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Genetic structure of local populations and divergence between growth forms in a clonal invertebrate, the Caribbean octocoral *Briareum asbestinum*

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Abstract Although the genetic structure of many populations of marine organisms show little deviation from panmixia, in those marine species with limited larval dispersal, patterns of microgeographic genetic differentiation may be common. The octocoral *Briareum asbestinum* should show local population differentiation because colonies reproduce asexually by fragmentation, most matings occur between colonies in very close proximity, and the sexually produced larvae and sperm appear to disperse only short distances. Variability in secondary chemistry of individual *B. asbestinum* colonies from different populations in close proximity also suggests local population differentiation. We determined the genetic composition of local populations by surveying allozyme variation of three shallow and two deep populations within a 300 m^2 area at San Salvador Island, Bahamas and at a site 161 km away on Little San Salvador, Bahamas in July 1990. As *B. asbestinum* occurs as either an erect branching form or an encrusting mat often at the same sites, we sampled both morphs to examine the extent of genetic exchange between them. Five of 21 loci were polymorphic and most populations showed a deficit of heterozygotes. Allele frequencies differed significantly between morphs at each site where they occurred together. The mean genetic distance (D=0.065) between morphs is consistent with the interpretation that the two morphs are genetically isolated. Despite the close spatial proximity of the San Salvador populations, both the branching and encrusting morphs showed significant genetic heterogeneity among neighboring populations. Similarly, pooled allelic frequencies for samples collected from the islands of San Salvador and Little San Salvador differed significantly at 1 locus for the branching morph and at 3 out of 5 loci for the encrusting morph.

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Introduction

Populations of marine organisms are often characterized as genetically "open" because habitats lack obvious subdivision and pelagic larvae with a high potential for dispersal are common among most fishes and invertebrates. However, the actual degree of population subdivision depends upon the mode of reproduction (e.g. sexual or asexual, brooding or broadcast spawning), the amount of time larvae spend in the plankton (Strathmann 1985, but see Wellington and Victor 1989), post-settlement selection (Koehn et al. 1973, 1976, Theisen 1978, Gartner-Kepkay et al. 1983, Ayre 1985, Hedgecock 1986), heterogeneity among larval recruits (Hedgecock et al. 1982, Johnson and Black 1982, 1984) and local hydrographic conditions (Sammarco and Andrews 1988). Mode of reproduction is of particular interest because of its potential to influence genetic differentiation due to both inbreeding and drift. For example, analyses of protein polymorphisms show that asexual propagation in corals and sea anemones such as colony fragmentation (Stoddart 1984 a, Willis and Ayre 1985), fission (Shick and Lamb 1977, Shick et al. 1979, Ayre et al. 1991) and pedal laceration (Hoffmann 1976, 1986, 1987) often produce local populations dominated by one or a few genotypes. Since, for most sessile animals matings occur largely between near neighbors (e.g. Grosberg 1991), low genotypic diversity will promote inbreeding and/or sexual reproductive failure. In addition, while the demographic population size of such assemblages may be large, the effective population size is often much smaller due to the over-representation of individuals from common clones (Stoddart 1984 a, Coffroth et al. 1992).

Population differentiation, due to restricted gene flow among populations, should be prevalent in those marine

species that produce non-feeding larvae which spend only a few minutes to a few hours in the plankton. Population differentiation is especially likely in those organisms that produce larvae with dispersal distances of the same magnitude as gamete dispersal distances. Under such conditions inbreeding becomes likely (Grosberg 1991). Such localized recruitment (Olson 1985, Young 1986, Grosberg 1987, Davis and Butler 1989) and its role in creating microgeographic genetic population structure have been demonstrated in only a few cases (Sabbadin and Graziani 1967, Grosberg 1987).

Consequently, colonial species, which typically have philopatric larvae (Jackson 1986) and often reproduce clonally, should have highly subdivided populations. However, colonial species often have very plastic morphologies that complicate efforts to determine genetic subdivision of populations. Individuals of some species change morphology in response to turbulent environments (de Weerdt 1981) and depth clines (Harvell et al. 1993 b), creating an appearance of subdivision that may not be genetically based. Electrophoresis has previously been used to map the population structure of such clonal species that are highly plastic in form. In the coral *Pavona cactus* (Willis and Ayre 1985, Ayre and Willis 1988), clonal genotypes were distinguished on the basis of morphology because plasticity was demonstrably small. This was not the case with more morphologically plastic species such as *Pocillopora damicornis* (Stoddart 1984 b). In other species, the degree of genetic differentiation between morphological and color variants suggested incipient speciation (McCommas and Lester 1980, Bucklin and Hedgecock 1982, Solé-Cava et al. 1985, Knowlton et al. 1992).

In this paper, we examine the local population genetic structure of the common Caribbean gorgonian *Briareum asbestinum* using allozyme data to examine intra- and inter-population patterns of genetic variability. The extent of microgeographic population differentiation in this species is of particular interest because: (1) the populations are expected to be highly subdivided since adult colonies not only reproduce asexually via fragmentation, but also produce philopatric larvae (Brazeau and Lasker 1990); (2) sclerite and branch morphology (Harvell et al. 1993 b) and defensive chemistry differ significantly among populations (Harvell al. 1993 a), again suggesting local population subdivision; (3) *B. asbestinum* has two distinct morphs that occur together at a number of sites throughout the Caribbean and which are currently described as the same species (Bayer 1961). One morph forms an encrusting mat while the other produces upright branches that may reach 1 m in height.

Materials and methods

Sample collection

We collected samples off the islands of San Salvador (24°03′N; 24°33′W) and Little San Salvador (24°14′N; 25°41′W) Bahamas, in July 1990. The most comprehensive collection was from Bonefish Bay, San Salvador, a broad west-facing bay with scattered patch reefs, adjacent to a sharp dropoff. The scattered, shallow (3 to 6 m) patch reefs are dominated by the encrusting and branching morphs of *Briareum asbestinum*, as well as other more arborescent gorgonian colonies of *Pseudopterogorgia americana*, *P. hummulincki* and *Gorgonia ventalina*. We collected shallow colonies from three contiguous populations, each ≃75 m wide and separated by ≃50 m. Forty colonies (20 of each morph) were collected from each site (here called Shallow 1, 2 and 3). Colonies of the branching morph of *B. asbestinum* often consist of large clumps of 10 or more branches (Brazeau and Lasker 1992 b). Since it is often difficult to determine if the branches in a clump are connected, we collected a single branch tip from each clump of branches, and did not collect more than one sample from clumps closer than 30 cm to each another.

We chose the site at Bonefish Bay because of the proximity of shallow populations of *Briareum asbestinum* to deep populations. The dropoff was ≃300 m seaward from the shallow populations and declined abruptly from 12 m to over 45 m. We sampled two neighboring populations at 30 to 33 m (Deep 1 and 2). The same sampling rules applied. Densities of *B. asbestinum* colonies at the deep sites were lower than at the shallow sites; thus, the 20 colonies sampled at each deep site represented nearly all the colonies present. The encrusting morph of *B. asbestinum* did not occur in deep water.

In addition, we collected 20 samples each of the branching and encrusting morphs from shallow (5 m) patch reefs at Little San Salvador (LSS), an island ≃161 km west of San Salvador. All samples came from a single patch reef, on the wave-exposed north end of the island, where both the branching morph and the encrusting morph were abundant and in close proximity.

Electrophoresis

Samples of 20 colonies of each morph from each site (except the deep sites where only the branching morph occurred) were individually electrophoresed and stained for 14 enzymes: leucine aminopeptidase (LAP: EC 3.4.11.-), 6-phosphogluconate dehydrogenase (6PGDH: EC 1.1.1.44), glycerol-3-phosphate dehydrogenase (αGPDH: EC 1.1.1.8), leucyl-alanine peptidase (PEPL: EC 3.4.11.-) superoxide dismutase (SOD: EC 1.15.1.1), malate dehydrogenase (MDH: EC 1.1.1.37), hexokinase (HK: EC 2.7.1.1), isocitrate dehydrogenase (IDH: EC 1.1.1.42), malic enzyme (ME: EC 1.1.1.40), creatine kinase (CK: EC 2.7.3.2), pyruvate kinase (PK: EC 2.7.1.40), phosphoglucose isomerase (PGI: EC 5.3.1.9), glucose-6-phosphate dehydrogenase (G6PDH: EC 1.1.1.49) and fumarate hydratase (FUM: EC 4.2.1.2). Small sections (≃0.5 cm in length) of individual colonies were coarsely ground with a spatula in 200 µl of grinding buffer [1 m*M* Tris, 1 m*M* EDTA and NADP (4 mg/100 ml), pH 7.0]. Samples were spun in a microcentrifuge for 5 min to pellet the symbiotic zooxanthellae; 10 µl of the supernatant (coral portion) was used to load up to five Titan III cellulose acetate gel plates (Helena Laboratories, Beaumont, Texas). Gels were run at 200 V in a Tris-glycine buffer (25 m*M* Tris, 0.2 *M* glycine, pH 8.5) at room temperature for 15 to 20 min. Gels were stained using an agar overlay and standard staining solutions. Electrophoresis buffers and stain protocols were according to Hebert and Beaton (1989).

Bands visible on the gels were considered to be the products of single enzyme loci if the banding patterns were consistent with the known quaternary structure of the enzyme (Harris and Hopkinson 1976). Alleles were labeled as percent mobility relative to the most common allele. To test departures of genotypic frequencies from Hardy-Weinberg equilibrium we used the χ^2-test comparing expected frequencies corrected for small sample size following Levene's method (1949). Variations in allele frequencies between morphs and among populations were tested using the *G*-test for heterogeneity (Sokal and Rohlf 1981). Means of pooled allele frequencies were used in comparisons between shallow and deep populations and between San Salvador and Little San Salvador populations. In order to reduce the probability of a Type I error due to the multiple statistical tests, significance levels were determined using the sequential Bonferroni procedure (Rice 1989). Levels of genetic differentiation among populations at each locus were also analyzed using Wright's (1978) fixation indices calculated using the BIOSYS-1 program (Release 1.7, Swofford and Selander 1981). Standard genetic distances

(*D*) between populations and morphs were calculated for all relevant pairwise combinations using Nei's (1987) method for small sample sizes.

Results

We estimated genetic variation in *Briareum asbestinum* at five neighboring sites in San Salvador and one site in Little San Salvador, Bahamas. From the 14 enzymes resolved, we detected 21 loci, of which 16 were monomorphic at all sites (LAP-1, LAP-2, 6PGDH, αGPDH, PEPL-1, PEPL-2, PEPL-3, SOD, MDH-1, MDH-2, HK-1, HK-3, IDH, PK, ME-2 and FUM). Five loci were polymorphic in at least some of the populations (CK, G6PDH, HK-2, ME-1 and PGI; Fig. 1). Electrophoresis and staining of the zooxanthellae for these same 14 enzyme systems revealed either no isozyme activity or poorly defined bands that did not correspond to the bands detected from coral extracts. Allele frequencies for the polymorphic loci for each population are given in Table 1. There were significant deviations from Hardy-Weinberg equilibria within some populations for PGI in the branching morph and for CK, G6PDH and HK-2 in the encrusting morph (Table 2). In all cases the deviations were due to heterozygote deficiencies.

Genotypic diversity within populations was high. At four of the sites (branching morph: Shallow 1, Deep 2 and LSS; encrusting morph: Shallow 1), the number of genotypes observed equaled the number of colonies sampled. At all other sites except one (branching morph: Deep 1), the number of genotypes observed was one less than the number of colonies sampled (19 genotypes identified out of 20 colonies at each site). For the branching morph at the Deep 1 site, we found 18 genotypes in the 20 samples. These data indicate that for the populations sampled,clonal (asexual) reproduction contributed little to overall pop-

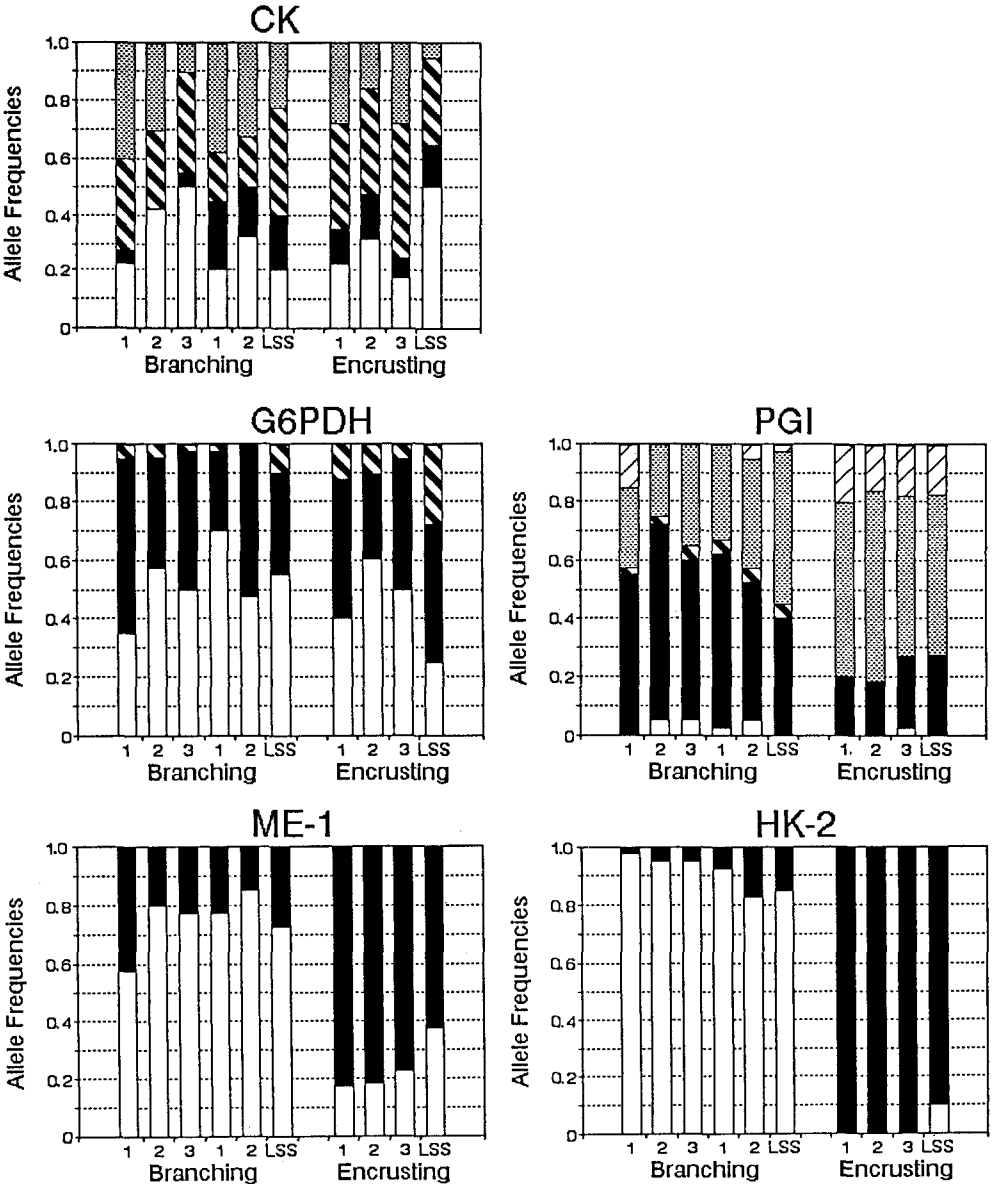

Fig. 1 *Briareum asbestinum.* Allele frequencies of branching and encrusting morphs in the 10 populations sampled: shallow (1, 2, 3) and deep (1, 2) populations at San Salvador, Bahamas, and one shallow population at Little San Salvador, Bahamas (LSS). Each allele frequency is represented by different bar pattern going from least to greatest mobility for each locus. See Table 1 for relative mobilities

Table 1 *Briareum asbestinum.* Allele frequencies of five enzyme encoding loci (monomorphic loci not shown) for shallow and deep populations at San Salvador, Bahamas; LSS: Little San Salvador, Bahamas. (*N*): no. of colonies sampled per site. –: Allele not present

Population	(*N*)	CK alleles:				G6PDH alleles:			HK-2 alleles:		ME-1 alleles		PGI alleles				
		60	70	100	170	100	170	185	100	120	80	100	35	70	90	100	120
Branching morph																	
Shallow 1	(20)	0.22	0.05	0.33	0.40	0.35	0.60	0.05	0.97	0.03	0.57	0.43	–	0.55	0.03	0.27	0.15
Shallow 2	(20)	0.42	–	0.28	0.30	0.58	0.37	0.05	0.95	0.05	0.80	0.20	0.05	0.68	0.03	0.24	–
Shallow 3	(20)	0.50	0.05	0.35	0.10	0.50	0.48	0.02	0.95	0.05	0.78	0.22	0.05	0.55	0.05	0.35	–
Mean shallow	(60)	0.38	0.03	0.32	0.27	0.48	0.48	0.04	0.96	0.04	0.72	0.28	0.03	0.60	0.03	0.29	0.05
Deep 1	(20)	0.20	0.25	0.18	0.37	0.70	0.27	0.03	0.92	0.08	0.78	0.22	0.03	0.60	0.04	0.33	
Deep 2	(20)	0.33	0.17	0.18	0.32	0.48	0.52	–	0.82	01.8	0.85	0.15	0.05	0.48	0.05	0.38	0.04
Mean deep	(40)	0.26	0.21	0.18	0.35	0.59	0.40	0.01	0.87	0.13	0.81	0.19	0.04	0.54	0.04	0.35	0.03
LSS	(20)	0.20	0.20	0.38	0.22	0.55	0.35	0.10	0.85	0.15	0.73	0.27	–	0.40	0.05	0.53	0.02
Mean branching	(120)	0.31	0.12	0.28	0.29	0.53	0.44	0.03	0.91	0.09	0.75	0.25	0.03	0.54	0.04	0.35	0.04
Encrusting morph																	
Shallow 1	(20)	0.21	0.13	0.38	0.28	0.40	0.48	0.12	–	1.00	0.18	0.82	–	0.20	–	0.60	0.20
Shallow 2	(19)	0.32	0.16	0.37	0.15	0.61	0.28	0.11	–	1.00	0.18	0.82	–	0.18	–	0.66	0.16
Shallow 3	(20)	0.18	0.07	0.48	0.27	0.50	0.45	0.05	–	1.00	0.23	0.77	0.03	0.25	–	0.55	0.17
Mean shallow	(59)	0.24	0.12	0.42	0.22	0.50	0.41	0.09	–	1.00	0.19	0.81	0.01	0.21	–	0.60	0.18
LSS	(20)	0.50	0.15	0.30	0.05	0.25	0.48	0.27	0.10	0.90	0.38	0.62	–	0.28	–	0.55	0.17
Mean encrusting	(79)	0.31	0.13	0.38	0.18	0.44	0.42	0.14	0.03	0.97	0.24	0.76	0.01	0.23	–	0.59	0.17

Table 2 *Briareum asbestinum.* Probability values for chi-squared tests for fit to Hardy-Weinberg expectations for shallow and deep populations at San Salvador, Bahamas; LSS: Little San Salvador, Bahamas. Significant deviations determined by sequential Bonferroni test (Rice 1989). Table-wide α levels indicated as $*P<0.05$, $**P<0.01$, $***P<0.001$.
–: fixed for one allele

Popluation	CK (6 *df*)	G6PDH (3 *df*)	HK-2 (1 *df*)	ME-1 (1 *df*)	PGI (10 *df*)
Branching morph					
Shallow 1	0.085	0.343	1.0	0.166	<0.001***
Shallow 2	0.787	0.696	0.869	0.300	0.378
Shallow 3	0.389	0.729	0.862	0.920	<0.001***
Deep 1	0.889	0.861	0.764	0.225	<0.001***
Deep 2	0.211	0.014	0.368	0.254	<0.001***
LSS	0.092	0.128	0.475	0.502	<0.001***
Encrusting morph					
Shallow 1	<0.001***	<0.001**	–	0.386	0.013
Shallow 2	0.028	0.033	–	0.368	0.284
Shallow 3	0.467	<0.001***	–	0.225	0.020
LSS	0.183	0.013	<0.001***	0.764	0.082

ulation structure. Subsequent analyses assumed that colonies were genotypically distinct.

At the four sites where they co-occurred, branching and encrusting morphs showed significant morph-specific differences in allele frequencies at PGI, HK-2, ME-1 and CK (with the exception of Shallow Site 1: Table 3). At HK-2, the two morphs were nearly fixed for different alleles (Fig. 1). The mean (all 21 loci) standardized genetic distance between the branching and encrusting morph is an order of magnitude greater than that observed in comparisons among either the branching or encrusting morphs alone (between morphs, $D=0.065$, 99% CI=0.060 to 0.070, $N=24$ pairwise comparisons; intramorph comparisons: branching, $D=0.0033$, 99% CI=0.002 to 0.005, $N=15$; encrusting, $D=0.0028$, 99% CI=0.0 to 0.006, $N=6$). The morphs are clearly separated by an unweighted pair-group method with arithmetic means (UPGMA) cluster analysis, supporting the conclusion that the two morphs are genetically distinct (Fig. 2).

Six of the populations sampled showed significant heterozygote deficiencies (Table 4). For the other four populations, three had heterozygosities less than those expected, although the difference was not significant. Pooling all 21 loci yielded a mean heterozygosity of 0.091 (0.0017 SD) for the branching morph and 0.077 (0.011 SD) for the encrusting morph.

Despite the proximity of the sites, allelic frequencies varied significantly for some loci among the San Salvador and Little San Salvador populations (Table 5). Comparisons of pooled allelic frequencies showed significant differences between shallow and deep sites for CK and marginal significance for HK-2. Similarly, pooled allelic frequencies for San Salvador and Little San Salvador were significantly different for CK for the branching morph (again HK-2 was marginally significant) and at three loci for the encrusting morph (Table 5).

The patterns of genetic differentiation among populations as measured using Wright's fixation indices agree

Table 3 *Briareum asbestinum.* Probability values for *G*-tests for allele frequency differences between morphs at each site where both morphs occur together in shallow populations at San Salvador, Bahamas; LSS: Little San Salvador, Bahamas. Significant deviations determined by sequential Bonferroni test (Rice 1989). Table-wide α levels indicated as $*P<0.05$, $**P<0.01$, $***P<0.001$

Site	CK (3 *df*)	G6PDH (2 *df*)	HK-2 (1 *df*)	ME-1 (1 *df*)	PGI (3 *df*)
Shallow Site 1	0.494 NS	0.360 NS	<0.001***	<0.001***	0.004**
Shallow Site 2	0.009*	0.535 NS	<0.001***	<0.001***	<0.001***
Shallow Site 3	0.012*	0.831 NS	<0.001***	<0.001***	0.004**
LSS	0.013*	0.012*	<0.001***	0.001**	0.032*

Table 4 *Briareum asbestinum.* Mean observed (obs.) heterozygosities of the five polymorphic loci and all 21 loci. *G*-tests are for departures from expected (exp.) values for the five polymorphic loci of shallow and deep populations at San Salvador, Bahamas; LSS: Little San Salvador, Bahamas

Population	Five polymorphic loci					All 21 loci,
	obs.	exp.	*G*	(*df*)	*P*	obs.
Branching morph						
Shallow 1	0.270	0.478	24.65	(4)	<0.001	0.064
Shallow 2	0.430	0.425	1.30	(4)	NS	0.102
Shallow 3	0.390	0.442	8.27	(4)	NS	0.093
Deep 1	0.400	0.447	6.17	(4)	NS	0.095
Deep 2	0.350	0.492	17.46	(4)	<0.01	0.083
LSS	0.480	0.501	3.53	(4)	NS	0.114
Encrusting						
Shallow 1	0.130	0.238	24.08	(3)	<0.001	0.062
Shallow 2	0.277	0.421	14.69	(3)	<0.01	0.075
Shallow 3	0.329	0.441	11.44	(3)	<0.01	0.086
LSS	0.380	0.459	20.45	(4)	<0.001	0.083

with the χ^2-tests for heterozygote deficiencies and population differentiation (Table 6). The majority of the variance in F_{IT} is contributed by F_{IS}, as expected given the overall heterozygote deficiencies. Values of F_{ST}, although small, are significantly different from zero for CK, G6PDH, HK-2 and PGI for one or both morphs, indicating some differentiation among populations

Discussion

Like a number of other species with distinct, sympatric morphotypes (McCommas and Lester 1980, Bucklin and Hedgecock 1982, Solé-Cava et al. 1985, Knowlton et al. 1992), the two morphotypes of *Briareum asbestinum* are associated with genetic differences at electrophoretically detectable loci. The consistent differences in allele frequencies between the branching and encrusting morphs of *B. asbestinum* which occur in the same populations strongly suggests that gene flow between the morphs is absent or greatly restricted. This is surprising, since the reproductive biology of the two morphs is similar. Males of both

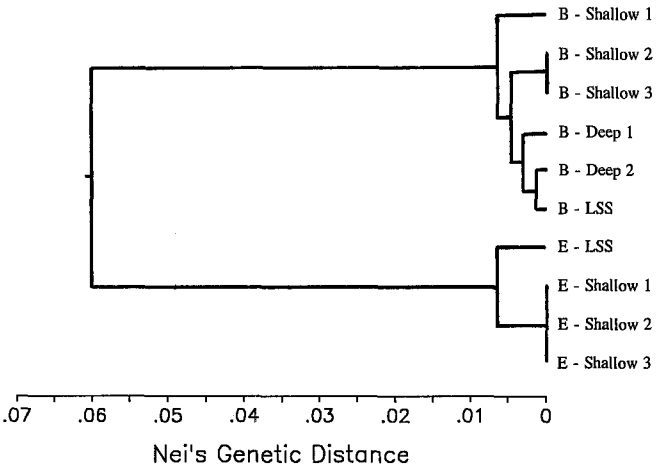

Fig. 2 *Briareum asbestinum.* UPGMA dendrogram summarizing unbiased estimates of Nei's standardized genetic distance (*D*) among 10 subpopulations of branching (B) and encrusting (E) morphs in shallow and deep populations at San Salvador, Bahamas and shallow population at Little San Salvador, Bahamas (LSS)

morphs spawn near the full moons in the summer months and the embryos are externally brooded (Brazeau unpublished data). The mean genetic distance (D=0.065) calculated for these morphs is an order of magnitude higher than the mean genetic distances calculated between populations within each morph, and exceeds the value suggested by Nei (1987) as indicative of subspecies status (D>0.05). Other aspects of the biology of the branching and encrusting morphs are consistent with the notion that they are reproductively isolated. The sclerites and secondary chemistry, both characters of systematic significance in Octocorallia, are distinctly different (Harvell and Fenical unpublished data). While further allozyme surveys at other locations across the ranges of both morphs are necessary to define the systematic relationships between these morphs, at the sites studied here, the populations of the two morphs appear to be reproductively isolated.

In contrast to a number of studies which have shown reduced genotypic diversity in anthozoans that reproduce asexually (Black and Johnson 1979, Ayre 1984, Stoddart 1984a, Hoffmann 1987, Ayre et al. 1991, Coffroth et al. 1992), the *Briareum asbestinum* populations studied here show genotypic diversities equal to those expected for a population dependent solely on sexual reproduction. This result is surprising given *B. asbestinum*'s propensity to spread vegetatively at other sites across distances greatly exceeding the 30 cm limit imposed here (Lasker 1983). Lasker estimated from visual surveys that 24 to 60% of the *B. asbestinum* colonies at Marsarkantupo Reef in the San Blas Islands, Panamá and 18 to 67% of the colonies at Carrie Bow Cay, Belize, were derived from fallen branches which had reattached to the substrate. Similarly, 62% of 118 colonies monitored for 2 yr in the San Blas Islands, Panamá, had branches that had broken off and subsequently reattached to the substratum (Brazeau and Lasker 1992b). Although we did attempt to avoid resampling the same colony by collecting only one sample from clumps

Table 5 *Briareum asbestinum.* Probability values for *G*-tests for allele frequency heterogeneity for each locus among populations of each morph in Shallow and deep populations at San Salvador (SS), Bahamas; LSS: Little San Salvador, Bahamas. Significant deviations determined by sequential Bonferroni test (Rice 1989). Table-wide α levels indicated as * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Population	CK	G6PDH	HK-2	ME-1	PGI
Branching morph					
Shallow sites	0.013*	0.305 NS	0.799 NS	0.054 NS	0.005*
Deep sites	0.598 NS	0.043 NS	0.169 NS	0.389 NS	0.279 NS
Shallow vs deep	<0.001***	0.179 NS	0.029 NS	0.118 NS	0.591 NS
LSS vs SS	0.007*	0.140 NS	0.029 NS	0.920 NS	0.024 NS
Encrusting morph					
Shallow sites	0.528 NS	0.008*	1.0 NS	0.839 NS	0.787 NS
SS vs LSS	0.002*	0.004*	0.001**	0.025 NS	0.748 NS

Table 6 *Briareum asbestinum.* Wright's *F*-statistics for each locus of branching and encrusting morphs. F_{IS}: genetic variance within populations; F_{IT}: genetic variance in total populations (whole set of samples); F_{ST}: genetic variance among populations

	CK	G6PDH	HK-2	ME-1	PGI
Branching morph					
F_{IS}	0.117	0.135	–0.140	0.028	0.361
F_{IT}	0.163	0.174	–0.096	0.067	0.382
F_{ST}	0.053*	0.046*	0.038	0.040	0.033*
Encrusting morph					
F_{IS}	0.368	0.237	1.0	–0.150	0.417
F_{IT}	0.394	0.275	1.0	–0.110	0.421
F_{ST}	0.041*	0.050*	0.077*	0.035	0.006

* $F_{ST}>0$ at $P\geq0.95$ (χ^2 test of Workman and Niswander 1970)

of branches within a 30 cm radius, the high ratio of distinct genotypes to sample size at our sites indicates that the contribution of asexual reproduction to the genetic structure of these sites is negligible. Ayre (1984) noted similar habitat-dependent differences in genotypic diversities in the sea anemone *Actinia tenebrosa*, where genotypic diversities were significantly higher on unstable boulder beaches than on stable rock platforms. In addition to the apparent differences in asexual reproduction reported for *B. asbestinum* in earlier studies and the results of the present study, success at sexual reproduction (number of embryos/female colony; Brazeau and Lasker 1992 a) as well as growth rates (Brazeau and Lasker 1992 b) also vary significantly between sites. Thus, the genotypic diversities of colonial species such as *B. asbestinum* may be particularly sensitive to habitat-specific factors that influence rates of both sexual (i.e., fertilization success and recruitment) and asexual (i.e., rates of growth, fragmentation and reattachment success of fragments) processes, because the products of both modes of reproduction often recruit locally (Jackson 1986).

The significant values for F_{ST} for 3 of 5 loci for both morphs of *Briareum asbestinum* indicate that even neighboring sites within 50 to 100 m on the same reef exhibit some genetic differentiation. Both the larval biology and the mating biology of the branching morph of *B. asbestinum* should promote the genetic isolation observed among populations here. *B. asbestinum* broods its larvae externally; when released, the larvae settle to the bottom (Brazeau and Lasker 1990). Often the larvae are trapped in mucous sheets secreted by the colonies and are carried to the base of the colony as the sheets become fouled and are shed. Thus, dispersal from the natal colony is often extremely limited. In addition, female fertilization success is dependent upon the number and proximity of nearby males (Brazeau and Lasker 1992 a). These conditions (philopatry and nearby colony matings) should yield populations with high levels of inbreeding, since at least some of the males around a female colony will be her sons, and sons and daughters will also be in close proximity. The data presented here support this conclusion. The large values of F_{IS} together with significant heterozygote deficiencies for a number of populations indicate that local populations may comprise a number of family groupings with limited gene flow between populations separated by distances as little as 50 to 100 m. This study is similar to that of Grosberg (1991), in which a sessile marine invertebrate, *Botryllus schlosseri*, with similar reproductive traits (i.e., limited dispersal of gametes and larvae) had high values of F_{IS} and F_{IT}. However, in contrast to the data reported here, Grosberg did not find significant genetic differentiation (F_{ST} values) on a microgeographic scale, presumably due to the small spatial and temporal scales of his study. However, microgeographic differentiation in *B. schlosseri* has been found elsewhere (Sabbadin and Graziani 1967).

Another factor that affects the overall genetic diversity within populations and increases the random divergence among populations is the genetically effective population size. While little is known about the effective population size of any marine organism, several features of the reproductive biology of *Briareum asbestinum* should reduce effective population size, such as the male-biased sex ratios observed in a large number of populations (Brazeau and Lasker 1990), the low reproductive success (Brazeau and Lasker 1990) and the non-random patterns of mating (i.e., females with nearest males). While non-random patterns of matings may be particularly common among sessile colonial marine invertebrates (Grosberg 1987, 1991, Smith and Potts 1987), the most significant factor affecting effective population size in *B. asbestinum* is likely to be low reproductive success. Among sessile marine invertebrates, variation in breeding success has been suggested to be the most important determinant of effective population size (Hedgecock 1982). Reproductive success of 31 to 40 colonies was monitored over 3 yr for the branching morph of

B. asbestinum on two reefs in the San Blas Islands, Panamá. The percentage of female colonies observed to release embryos ranged from 47% in 1986 to 5% in 1988 (Brazeau and Lasker 1992 a). On average, 73% of the reproductive female colonies experienced total reproductive failure during each year of the study. Even among colonies that released some embryos, <20% of the mature eggs present in each colony were released as developing embryos, due to sperm limitation (Brazeau and Lasker 1992 a). Thus, simple counts of the number of adults present in a population would greatly exaggerate the effective population size in this invertebrate. All of these factors taken together suggest that *B. asbestinum* populations are composed of many small, isolated demes that tend to diverge from one another via random drift, inbreeding and perhaps due to variation in local selection regimes.

One consequence of a subdivided population structure driven by local matings and local dispersal is the potential for these small populations to respond rapidly to local selection regimes. Shields (1982) argued that philopatry and inbreeding enhance individual fitness by transmitting locally successful parental genotypes to offspring. This advantage of inbreeding sex is greatest for low-fecundity, long-lived organisms in stable environments. Compared to most marine invertebrates, *Briareum asbestinum* has low fecundity (particularly actual or realized fecundity) and the genet is probably long-lived. Jackson (1986) noted that, compared to aclonal invertebrates, most clonal organisms have low fecundities and are long-lived, and he suggested inbreeding as one explanation for the apparent commonness of short-distance dispersal in clonal sessile animals. Variation in the secondary chemistry of *B. asbestinum* has been detected between the same shallow and deep reefs sampled in San Salvador. Indeed, the secondary chemistry of *B. asbestinum* is highly variable throughout the Bahamas and at sites in the Virgin Islands, over short spatial scales (Harvell et al. 1993 a). Short-term transplant experiments support the electrophoretic evidence that the differences in the chemistry are genetically-based, since transplanted colonies – at least over the short term – do not change chemistry to match new environments (Harvell et al. 1993 a). It is possible that these differences in chemistry are the result of selection for different chemotypes in different habitats.

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