiation. Geographic distance among the sampled localities was determined using the shortest distance over water among all pairs of localities.

Clones that showed significant heterogeneity in frequency among localities were subjected to a spatial autocorrelation analysis to determine the pattern of variation. This analysis correlates the value of a variable (in this case clone frequency) among localities at specified distances apart. Positive autocorrelation for a specified distance class indicates that the localities in this distance class have a similar value for the variable. Nonsignificant autocorrelation indicates the absence of predictable spatial trends in the data. This analysis has proven useful for detecting geographic patterns such as clines in gene frequency (Sokal and Oden, 1978a, b; Archie *et al.*, 1985). Spatial autocorrelation was analyzed using the SAAP program developed by D. Wartenberg which calculated Moran's autocorrelation coefficient (I). A description of this autocorrelation coefficient can be found in Karlin *et al.* (1984). Four distance classes were chosen so that each autocorrelation coefficient was calculated from 30 point pairs for each distance class.

Within-locality variation

Variation within and among localities was analyzed by calculating the genetic distance ($1-I_H$) among all 33 samples (Table 4) and summarizing the pattern using a principal coordinate analysis on the distance matrix (Gower, 1966; Sneath and Sokal, 1973). Genetically similar samples cluster together when projected onto the two principal axes.

Salinity experiments

Six individuals representing two clones were compared for growth at two salinities (28 and 4‰ S) which represented the extremes found among the sampled localities. Three individuals of Clone 9 were collected from the MP locality and three individuals of Clone 1 were collected from the adjacent MM locality (Fig. 1). These experiments used a single batch of filtered, enriched (Von Stosch media, McLachlan, 1973) and sterilized sea water from SB with a salinity of 28‰ S. For the low salinity treatment, sea water was diluted to 4‰ S with distilled water before enrichment and sterilization. Individuals from the field were allowed to release spores into the 28‰ S culture medium. Settled spores germinated and grew in 5-cm diameter petri dishes with 10 ml of medium as previously described (Innes and Yarish, 1984). Germlings were selected for experiments when they had reached a length of about 1 mm. Ten germlings from each of the six individuals were then placed in petri dishes with 10 ml of either 28 or 4‰ S sterile medium. Two replicates were used for each individual at each salinity treatment. Since growth was primarily in one dimension, increases in length were used as an estimate of growth. Measurements were made by digitizing the projected image from a camera lucida of a dissecting microscope. Lengths of the ten germlings within each petri dish were measured on Days 0, 2, 4 and 6. These data were analyzed using a linear regression procedure on the log transformed lengths (Sokal and Rohlf, 1981). The regression coefficients (b) were used as an estimate of growth rate and analyzed using analysis of variance (ANOVA).

Results

Clonal diversity

A total of 13 clones were found in the samples from all 16 localities (Table 1). This represents no additional clones

from those originally detected in a sample of only four of the localities (Innes and Yarish, 1984). The number of clones at each locality ranged from 2 to 8 with a mean of 4.4 (Table 2). Variation in the number of clones detected at each locality is partially affected by the number of samples taken, although there was no apparent relationship between sample size and number of clones detected. For example, samples of 43 and 240 individuals at the SB locality (Table 4) contained the same five clones. The evenness of the distribution of clones within each locality ranged from 0.32 to 0.77 (mean 0.58). The CA locality had the highest number of clones but the lowest evenness value due to a single common clone and several less abundant clones.

There was variation in the distribution of each clone among the localities. Clone 1 was found in 14 of the 16 localities and Clones 4, 10 and 12 occurred at only single localities (Table 3). The remaining clones showed an intermediate level of distribution for an average of 5.5 localities per clone for all 13 clones.

Population differentiation

The frequency of each clone varied among the 16 localities with variation also observed between samples within some of the localities (Table 4). Significant heterogeneity ($G=614$, $df=50$, $p<0.001$) in clone frequency was found among localities for samples collected in the spring over the period April 5 to May 24 (Table 4). Significant heterogeneity ($G=653$, $df=65$, $p<0.001$) in clone frequency was also found among localities for samples collected in the late summer over the period August 18 to September 14 (Table 4). Unweighted pooling of samples within each locality summarized differences in clone frequency among localities, followed by an analysis of within- and between-locality variation (see below). The row (locality) × column (clone) G-test on these data showed highly significant ($G=2\,196$, $df=90$, $p<0.001$) among-locality heterogeneity for clone frequency. The maximum level of differentiation is evident in Table 4 where some localities (SH, NY, NG, OP, MS, HB) shared no clones with others (NW, MT). Four of the more widely-distributed clones (Clones 1, 2, 3 and 6) were each tested for heterogeneity in frequency among the 16 localities using a conservative simultaneous test procedure (Sokal and Rohlf, 1981). Clones 1, 2 and 6 showed significant ($p<0.01$) heterogeneity ($G=738$, 261, 506, respectively), while Clone 3 showed borderline significance ($G=212$, $p=0.05$).

There was a significant ($r_s=0.21$, $p<0.01$) but very weak relationship between genetic distance and geographic distance (Fig. 2). For example, three pairs of localities (MP–MM, NW–N1, CA–CB) with the closest geographic proximity (Fig. 1) showed large differences in the relative frequency of each clone (Table 4).

Lack of spatial patterns related to geographic distance was also found for the four more widely-distributed clones (1, 2, 3 and 6). Most of the autocorrelation coefficients for

Table 1. *Enteromorpha linza.* Thirteen clones from Long Island Sound as determined from five-locus phenotypes

	Locus				
	GOT-1	GOT-2	AMY	PGM	TO
1.	SS	FF	SS	FF	SS
2.	SS	FF	SF	FF	SS
3.	SS	FF	SS	FS	SS
4.	SM	FF	SS	FF	SS
5.	SF	FF	SF′	FF	SS
6.	SS	FM	SS	FF	SF
7.	S′S	FM	SS	FF	SF
8.	SS	MS	SS	FF	FF
9.	SS	MM	SS	FF	FF
10.	S′S	MM	SS	FS	FF
11.	SS	MM′	SS	FF	FF
12.	SS	FF	SS	FF	FF
13.	SS	FM′	SS	FF	SF

Table 2. *Enteromorpha linza.* Number of clones in samples from 16 localities in Long Island Sound (see Fig. 1). Clonal diversity as measured by G_O and evenness (see Materials and methods)

Locality	No. of clones	G_O	Evenness
SB	7	3.77	.54
NE	5	2.72	.54
NW	4	1.85	.46
SH	3	1.41	.47
HB	3	2.32	.77
CB	6	3.43	.57
CA	8	2.53	.32
MS	2	1.09	.54
OP	2	1.15	.58
FP	4	2.83	.71
MP	7	3.45	.49
NG	3	2.28	.76
NY	2	1.18	.59
MT	4	2.05	.51
MM	6	3.88	.65
N1	5	3.77	.75
Mean	4.4	2.48	.58

Table 3. *Enteromorpha linza.* Total abundance and distribution of each clone among localities in Long Island Sound

Clone	Total no. of individuals	%	No. of localities
1	485	29.2	14
2	261	15.7	13
3	113	6.8	8
4	16	1.0	1
5	106	6.4	5
6	426	25.7	9
7	30	1.8	6
8	21	1.3	4
9	140	8.4	4
10	25	1.5	1
11	31	1.9	3
12	1	0.1	1
13	4	0.2	2
Total	1 659	100.0	Mean 5.5

Table 4. *Enteromorpha linza.* Frequency of 13 clones in samples from 16 localities in Long Island Sound (Fig. 1) collected in 1982

Locality	Date	Salinity (‰S)	*N*	Clone												
				1	2	3	4	5	6	7	8	9	10	11	12	13
SB	3/6	–	25	.080	.520	.160		.240								
	3/15	25	52	.231	.538	.038		.058	.135							
	4/14	22	43	.047	.419	.093		.093	.349							
	5/28	–	240	.125	.217	.071		.138	.450							
	8/21	22	64	.156	.125	.063			.547	.016	.094					
NE	4/5	25	62	.581	.065	.032			.129	.194						
	8/24	24	48	.500	.042	.021			.271	.167						
NW	5/24	11	28						1.000							
	8/24	0	55						.509			.436		.036		.018
SH	4/19	30	67	.746	.060		.194									
	9/7	29	71	.915	.042		.042									
HB	9/14	25	24	.167	.583	.250										
CB	5/3	24	24	.292	.083	.375		.083	.167							
	9/14	24	30	.367	.200	.400									.033	
CA	5/3	5	47		.021	.021			.660		.021	.277				
	9/14	19	30	.133	.133	.067			.500	.067						.100
MS	9/13	28	24	.958	.042											
OP	9/8	30	28	.929	.071											
FP	3/23	28	95	.368	.274	.326				.032						
	8/18	24	64	.625	.203	.172										
MP	4/22	0	34						.735	.029		.059	.147	.029		
	6/10	4	30						.167		.033	.567	.033	.200		
	7/19	3	148	.014					.209	.007	.041	.473	.128	.128		
NG	5/3	25	32	.031				.969								
	9/13	28	24	.583	.333			.083								
NY	5/3	22	24	.917	.083											
	9/14	27	24	.917	.083											
MT	5/16	–	23						1.000							
	9/13	0	48						.500		.146	.292		.063		
MM	6/10	22	28	.214	.571			.143	.071							
	7/21	24	68	.324	.221	.044		.265	.103	.029						
N1	5/24	–	28	.143	.286	.143		.107	.321							
	8/24	28	30	.433	.300				.267							

each clone were nonsignificant (Table 5). The absence of significant autocorrelation for any of the clones in the first distance class again demonstrates that the level of genetic similarity between localities separated by short distances cannot be predicted. The second and fourth distance classes for Clone 1 showed significant positive and negative autocorrelation coefficients, respectively (Table 5). This pattern appeared to be due to differences in the frequency of Clone 1 between two groups of localities. Four localities (NY, MS, OP, SH) with the highest Clone 1 frequency (Table 4) were close to the Atlantic Ocean at the entrance to Long Island Sound (Fig. 1). Most of the remaining localities were closer together in the western part of the Sound and were characterized by a lower frequency of Clone 1 (Table 4). Localities within these two groups fell into the second distance class, explaining the positive auto-

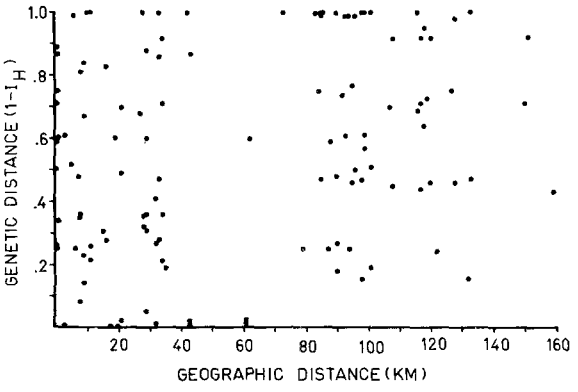

Fig. 2. *Enteromorpha linza.* Genetic distance between all pairs of localities plotted against the geographic distance between the same pairs of localities. Spearman's rank correlation coefficient = 0.21 ($p < 0.01$)

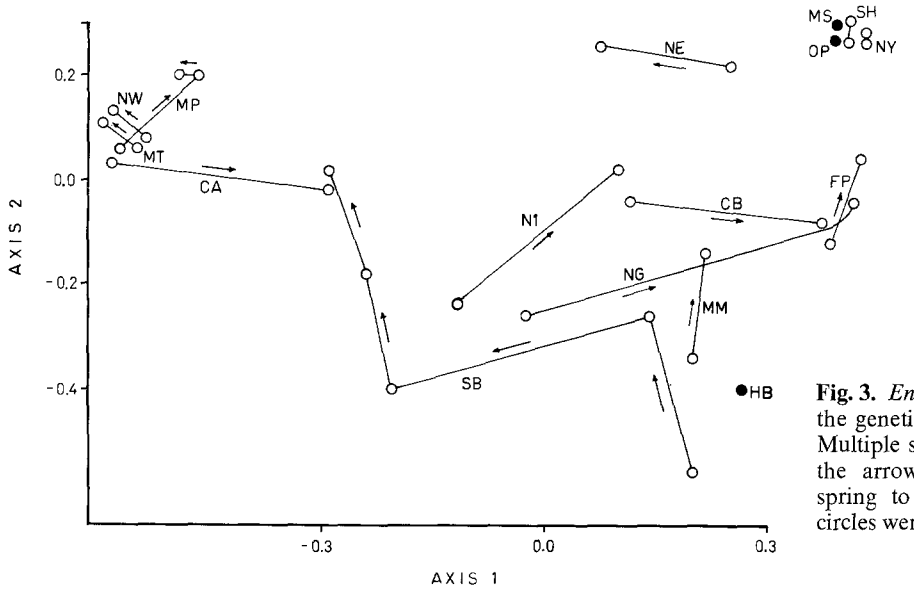

Fig. 3. *Enteromorpha linza.* Principal coordinate plot of the genetic distance between all 33 samples in Table 4. Multiple samples from each locality are connected and the arrows show the direction of change from the spring to the summer samples. Localities with solid circles were only sampled on a single occasion

Table 5. *Enteromorpha linza.* Spatial autocorrelation analysis of four clones distributed among several localities in Long Island Sound. Table entries are Moran's I

	Distance class (km)			
	0 to 16	16 to 43	43 to 98	98 to 159
Clone 1	.188	.437**	−.309	−.583**
Clone 2	−.200	.057	.048	−.172
Clone 3	.050	−.056	.090	−.350*
Clone 6	−.041	.162	−.110	−.278

* $p < 0.05$
** $p < 0.001$

correlation. The fourth distance class paired the eastern localities (high Clone 1 frequency) with the western localities (low Clone 1 frequency), resulting in significant negative autocorrelation. The absence of Clone 3 in the four eastern localities and its presence in most of the western localities (Table 4) resulted in the significant negative autocorrelation observed in the fourth distance class for this clone (Table 5). The general tendency for negative autocorrelation coefficients in this fourth distance class suggests that the two groups of localities are genetically differentiated with respect to these four clones.

Within-locality variation

Multiple samples from many of the localities showed differences in clone frequency (Table 4) that may obscure among-locality variation. A principal coordinate analysis of all 33 samples was used to compare within- and between-locality variation and show the genetic distance relationship among the localities. A projection of the samples onto the first two principal axes (explaining 76% of the total variation) displays the observed pattern of variation (Fig. 3). Samples from MS, OP, SH and NY formed a tight cluster, separated by a large distance on the first axis from a cluster made up of NW, MP and MT. Samples from the remaining localities were spread over the area between these two clusters. Significant differentiation was evident among some of these localities despite the variation within localities, but they formed no obvious clusters. Many of the localities showed shifts in clone frequency between the spring and summer samples (Table 4, Fig. 3) with shifts often involving different clones. For example, at five localities (N1, NG, MM, CB, FP) the relative frequency of Clone 1 increased, while at NE and SB, Clone 6 increased and Clone 2 decreased in relative frequency (Table 4). The presence of Clone 1 and the absence of Clone 9 in the fall sample compared with the spring sample at the CA locality (Table 4) explained the increased similarity of this locality with the last SB sample (Fig. 3). The greatest shift in clone frequency was observed at the SB locality where Clone 2 decreased and Clone 6 increased in relative frequency (Fig. 4).

Prior to resolving all five enzyme-loci, data on temporal variation for GOT-2 phenotype frequencies were collected from NM, NE and SB over a four-year period (1979 to 1982). Most of the variation involved differences in frequency of the FM (probably Clone 6) and FF (Clones 1, 2, 3 or 5) phenotypes. Variation at SB was markedly seasonal with a low frequency of GOT-2 FM individuals in the spring (and fall of 1981) followed by an increased frequency in the late summer (Fig. 5). An approximately similar pattern was observed at NE (Fig. 5). Less variation was observed at NW with GOT-2 FM individuals near 100% most of the time (Fig. 5).

Salinity experiments

The low salinity localities (NW, MP, MT including one sample from CA) clustered together in the principal coordinate analysis (Fig. 3). These localities had salinity values of 0 to 11‰ S and were characterized by the presence of Clones 6 and 9 and the absence or low fre-

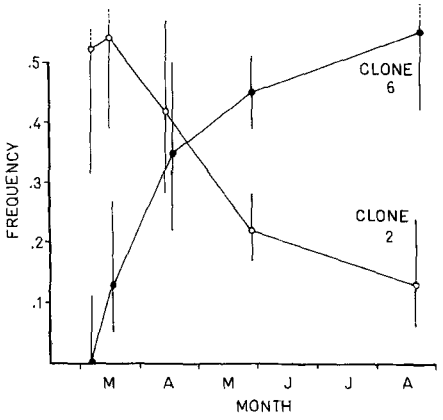

Fig. 4. *Enteromorpha linza.* Change in frequency of Clones 2 and 6 at the SB locality in 1982. Vertical bars are 95% confidence intervals (Rohlf and Sokal, 1981)

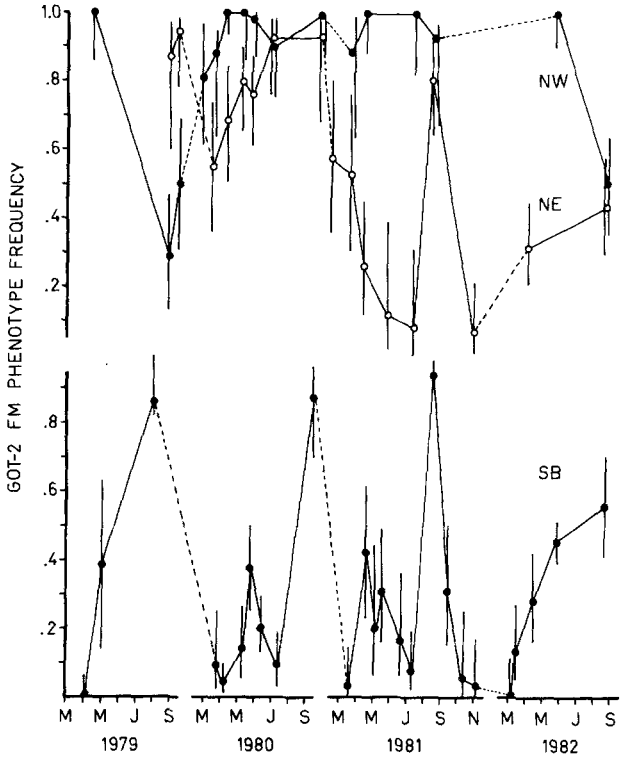

Fig. 5. *Enteromorpha linza.* Seasonal variation in the frequency of the GOT-2 FM phenotype at the NW, NE and SB localities for 1979, 1980, 1981 and 1982. Vertical bars are 95% confidence intervals

Table 6. *Enteromorpha linza.* Analysis of variance for growth rate between Clones 1 and 9 at two salinities (4 and 28‰S)

Source of variation	DF	Mean square	F	*p*
Clone (C)	1	.0312	9.05	<0.05
Individuals within clones [C (I)]	4	.0035	57.50	<0.001
Salinity (S)	1	.0335	17.70	<0.05
C×S	1	.0017	0.91	NS
C (I)×S	4	.0019	29.38	<0.001
Error	12	.00006		

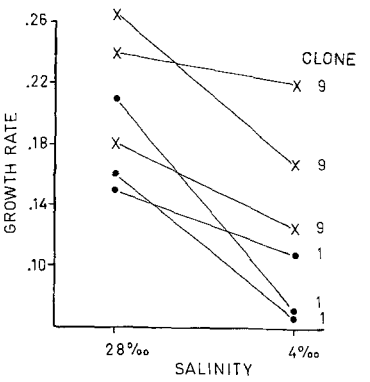

Fig. 6. *Enteromorpha linza.* Growth rate for three individuals each of Clones 1 and 9 grown at two salinities. Each value is the mean of two replicates (see Materials and methods). Growth rate is the regression coefficient (b) for log transformed lengths measured after 0, 2, 4 and 6 d growth (see Materials and methods). Data analyzed in Table 6

quency of Clones 1, 2 and 3 (Table 4). The salinity of the remaining localities ranged from 19 to 30‰ S and were characterized by a high frequency of Clones 1 or 2 (Table 4). Laboratory experiments showed that at 4‰ S, Clone 9 individuals had a higher growth rate than Clone 1 individuals (Fig. 6). Both clones increased their growth rate at 28‰ S, with two of the three Clone 9 individuals showing the highest growth rate at both salinities (Fig. 6). All sources of variation were significant except the clone×salinity interaction (Table 6).

Discussion

The genetic structure of populations of *Enteromorpha linza* in Long Island Sound was similar to that found for other asexually reproducing organisms (Levin and Crepet, 1973; Solbrig and Simpson, 1974; Levin, 1975; Shick and Lamb, 1977; Black and Johnson, 1979; Ellstrand and Levin, 1982; Hebert and Crease, 1983). A lower level of genotypic diversity than expected for sexually reproducing species was found within the sampled localities along with marked differentiation among localities, even between localities separated by less than a few hundred meters. This was unexpected since these localities are connected by water with no obvious barriers to dispersal. It is possible that spore dispersal in *E. linza* is extremely limited, as found for some other species of benthic algae (Anderson and North, 1966; Dayton, 1973; Paine, 1979). However, this seems unlikely, since spores were observed being shed into the water column in the field and were free-swimming for several hours or more in the laboratory (personal observation). Some evidence of the colonizing ability of this species was observed at the SH locality where rocks had been added to the water to extend the existing breakwater. After a few months this substrate was covered with a dense population of *E. linza*. A likely source of spores was from a large population on the original breakwater, a few hundred meters away, since the same clones were found on both parts of the breakwater. Colonization of introduced substrate was also observed at the SB locality (unpublished

data). This does not prove that *E. linza* spores are highly dispersed, but it does show that colonization by spores can occur in nature and it is probable that these spores can travel at least a few hundred meters.

The differentiation observed between the localities at the eastern end of Long Island Sound and localities further west could be due to the large distance separating these areas. However, one of the eastern localities (NG) was genetically distinct from nearby localities and was genetically similar to some of the localities in the western end of the Sound. One possible explanation for this observed pattern of genetic differentiation is the association of clones with patchily distributed environmental factors. The distribution of some clones appeared to be associated with habitat salinity or environmental factors correlated with habitat salinity. Low salinity areas in Long Island Sound occur in small patches where freshwater creeks or seepages flow into the Sound. The localities situated on these low-salinity patches were found to be genetically similar. Clone 9 was restricted to these areas and Clones 1, 2 and 3 were usually absent. The low salinity localities were situated adjacent to high salinity areas with tidal currents providing opportunities for the exchange of spores between the differentiated areas. Maintenance of this differentiation suggests that some clones may be adversely affected by the different salinity conditions or other environmental factors associated with these areas. Evidence for genetically based adaptations to different salinity conditions has been found for *Enteromorpha intestinalis* from areas differing in salinity (Reed and Russell, 1979).

The laboratory experiments comparing growth of Clone 1 from a high salinity area and Clone 9 from a low salinity area revealed growth-rate differences between the two clones but no interaction between clone type and salinity condition. Both clones showed decreased growth at 4‰ S, with growth significantly inhibited for Clone 1. At 28‰ S, both clones showed increased growth, with Clone 9 growing faster than Clone 1 under these conditions. These results suggest that the absence of Clone 1 from low salinity patches could be partially explained by the adverse effect of low salinity on growth. However, reduced growth may be due to the dilution of specific nutrients or growth factors since both clones showed a decreased growth at 4‰ S. The absence of Clone 9 from high salinity areas cannot be explained by any adverse effects of high salinity, at least during the early stages of growth. It is possible that high salinity may limit the long-term survival in this clone, since the laboratory experiments examined only the first few days of growth. Other environmental and biological factors need to be considered to explain the absence of specific clones from different salinity habitats.

The present study documents significant differentiation among individuals of *Enteromorpha linza* separated by very short distances. Although environmental variables such as salinity may contribute to this differentiation, other factors such as competitive interactions among clones and limited recruitment could also be important. Species of *Enteromorpha* have been classified as annuals since they are usually absent during the winter (Lubchenco, 1978; Mathieson, 1979). However, it is possible that these species overwinter as microscopic holdfasts which regenerate new plants the following spring. This appears to occur in species of red algae where individuals die back to a few cells which regenerate a new thallus in the spring (Dixon, 1965). If most of the available substrate is occupied by perennial individuals with long life-spans, then recruitment would be severely limited and the genetic composition of a given year would remain relatively stable. The temporal variation observed at some localities could be explained by the presence of microscopic holdfasts of several clones and a seasonal dependence for the regeneration of new macroscopic plants by each clone. This process could explain the regular seasonal trends observed at the SB locality with Clone 6 stimulated to regenerate during the warmer summer months. Some evidence for this process was observed during preliminary attempts to monitor recruitment at the SB locality. Artificial substrate was introduced in the early spring when Clone 2 was predominant. Samples taken several weeks later showed that this was the only clone that had settled on this substrate. However, samples from rocks around the introduced substrate indicated that the population had shifted to predominantly Clone 6, suggesting that these individuals had regenerated from microscopic holdfasts rather than newly settled spores. Shifts in clonal frequency could also be due to some recruitment and the clonal composition of the area resulting from competitive interactions among clones. The distribution of Clone 9 may be due to its competitive inferiority to other clones, except under low salinity conditions. Studies on the genetic composition of the spore population in the water column and the longevity of individuals in the population would clarify the relationship between potential and actual recruitment of new clones to a given area and determine the origin of individuals initiating the populations in the early spring. Overwintering individuals in the subtidal may be producing spores which colonize the intertidal zone during the spring.

No single process can explain the pattern of distribution of clones of *Enteromorpha linza* in Long Island Sound. Variation in environmental factors such as habitat salinity are potentially important selective forces influencing clonal distributions. The results of this study show that environmental factors other than habitat salinity need to be considered as possible explanations for the marked differentiation observed between different salinity habitats. Some of these factors may include microgeographic variation in nutrient conditions in the water. In addition, limitations on recruitment could maintain stable microgeographic differentiation despite the exchange of spores between differentiated areas. Detailed studies on the demographic genetics of *E. linza* will be necessary to assess the relative importance of recruitment and mortality on the rate of turnover of clones in the intertidal populations. The present study has shown that differentiation over

short distances of a few hundred meters can be as great as differentiation over several to tens of kilometers. This suggests that factors operating on a microgeographic scale probably have a dominant influence on the genetic structure of *E. linza*.

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