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Clonal diversity in introduced populations of an Asian sea anemone in North America

Joy H. Ting[1,2] & Jonathan B. Geller[3,*]

[1] *Department of Biological Sciences, Center for Marine Science Research, University of North Carolina, Wilmington, NC 28403, USA;* [2] *Present address: Department of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332-0230, USA;* [3] *Present address: Moss Landing Marine Laboratories, P.O. Box 450, Moss Landing, CA 95039, USA;* [*] *Author for correspondence (e-mail: geller@mlml.calstate.edu; fax: +1-831-632-4436)*

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Abstract

Previous reports hypothesized that introduced populations of the Asian sea anemone *Diadumene lineata* (Verill, 1870) which reproduces by fission, are often monoclonal or to be composed of few clones. To test this hypothesis, sea anemones were collected from thirteen sites in three non-native regions and one native region: Chesapeake Bay, New England, central California, and Japan. The internal transcribed spacer (ITS) region separating nuclear ribosomal RNA genes was amplified from each individual using PCR and surveyed for DNA sequence variation using single strand conformational polymorphism analysis (SSCP). Fifty-six distinct electrophoretic banding patterns were found in 268 anemones, and each pattern was considered a different genotype. The number of genotypes in a population ranged from one to thirteen. Only one sample (York River, Chesapeake Bay, $n = 10$) was monoclonal, although six populations were dominated (>50%) by single genotype. Only four genotypes were found in more than one population, and these were confined to single regions. Walker Creek, California was sampled in 1995 and 1997 and no genotypes were found in both years, suggesting rapid shifts in genotype frequency. We conclude that multiple genotypes of *D. lineata* have invaded North America and that the primary importance of clonal growth for introduced populations is the production of colonizing propagules.

Introduction

Coastal ecosystems throughout the world are being rapidly invaded by hundreds of nonindigenous species (e.g., Carlton 1989; Carlton and Geller 1993; Cohen and Carlton 1998). Recent marine invasions are most often a consequence of the transport of larvae or adults in ballast water (Carlton and Geller 1993). Ballast water transport is unselective of organisms with regard to life history and ecological characteristics except that some life stage must be found in the water column to be entrained into ballast tanks (Carlton and Geller 1993). In contrast, invasion success is likely to depend on these characteristics, although the contribution of specific traits to invasion success are not well known (Lodge 1993). The ability to reproduce asexually may confer several advantages. For example, asexual reproduction can allow population growth even when individuals are too dispersed for successful mating or external fertilization (a condition sometimes referred to as the Allee effect). In such situations, asexual reproduction may also generate dispersing propagules that allow the persistence of a metapopulation of invaders even when local populations frequently become extinct (Shick et al. 1979). In both these cases, populations might consist of monoclonal stands of genetically identical

individuals. These considerations apply equally well to native species, but the prevalence of clonal taxa among marine introduced species (Cohen and Carlton 1996; Coles et al. 1997; Ruiz et al. 1996) suggests a specific role for asexual reproduction for invasion success. In this study, we ask whether populations of *Diadumene lineata* (Verill 1869), a species of sea anemone with clonal growth, are often monoclonal.

Diadumene lineata was described by Verill (1870) in 1869 from Hong Kong as *Sagartia lineata*, and again from Massachusetts as *S. luciae* (Verill, 1898) as an inadvertent synonym. *S. luciae* was renamed *D. luciae* by Stephenson (1925) and *Haliplanella luciae* by Hand (1955). Accepting Stephenson's genus assignment and recognizing the original species description, this species is properly named *D. lineata* (Hand 1989). This sea anemone is thought to be native to Japan (McMurrich 1921; Uchida 1932; Stephenson 1935) but has been reported from the coasts of the Asian Pacific, New Zealand, Brazil, North America, and Europe (Verill 1870; Omori 1895; Parker 1902; Walton 1908; Davis 1919; McMurrich 1921; Pax 1921; Uchida 1932, 1936; Ricketts and Calvin 1939; Carlgren 1952; Hand 1955; Amanieu 1967; Kiener 1971; Perkins and Larson 1975; da Costa-Belem and da Cruz Monteiro 1977; Shick and Lamb 1977; Dunn 1982; see also Carlton 1979). *D. lineata* is a dioecious broadcast spawner that also grows clonally by longitudinal fission and occasionally by pedal laceration (Atoda 1973, 1976; Minasian 1976, 1979, 1982; Johnson and Shick 1977). Sexual reproduction in native populations implies that those populations consist of multiple clones (Atoda 1973, 1976; Fukui 1991). In contrast, two lines of evidence suggest that introduced populations are often monoclonal.

First, previously described introduced populations were composed of individuals of a single sex. Shick (1976) examined 213 specimens in a population at Blue Hill Falls, Maine, and found all to be male. R.B. Williams (cited in Shick 1976) found only males in Wells, England and only females in Plymouth, England. Exclusively female populations were found in Selangor, Malaysia (Dunn 1982). In fact, no mixed sex populations have been explicitly reported in non-native regions. Second, Shick (1976) found fixed heterozygosity, indicating a lack of recombination, in the Maine population mentioned above, and Shick and Lamb (1977) found a fixed heterozygosity at two loci in a Barnstable, Massachusetts population. Two of the four populations studied by Shick and Lamb (1977) were composed of a single genotype.

Following from the above observations, we hypothesize that the majority of North American populations of *D. lineata* are monoclonal. The null hypothesis therefore is that a majority of populations are polyclonal. Thus, we can reject the null hypothesis if $<50\%$ of North American sites contain >1 clone. A clone of *D. lineata* is defined as all polyps descended by fission events from a single polyp which resulted from one zygote. We assume that somatic mutations are unlikely to segregate in sister polyps, thus anemones that have different genotypes cannot be clonemates. In this study, we shall also assume that anemones that have the same genotype belong to a single clone. This assumption is permissive because it biases us towards rejection of the null hypothesis. This is because no method of genotyping, short of full genome sequencing, can detect all genetic variation between genomes. On the other hand, if the null hypothesis survives this bias, the hypothesis of monoclonality is easily rejected. We used the single strand conformational polymorphism (SSCP) assay (Orita et al. 1989) to detect variation in the internal transcribed spacer (ITS) region which is located between tandemly arranged ribosomal genes (Hillis and Dixon 1991). SSCP assays are based on electrophoretic mobility shifts due to sequence-dependent folding differences in single stranded molecules of equal length (Orita et al. 1989; Lessa and Applebaum 1993).

Materials and methods

Collection of samples

Anemones were collected from populations on the east and west coasts of North America and from Japan. Anemones were kept alive until processed, or were minced and stored individually in 70% ethanol or DNE (20% DMSO, 250 mm EDTA, NaCl to saturation) until processed. Except for samples taken from Walker Creek in 1997 and Chesapeake Bay, collections were haphazard within the sites described below and individuals were separated by 1 cm to 10 m.

From eastern North America, three locations were sampled in 1996 within the Chesapeake Bay area near Norfolk, Virginia (Mobjack Bay, the York River, and Lynnhaven Bay). These samples were taken from four

10 × 10 cm settlement plates suspended from the water surface that were submerged at each site for one year. The Mobjack Bay location was further sampled at four separate sites spaced at approximately 1.6 km intervals. A second eastern location was Mystic, Connecticut, where anemones were collected from a floating dock in 1996.

From western North America, anemones were collected in California at Tomales Bay and San Francisco Bay. In Tomales Bay, samples were taken from two locations: *Salicornia* marshes near Millerton (1995) and Walker Creek (sampled twice, in 1995 and in 1997). In 1997 at Walker Creek, anemones were collected from three 30 × 50 cm quadrats at 2 m intervals along a transect in a tidal channel in a *Salicornia* marsh and in a fourth quadrat 100 m distant from the others. Within a quadrat, anemones on the same pebble or within 2.5 cm of each other were recorded as belonging to a group. Not all anemones in a quadrat were taken. Samples were also taken from Bodega Bay, a distance of about 15 km away from Walker Creek.

Anemones were collected from four locations in San Francisco Bay. Berkeley Marina (floating docks) and Hayward Marsh (*Salicornia* marsh) were sampled in 1996, while Emeryville (mud covered rock), and Redwood City (floating docks) were sampled in 1995. Samples were also taken from small rocks (10–20 cm) in intertidal sandy mudflats in Tokyo Bay and Hamanako Bay in Japan in 1996.

Isolation of DNA

Total nucleic acids were isolated from whole or partial individuals (approximately 0.1 g) of *D. lineata*. Tissues were homogenized in 500 µl 1× CTAB extraction buffer [50 mM Tris–HCl (pH 8.0), 0.7 m NaCl, 10 mM EDTA, 1% CTAB (hexadecyltrimethylammonium bromide), 0.1% 2-mercaptoethanol] and incubated 3–16 h in a 56 °C water bath. Homogenates were extracted two or three times with equal volumes of chloroform : isoamyl alcohol (24 : 1) and nucleic acids were precipitated with 1/10 volume 3 m sodium acetate and 2 volumes cold 100% EtOH. Precipitated nucleic acids were kept at −20 °C for 1 h to overnight, then centrifuged at 12,000 rpm for 10 min to pellet the DNA. Pellets were washed with 300 µl 70% EtOH, then dried under heat and vacuum. Pellets were resuspended in 100–500 µl TE (10 mm Tris and 0.1 mm EDTA, pH 8.0) and stored at −70 °C until used.

PCR and restriction digestion

The entire ITS region of ribosomal DNA was amplified with the polymerase chain reaction (PCR) using the primers 28-5 (5′-GAT TAC GTC CCT GCC CTT TGT ACA CAC CGC-3′) and D1-3 (5′-CTT TCC CTC ATG GTA CTT GTT TGC TAT CG-3′) designed by R. Rowan (pers. comm.). These primers take advantage of the conserved 3′ end of the 18S rRNA gene and the 5′ end of the 28S rRNA gene that flank the ITS region. PCR mixes [10 mm Tris (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% Triton-X 100, 0.01% gelatin, 0.01% Nonidet P-40, 200 µm of each dNTP, 0.3 units *Taq* DNA polymerase, and 25 pmole of each primer] contained 0.5 µl of genomic DNA as template in a 25 µl volume. The PCR profile consisted of an initial 94 °C melting step for 2 min, and thirty cycles of 98 °C for 10 s, 56 °C for 30 s, 72 °C for 2 min. Reactions were checked for successful amplification on 1.2% agarose gels stained with ethidium bromide. Ten microliters of each product were digested with the endonuclease *Hinf*I (New England Biolabs, Beverly, MA). Reactions were incubated at 37 °C for 3–16 h and checked for complete digestion on a 1.2% agarose gel stained with ethidium bromide, then were used in SSCP analysis.

Several alterations to the standard amplification procedure described above were made to test the consistency of SSCP patterns. DNA from one sample (individual 4) from Bodega Bay was amplified over a range of template concentrations (525, 262.5, 131.25, 26.25, and 13.125 ng), primer concentrations (4, 6, 8, and 20 µm), and extension times of 2, 4, and 10 minutes (using 262.5 ng of DNA and 2 µm of each primer). Individuals 4–8 from Bodega Bay were amplified twice under standard conditions, as well as once with 4 and 10 min extension times.

SSCP and silver staining

One-fifth (3 µl) of the restriction digest was mixed with 1.8 µl denaturing loading dye (95% formamide, 0.5% bromophenol blue, 0.5% xylene cyanol) and heated to 95 °C for 5 min to denature the double stranded DNA products. After heating, reactions were snap cooled on ice and 2 µl were loaded onto a 45 × 35 cm × 0.4 mm MDE polyacrylamide gel (AT Biochem, Mavern, Pennsylvania). Single stranded DNA was electrophoresed for 14 h at 6 W in 0.6× TBE buffer (Tris Boric Acid, EDTA). Silver staining was

carried out according to the instructions of the kit's manufacturer (Promega, Madison, WI) except that the developing solution was cooled to 12–14 °C rather than 10 °C and we used an additional post-staining water wash. After staining, gels were air dried and photographed with Silver Sequence automatic processor film (Promega).

Analysis of SSCP patterns

Genotypes were assigned based on the presence or absence of bands in the profile. Any individuals with the same banding profile were assigned the same genotype. Genotypes were named alphabetically in order of discovery. A representative of every unique genotype from each population except Walker Creek (1997) and Bodega Bay was run on a single gel to facilitate comparisons and to determine if populations shared genotypes. Simpson's indices of dominance and diversity for nonrandom samples (Brower et al. 1990):

$$\lambda = \frac{\sum n_i^2}{N^2} \quad (1)$$

$$\Delta_s = 1 - \lambda \quad (2)$$

were used to determine clonal diversity and clonal dominance within each sample, where N is the total number of individuals in a sample and n_i is the abundance of the ith genotype in the sample. This measure of dominance (λ) represents the probability that two individuals sampled from a single population will have the same genotype. The diversity measure (Δ_s) is the probability of sampling two individuals of a different genotype. We tested heterogeneity of genotype distribution within and between quadrats at Walker Creek (1997) using GENEPOP 3.1, which performs the equivalent of Fisher's exact G test (Raymond and Rousset 1995).

Results

PCR amplification of the ITS region yielded a single 1250 base pair product. Restriction digestion of PCR products with *Hinf*I yielded co-migrating fragments of 175 and 300 base pairs in length, within the 100–400 base pair range best resolved by SSCP. Each individual showed a profile of 9–11 visible bands under SSCP conditions (jpeg gel image files are available from the second author). Amplification of the same individual several times followed by SSCP yielded identical results regardless of primer concentration and template DNA concentration. Increased elongation time in PCR produced two new, longer amplification products in all tested individuals, but no new variants were detected nor were any genotype assignments changed following SSCP. Thus, this assay was robust and results were repeatable despite deliberate variation of quantities and timing that exceeded the experimental error expected to arise during DNA extraction, amplification, and SSCP.

Although sampling effort was comparable among populations (with the exception of settling plates and quadrats for which sampling was quite different), it cannot be assumed that genotypic diversity was similarly sampled in each population. For example, spatial relationships of clones may be influenced by habitat type, which differed considerably among sites. Our estimates of clonal diversity express evenness of clone frequencies within samples, and do not necessarily reflect patterns of diversity in entire populations or regions. Despite this limitation, the hypothesis that most populations were monoclonal was refuted by the presence of >1 genotype in twelve of the thirteen samples.

Eastern North America

The York River sample was monoclonal ($\Delta_s = 0.0$, $n = 10$). Only two anemones were recovered from Lynnhaven, each a different genotype. Mobjack Bay contained 13 genotypes and showed the highest diversity of all sites sampled ($\Delta_s = 0.85$, $n = 23$). No genotypes were found in more than one site within Mobjack Bay, but one genotype (AO) was found in both the York River and at Lynnhaven (Table 2). In two of the three locations in the Chesapeake Bay, more than 50% of the individuals belonged to a single genotype. Two genotypes were found in the sample from Mystic, CT ($\Delta_s = 0.39$, $n = 15$).

Western North America

The Walker Creek (1995) sample from Tomales Bay contained 3 genotypes and $\Delta_s = 0.59$. The Walker Creek (1997) sample contained 9 genotypes with a $\Delta_s = 0.71$, which was the highest value in west coast populations (Table 1). No genotypes were found in both the 1995 and 1997 collections at Walker Creek even though the same general region was sampled. The 1997 sample was made in four quadrats on a transect, with anemones collected from pebbles at or just below the mud surface. Pebbles (which were

Table 1. Numbers of genotypically distinct clones of *D. lineata* detected by SSCP analysis of the PCR-amplified ribosomal ITS region from two native and 11 introduced populations. λ and Δ_s are Simpson's indices of dominance and diversity (see text).

Regions	Populations of genotypes n	Number	λ	Δ_s	
Chesapeake Bay, Virginia		35	14	0.16	0.84
	Mobjack Bay	23	13	0.15	0.85
	York River	10	1	1	0
	Lynnhaven	2	2	0.5	0.5
New England					
	Mystic	15	2	0.61	0.39
Tomales Bay and Bodega Bay, California		100	17	0.12	0.88
	Walker Creek (1995)	19	3	0.41	0.59
	Walker Creek (1997)	52	9	0.29	0.71
	Millerton	20	5	0.41	0.59
	Bodega Harbor	9	3	0.43	0.57
San Francisco Bay, California		97	16	0.2	0.8
	Berkeley	25	5	0.59	0.41
	Hayward Marsh	26	2	0.93	0.07
	Emeryville	23	4	0.44	0.56
	Redwood City	23	7	0.33	0.67
Japan		21	7	0.26	0.74
	Tokyo Bay	13	4	0.44	0.56
	Hamanako Bay	8	3	0.59	0.41
Total		268	56		

oblong and *ca* 3 cm in longest dimension) held 1–4 anemones. There was no evidence for a nonrandom distribution of genotypes to pebbles within quadrats (G tests, Quadrat 1: $P = 0.23$ n.s.; Quadrat 2: $P = 0.12$ n.s.; Quadrat 3: $P = 0.78$ n.s.), but the distribution of genotypes among quadrats was significantly nonrandom (G test, $^{***}P < 0.0001$). For example, three of four quadrats were dominated (i.e., nearly 50% of individuals) by a single genotype which was different for each quadrat. Likewise, when anemones on pebbles were considered regardless of the quadrat in which they occurred, the distribution of genotypes was significantly nonrandom (G test, $^{***}P < 0.0001$). These results suggest that clones were localized along the transect but were not segregated within quadrats.

Genotypic diversity in other west coast populations ranged from a low of $\Delta_s = 0.07$ at Hayward Marsh ($n = 26$) to a high of $\Delta_s = 0.67$ at Redwood City ($n = 23$) (Table 2). In all regions, only the monoclonal York River had a diversity index lower than Hayward Marsh, in which one genotype represented 96% of the population. Three genotypes were found in the population at Bodega Harbor ($\Delta_s = 0.57$, $n = 9$).

Japan

Four genotypes were found in Tokyo Bay ($\Delta_s = 0.56$, $n = 13$) and three genotypes were found in Hamanako Bay ($\Delta_s = 0.41$, $n = 8$). No genotypes were found in both samples (Table 2).

Widespread genotypes within regions

Most genotypes were found only in a single population, but four genotypes were found in more than one geographic location within a region (Table 2). Two genotypes (Ah and Ai) were found in both the Walker Creek and Bodega Bay populations, which were separated by a distance of about 15 km. Genotype AO, the only genotype found in the York River, was also found at Lynnhaven, 48 km distant. In San Francisco Bay, one genotype (C) was shared by populations in

Table 2. Identification of unique clones (with numbers in parentheses) of *D. lineata* detected by SSCP. Clones were named alphabetically in order of discovery (A–Z, AA–AZ, Aa–Ai). Sites in Mobjack Bay represent totals from four settlement plates. Data from Walker Creek (1997) are pooled from four quadrats.

Region	Population	Genotype (n)
Chesapeake Bay (Virginia)	Mobjack Bay	
	Site WR	AB(1), AC(1), AD(7), AE(1), AF(1)
	Site MO	AG(3), AH(1), AI(1)
	Site GD	AJ(1), AK(1), AC(1)
	Site ZM	AM(1), AN(3)
	York River	AO(10)
	Lynnhaven	AO(1), AP(1)
Tomales Bay and Bodega Bay, California	Walker Creek 1997	Aa(1), Ab(1), Ac(21), Ad(1), Ae(17), Af(5) Ag(1), Ah(4), Ai(1)
	Walker Creek 1995	S(2), U(8), V(9)
	Millerton	W(3), X(1), Y(12), Z(3), AA(1)
	Bodega Bay	Ai(5), Af(3), Ah(1)
San Fancisco Bay, California	Berkeley Marina	A(1), B(1), C(2), D(2), E(19)
	Hayward Marsh	G(1), H(25)
	Emeryville	C(14), I(1), J(3), K(5)
	Redwood City	C(12), M(3), N(1), O(1), P(1), Q(1), R(4)
New England	Mystic, CT	AX(11), AY(4)
Japan	Tokyo Bay	AQ(1), AR(3), AS(1), AT(8)
	Hamanako Bay	AU(6), AV(1), AW(1)

Redwood City, Emeryville and Berkeley Marina, spanning a distance of 43 km. This genotype was absent in other locations within San Francisco Bay, as well as those in nearby Tomales Bay and Bodega Bay.

Discussion and conclusion

As in other recent studies of natural populations (Bagley et al. 1997; Orti et al. 1997; Li and Hedgecock 1998), SSCP of PCR products was a sensitive and robust method to detect genetic variation. Fifty-six different ITS genotypes were found in a total of 268 anemones assayed with SSCP. This number may be an underestimate of clonal diversity for two reasons. First, SSCP may not have revealed all sequence differences that were present despite its sensitivity. The SSCP method has been thoroughly investigated, and reports agree that about 90% of known mutations in DNA molecules are detected (e.g., Orita et al. 1989; Broly et al. 1995; Jensen et al. 1996; Spire-Vayron del la Moureyre et al. 1998; Gross et al. 1999), leaving 10% undetected. Second, members of different clones could potentially have the same ITS genotype. Furthermore, our sampling of each site was not exhaustive, therefore members of additional clones may not have been collected. It should be emphasized that an underestimate of clonal diversity biases results against the null hypothesis of frequent multiclonal populations which was nonetheless accepted.

The high number of ITS genotypes detected in our study contrasts the few other reports of ITS in other cnidarians (Beauchamp and Powers 1996; Chen and Miller 1996), prompting one reviewer to question whether different SSCP electromorphs in this study actually correspond to different sequences. The theoretical basis for SSCP precludes multiple electromorphs from single sequences under identical electrophoretic conditions (Nataraj et al. 1999). Impirical investigations of the SSCP method have never, to our knowledge, revealed the same DNA molecule under constant electrophoretic conditions to give different banding patterns nor different bands to have the same sequences (Orita et al. 1989; Broly et al. 1995; Jensen et al. 1996; Spire-Vayron del la Moureyre et al. 1998; Gross et al. 1999; Kutach et al. 1999). Thus, if the number of genotypes was overestimated, a more likely cause would be PCR artifacts wherein different loci were amplified in clonemates. This is unlikely for two reasons. First, we used gene-specific primers that were robust to PCR conditions and produced amplicons of a single size. Second, we digested all PCR products

Table 3. Distribution of clones (with numbers in parentheses) of *D. lineata* found on pebbles in quadrats at Walker Creek (1997) in Tomales Bay, California. Quadrats 1–3 were separated by 2 m intervals along a transect, while quadrat 4 was 100 m removed. Anemones on different were not counted separately in quadrat 4.

Quadrat	Pebble	Genotype
1	1	Aa(1), Ab(1), Ac(2)
	2	Ad(1)
	3	Ae(3)
	4	(Ae(4)
	5	Ae(2), Ac(1)
	6	Ac(1), Ae(1)
	7	Ae(1)
	8	Ac(1), Ae(1)
	9	Ae(2)
	10	Ac(1), Ae(1)
2	1	Af(1)
	2	Af(1), Ac(1)
	3	Ac(1)
	4	Ac(3)
	5	Ag(1)
	6	Ae(1)
3	1	Ae(1)
	2	Ah(1)
	3	Af(1)
	4	Af(1), Ac(1)
	5	Ae(1), Af(1), Ac(1)
	6	Ah(2), Ac(1)
4		Ac(6), Ah(1), Ai(1)

with *Hinf*I and detected a conserved restriction site in all amplicons, indicating homology of products. ITS is found in multiple copies in eukaryotic genomes, and copies are usually identical due to concerted evolution, a process in which copies are first homogenized within individuals and then within sexual populations (Li 1997). However, exceptions exist (Buckler et al. 1997), and it is not known whether ITS copies in individuals of *D. lineata* are homogeneous. If different copies of ITS were present in individuals, clonemates should produce identical, multilocus SSCP banding profiles barring other PCR artifacts (e.g., differential amplification of different copies in different samples of clonemates). Our manipulations of DNA template concentration, the factor most likely to vary among DNA samples isolated from clonemates, in PCR reactions did not alter SSCP results. We therefore conclude that our SSCP patterns indicate genotypic differences between individuals, and suggest that a more comprehensive survey of ITS variation in cnidarians is necessary to fully understand concerted evolution in these clonal organisms.

Most populations were characterized by multiple genotypes seen in no other regions. This result contrasts with previous reports of populations inferred to be monoclonal because of unisexuality, uniformity of color patterns, or genetic markers (Shick and Lamb 1977). Furthermore, regional diversity increased as more populations were added into the estimate. For example, San Francisco Bay had a Δ_s index of 0.80, Tomales Bay had a Δ_s index of 0.88 and Chesapeake Bay had a Δ_s index of 0.84. Of 56 genotypes identified in 14 populations, only 4 were found in more than one population, with no greater than 43 km separating individuals sharing a genotype. This seems to suggest multiple introductions, which is consistent with known high levels of invasion in these regions (Cohen and Carlton 1996; Ruiz et al. 1996). Contrary to our hypothesis, populations were not monoclonal and separated by distances that would preclude sexual reproduction. Instead, with one exception, our samples were composed of several clones that were intermixed even at the smallest spatial scales (on single pebbles or <2.5 cm). This is consistent with the finding of two genotypes on a single shell by Shick and Lamb (1977) (illustrated in Shick 1991). Fighting or catch tentacles (elongated oral tentacles used in aggressive interactions with conspecific individuals) have been reported for *D. lineata* (Williams 1975; Shick and Lamb 1977) but these data do not suggest that aggressive behaviors, if present, are associated with clonal aggregation as seen in other actiniarians (Purcell 1977; Purcell and Kitting 1982; Ayre 1982; Francis 1988).

Only one population, in the York River (Chesapeake Bay) appeared to be monoclonal. This sample was small (only 10 individuals), and all but one individual were found on the same settling plate. Monoclonality in this case was most likely due to a single settler on a very restricted substratum. Two of the samples, from Mystic and Hayward Marsh, contained only two genotypes and showed the lowest nonzero Δ_s values (0.391 and 0.074). This contrasts a mean of 5 genotypes in other populations in which an equal or lesser number of individuals were sampled. Hayward Marsh was nearly monoclonal; only a single individual was not in the dominant clone. The minority genotype was represented by 4 individuals at Mystic. Most populations were determined to be polyclonal even when sampling was not extensive.

Samples taken at Walker Creek two years apart revealed no overlap in genotypic composition. Three alternative explanations can be suggested. First, because we found differences in genotypes over short distances at Walker Creek in 1997, this difference might reflect spatial variation and not temporal variation. Second, genotypes frequencies might fluctuate over time such that rare genotypes, undetected in one year, may become common while other genotypes decline to levels undetectable by our sampling methods. Rare genotypes would be expected to occasionally decline to extinction, thus this second explanation does not fit with persistently multiclonal populations. Third, the genotypes seen in 1995 may have become extinct and the site may have been colonized by immigrants. However, the paucity of shared genotypes among all sites in this study argues against frequent gene flow. The first explanation is most consistent with both low gene flow and nonoverlapping genotypes at Walker Creek.

The difference in frequency of monoclonal populations (0.5 vs. 0.07) in this and the earlier study by Shick and Lamb (1977) is large and remains unexplained. The difference may simply be an effect of sample size, as only four sites were examined in the earlier study. Alternatively, it may be pertinent that both of the monoclonal populations described by Shick and Lamb (1977) were in the northeast of North America (Blue Hills Falls, Maine and Barnstable, Massachusetts) where *D. lineata* populations may be at the limits of its environmental tolerances. There are data to suggest that many non-native populations of *D. lineata* have suffered extinction (Parker 1902, 1918; Hausman 1919; Stephenson 1935; Williams 1973; Shick 1976). Where extinction rate is high and colonization occurs infrequently, populations might often consist of the asexual progeny of a single colonist or group of clonemates, as suggested for Blue Hills Falls (Shick and Lamb 1977). We hypothesize that the major difference between monoclonal and polyclonal populations is frequency of population bottlenecks or extinction. Future studies should focus on rates of extinction and colonization.

Because most populations were not monoclonal, we conclude that Allee effects are not of critical importance in present day North American *D. lineata* populations (assuming that multiclonal populations are not also all single sexed). The avoidance of Allee effects is only one possible advantage of clonal growth (Bell 1982; Hughes 1989; Barton and Charlesworth 1998), and single clones in fact dominated many of our samples. If the first groups of invaders in North America, over one century ago, were smaller, more dispersed, and had lower clonal diversity than modern populations, asexual reproduction may have been important for their growth and persistence. If clonal growth is important today for the success of *D. lineata* as an introduced species in North America, its role may be the vegetative production of propagules for dispersal which might prevent regional extinction despite potentially high rates of local extinction. For example, *D. lineata* polyps may disperse by rafting on floating material or on the hulls of ships where they are often observed (Gollasch and Riemann-Zuerneck 1996; Geller, pers. obs., R. Grosberg, pers. comm.).

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