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Genetic structure of populations of two closely related brittle stars with contrasting sexual and asexual life histories, with observations on the genetic structure of a second asexual species *

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Abstract

Ophiocoma pumila Lütken and *Ophiocomella ophiactoides* (H. L. Clark) are morphologically similar brittle stars with contrasting life histories, the former obligately sexual, the latter fissiparous (capable of both sexual reproduction and asexual proliferation by binary fission). Electrophoretic analysis of five polymorphic enzymes was used to assess the genetic consequences of these differing life histories and provide a genetic perspective on the taxonomic relationship between the two species. Genotypic diversity of *Ophiocoma pumila* collected at Discovery Bay, Jamaica, in 1985 conformed to expectations for a sexually reproducing population. In contrast, genotypic diversity of *Ophiocomella ophiactoides* at this site was significantly lower than expected for a sexually reproducing population, due largely to the predominance of clonal proliferation over larval recruitment. Large variation in clonal composition over a short (50 m) distance emphasized the very localized scale of clonal mixing in this species. Allozymic data are indicative of a close sibling species relationship between *Ophiocoma pumila* and *Ophiocomella ophiactoides* which suggests that the present generic separation of the two species should be re-examined. Electrophoretic analysis was also used to examine the genetic structure of sponge- and alga-dwelling populations of a second fissiparous brittle star, *Ophiactis savignyi* (Müller & Troschel), which was also collected at Jamaica in 1985. Striking differences in the allelic composition of sponge- and alga-dwelling *O. savignyi* were observed. Genotypic diversity of *O. savignyi* in sponges was very low, each sponge being dominated by a single genotype. Genotypic diversity of *O. savignyi* in algae was higher, although still significantly lower than expectations for a sexually reproducing population. In the light of the highly clonal composition of fissiparous brittle-star populations, the adaptive significance of clonal growth may be related to an increase in the overall fitness of dispersed clones (genets), compared to individuals of strictly sexual counterparts, through greater genotype-specific biomass and, hence, fecundity.

Introduction

Brittle stars comprise a diverse and successful lineage of marine benthic invertebrates that display a broad array of life histories and habits. The vast majority of the roughly 2 000 extant species reproduce sexually by means of planktotrophic, lecithotrophic or brooded offspring (Hendler 1975, Lawrence 1987). However, approximately 37 species from 11 of 16 families are fissiparous, having a mixed life history that includes both sexual reproduction and clonal proliferation by binary fission (Emson and Wilkie 1980, Mladenov et al. 1983 and in press, Mladenov and Emson 1984, 1988, Emson et al. 1985). Although derived from a systematically diverse background, fissiparous brittle stars share a suite of traits, including small adult body size, mainly hexamerous symmetry, a largely tropical and sub-tropical distribution, and an often epiphytic or epizoic habit, that binds them into an ecologically homogeneous group. Furthermore, sex in all fissiparous species studied results in the production of planktotrophic larvae (Mladenov and Emson 1984). Some fissiparous species are geographically widespread and locally very abundant, indicating that fissiparity can be a remarkably successful strategy. For example, the fissiparous *Ophiactis savignyi* is a "tropicopolitan" species (Müller and Troschel 1842, Clark 1933, Hyman 1955) that is often associated with seaweeds and sponges in great numbers (Boffi 1972, Mladenov and Emson 1988). Also, the fissiparous *Ophiocomella ophiactoides* is widespread in the Caribbean Sea (Parslow and Clark 1963) and can be locally abundant in seaweed (Mladenov et al. 1983, Mladenov and Emson 1988).

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The genetics of fissiparous brittle star populations has never been studied. Indirect evidence suggests, however, that clonal proliferation may make a significant contribution to the genetic structure of populations. First, information on size structure and frequency of fission in various populations of *Ophiocomella ophiactoides* indicates that rates of recruitment of planktonic larvae are low and that population growth and maintenance can be attributed mainly to fission (Mladenov et al. 1983, Mladenov and Emson 1988). Second, the occurrence of highly skewed sex ratios among aggregations of *O. ophiactoides* occupying algal clumps, and of all-male and all-female aggregations of *Ophiactis savignyi* living in sponges, is consistent with the notion that these microhabitats are occupied by a small number of clones (Mladenov and Emson 1988).

A pair of sympatric sibling species of ophiocomid brittle stars, one obligately sexual (*Ophiocoma pumila*) and the other fissiparous (*Ophiocomella ophiactoides*), provided an opportunity to (1) examine the level of genetic relatedness of these two forms, and (2) compare the population genetic consequences of contrasting sexual and asexual life-histories in echinoderms. The inclusion of a closely related obligately sexual species in the comparison provides the potential to separate the effects of asexual proliferation on genotypic diversity from other factors that can depress genotypic diversity in a population, such as the Wahlund effect (due to population mixing) or inbreeding.

Ophiocoma pumila Lütken is a large, five-armed, brittle star with a tropical amphi-Atlantic distribution (Hyman 1955, Devaney 1970); it is strictly sexual, broadcasting small, externally fertilized eggs that develop as long-lived planktotrophic ophioplutei (Mladenov 1985). *Ophiocomella ophiactoides* (H. L. Clark) is a small, mainly six-armed, brittle star known from the West Indies and Brazil (Parslow and Clark 1963); replication by fission is the major means of recruitment in this species (Mladenov et al. 1983, Mladenov and Emson 1988), but broadcast spawning of sexually produced, long-lived ophioplutei, that are virtually indistinguishable from those of *Ophiocoma pumila*, occurs in some populations (Mladenov and Emson 1984, 1988). Apart from the differences in size and symmetry, the two forms are morphologically very similar (Parslow and Clark 1963). Since early workers assumed that small, six-armed fissiparous brittle stars represented the juvenile phase of larger, sympatric, non-fissiparous brittle stars (see Parslow and Clark 1963, and Devaney 1970), *Ophiocomella ophiactoides* has sometimes been referred to *Ophiocoma pumila*. However, Parslow and Clark and Devaney suggested, on the basis of both morphological criteria and geographic distribution, that the two forms, although closely related, are indeed separate species. [Parslow and Clark and Clark (1967) further suggested that a generic distinction seemed too drastic.] Other traits distinguishing the two species include (1) distributional differences at the microhabitat level (Mladenov and Emson personal observations, Hendler and Litman 1986), (2) lack of evidence for a transformation of *Ophiocomella ophiactoides* from the six-armed to the five-armed condition (Mladenov et al. 1983), (3) the presence of sexual *O. ophiactoides*, which belies their status as a juvenile form of *Ophiocoma pumila* (Mladenov and Emson 1984), and (4) the extensive occurrence of a parasitic copepod on *Ophiocomella ophiactoides*, but not on *Ophiocoma pumila* (Emson and Mladenov 1987).

A second fissiparous brittle star, *Ophiactis savignyi* Müller & Troschel, provided additional opportunity to study the population genetic consequences of fissiparity. This species occupies both sponge and algal turf habitats in the West Indies (Mladenov and Emson 1988). The sponge-dwelling and alga-dwelling populations differ in a number of ways (Mladenov and Emson 1988). Mean body size and density of *O. savignyi* are greater in sponges than in algal turf. Moreover, sponge-dwelling populations contain sexual as well as asexual individuals, whereas epiphytic populations are almost totally asexual. Between-habitat comparisons of the population genetics of this fissiparous brittle star might thus provide information on the relative contribution of asexual proliferation in each habitat and determine whether the differing population characteristics mentioned above are rooted in genetic differences.

In this paper, we use electrophoretic techniques to compare the genetic structure of nearby populations of *Ophiocoma pumila* and *Ophiocomella ophiactoides* from Discovery Bay, Jamaica. We also compare the genetic structure of an algal-dwelling population of *Ophiactis savignyi* from Discovery Bay, Jamaica, with sponge-dwelling aggregations of *O. savignyi* from a mangrove swamp in Salt Marsh Bay near Falmouth, Jamaica. We will show that (1) *Ophiocoma pumila* is genetically distinct from *Ophiocomella ophiactoides*; (2) the *Ophiocoma pumila* population is genotypically as diverse as expected for a sexual population, whereas the *Ophiocomella ophiactoides* population is significantly less diverse than expected due to clonal proliferation; and (3) sponge-dwelling and alga-dwelling populations of *Ophiactis savignyi* are clonally structured but genetically distinct. Our findings lead ultimately to a consideration of the adaptive significance of clonal growth in brittle stars and other echinoderms.

Materials and methods

Collection of specimens

Specimens of *Ophiocoma pumila* Lütken were collected in January 1985 from Columbus Park Reef at the southern end of Discovery Bay (see Fig. 1 in Mladenov and Emson 1988 for site locations referred to here and below) with the assistance of SCUBA equipment. At this site, *O. pumila* is abundant on and beneath a mat of coralline red alga, *Amphiroa tribulis*, which overlies a layer of coral rubble at depths of 5 to 10 m. Divers carefully searched the algal and rubble layers and removed all individuals of *O. pumila* encountered.

Specimens of *Ophiocomella ophiactoides* (H. L. Clark) were collected in January, 1985 from (1) Blue Maze Cove, a small, shallow (< 2 m in depth) bay situated just west of the Discovery Bay Marine Laboratory, Discovery Bay, Ja-

maica, and (2) Clone Cove, a second small, shallow (<1 m in depth) bay located just east of the laboratory, very close to the intake for the laboratory sea water supply. These sites are separated by about 50 m of coastline. *O. ophiactoides* occurs in clumps of coralline red (principally *Amphiroa rigida* and *A. fragillisima*) and calcareous green (*Halimeda opuntia*) algae in these bays. Clumps of these algae were taken to the laboratory, where all individuals of *O. ophiactoides* were removed.

Specimens of *Ophiactis savignyi* (Müller & Troschel) were collected in January 1985 from within (1) two individual sponges (one was *Lissodendoryx isodictyalis*, the other *Tedania ignis*) located on mangrove prop roots in Salt Marsh Bay near Falmouth, Jamaica, and separated by a distance of 2 m, and (2) clumps of coralline red and calcareous green algae obtained from Wahle Cove, a small, shallow bay on the west side of Discovery Bay. Only small numbers ($n=18$) of *O. savignyi* were obtained from Wahle Cove because of the generally low densities of this species in algal turf habitats (Mladenov and Emson 1988).

Gel electrophoresis

Specimens of all three species were transported in dry ice to Mount Allison University (Canada) where they were stored in liquid nitrogen. Whole specimens of *Ophiocomella ophiactoides* and *Ophiactis savignyi* (specimens of these species had disc diameters of 4 mm or less) were homogenized in 20 µl of 0.5 *M* Tris-HCl buffer (pH 7.1). Portions of the stomach tissue of *Ophiocoma pumila* were homogenized in 200 µl of the same buffer. Electrophoresis was conducted on 12.5% horizontal starch gels (Sigma starch No. S-4501) at 4°C. Two buffer systems were used (Ayala et al. 1973, Tracey et al. 1975) – (1) discontinuous: a tris-citric acid gel buffer (pH 8.65) and a boric acid-NaOH electrode buffer (pH 8.1); (2) continuous: tris-citric acid-EDTA gel and bridge buffers (pH 7.1). Variation was examined in each species for four protein systems encoded by five presumptive loci (as indicated by discrete zones of activity): glucose phosphate isomerase (GPI; EC 5.3.1.9, discontinuous buffer), phosphoglucomutase (PGM; EC 2.7.5.1, discontinuous buffer), malate dehydrogenase (MDH-1, MDH-2; EC 1.1.1.37, continuous buffer) and malic enzyme (ME; EC 1.1.1.40, continuous buffer). The enzyme staining assays of Ayala et al. and Tracey et al. were employed. Mobility controls (samples from individuals with known allozyme mobility) were used in every electrophoretic run. In the case of the *Ophiocomella ophiactoides–Ophiocoma pumila* comparison, the controls included samples of individuals of both species with known allozyme mobilities. Alleles at each locus were designated by letters representing the relative electrophoretic mobilities of their allozymes (a = fastest, b = next fastest, etc.). Subunit structures of the enzymes were inferred from heterozygote banding patterns. These were: GPI, MDH-1 and MDH-2, dimers; PGM and ME, monomers. These designations conform with published subunit numbers for humans with the exception of ME, which is a tetramer in humans (Harris and Hopkinson 1976). However, heterozygotes at the *Me* locus in humans show double-banded staining patterns in starch gels (Harris and Hopkinson 1976) despite the tetrameric structure of the ME enzyme. Thus, a tetrameric structure for brittle star ME cannot be excluded.

Analysis

The degree of departure of genotypic frequencies from Hardy-Weinberg expectations at single loci was represented by the coefficient

$$H_d=(H_o-H_e)/H_e, \quad (1)$$

where H_o is observed heterozygosity and H_e is heterozygosity calculated from Hardy-Weinberg expectations (Selander 1970). Observed heterozygote deficiency is indicated by a negative value of H_d, whereas observed heterozygote excess is indicated by a positive value.

The extent of genetic differentiation between populations of *Ophiocoma pumila* and *Ophiocomella ophiactoides* was estimated by calculating genetic identity (I) and genetic distance (D) using all five loci studied (Nei 1972).

Success in recognizing clones by scoring five polymorphic loci was evaluated using a procedure developed by Hoffmann (1986). For each collection, the average number of unique genotypes (putative clones) recognized was determined using each locus individually, all possible two-way, three-way, and four-way combinations of loci and, finally, the five-locus genotypes themselves. A plot of number of unique genotypes against number of loci should reach an asymptote if all clones have been recognized. Failure to reach an asymptote indicates that some clones in the sample have not been distinguished.

Genotypic diversity was examined in two ways. First, the diversity measure, d, of Hoffmann (1986) was calculated. It is given by:

$$d=\text{no. of unique multi-locus genotypes}/n, \quad (2)$$

where n is the sample size. d may vary between nearly 0, for collections where only a single genotype is found, and 1, for collections where each individual has a unique genotype. Second, the index of observed genotypic diversity, G_o, was calculated (Black and Johnson 1979, Parker 1979). It is given by:

$$G_o=1\Big/\sum_{i=1}^{k} g_i^2, \quad (3)$$

where g_i is the frequency of the ith of k multilocus genotypes. G_o may vary between 1, where only one genotype is found, and a maximum of k, where all k genotypes occur at equal frequency.

To determine whether observed genotypic diversity values of brittle-star populations differed significantly from expectations under the conditions of Hardy-Weinberg equilibrium and random mating, the expected genotypic diversity, G_e, was determined by simulation using a computer program (SIMSD) provided by J. A. Stoddart. This program

Table 1. *Ophiocoma pumila* and *Ophiocomella ophiactoides*. Allele frequencies for populations at Discovery Bay, Jamaica. *N*: no. of individuals

Ophiocoma pumila						*Ophiocomella ophiactoides*				
N	Locus					*N*	Locus			
29	*Gpi*					134	*Gpi*			
	a	b					a	b		
	0.97	0.03					0.86	0.14		
29	*Pgm*					111	*Pgm*			
	a	b	c	d	e		a	b		
	0.05	0.12	0.74	0.07	0.02		0.55	0.45		
29	*Mdh-1*					72	*Mdh-1*			
	a	b	c	d			a	b	c	
	0.28	0.16	0.40	0.17			0.50	0.47	0.03	
29	*Mdh-2*					86	*Mdh-2*			
	a	b					a	b		
	0.02	0.98					0.30	0.70		
29	*Me*					63	*Me*			
	a	b	c	d			a	b		
	0.12	0.19	0.62	0.07			0.43	0.57		

calculates the mean and standard deviation of the expected genotypic diversity in a sample drawn from a 500-individual population, using 500 Monte Carlo simulations (Stoddart and Taylor 1988). The value of G_o was tested against expectation with a Student's *t*-test for comparison of a single observation with the mean of a sample (Sokal and Rohlf 1981).

Results

Genetic structure of *Ophiocoma pumila* and *Ophiocomella ophiactoides* populations

Putative allele frequencies for the five polymorphic loci in Discovery Bay populations of *Ophiocoma pumila* and *Ophiocomella ophiactoides* are presented in Table 1. Two alleles were resolved at the *Gpi* locus in both species. Their allozymes had the same electrophoretic mobility in each species. *Gpi* bb genotypes were not detected in either species. Five alleles were resolved at the *Pgm* locus in *Ophiocoma pumila*, three at low frequency. Two alleles were resolved at this locus in *Ophiocomella ophiactoides*; the two *O. ophiactoides* allozymes had the same electrophoretic mobilities as the common PGM b and PGM c allozymes of *Ophiocoma pumila*. Four alleles were resolved at the *Mdh-1* locus in *O. pumila*, and three at this locus in *Ophiocomella ophiactoides*. The MDH-1 a and MDH-1 b allozymes of *O. ophiactoides* had the same mobilities as the MDH-1 b and MDH-1 c allozymes of *Ophiocoma pumila*. Two alleles were resolved at the *Mdh-2* locus in *O. pumila*, the *Mdh-2* a allele at very low frequency. Two alleles were also resolved at this locus in *Ophiocomella ophiactoides*; the MDH-2 a allozyme had identical mobility to the MDH-2 a allozyme of *Ophiocoma pumila*, the other allozyme had a unique mobility. Four alleles were resolved at the *Me* locus in *O. pumila*, the *Me* d allele at low frequency. Two alleles were found at this locus in *Ophiocomella ophiactoides*; the ME a allozyme had the same mobility as the ME a allozyme of *Ophiocoma pumila*; the other had a unique mobility. In sum, a relatively high degree of genetic differentiation between *O. pumila* and *Ophiocomella ophiactoides* was evident at four of the five loci studied. Coefficients of genetic identity and genetic distance were 0.517 and 0.660, respectively.

For the *Ophiocoma pumila* sample, 26 unique five-locus genotypes were detected among the 29 specimens analyzed (Table 2). Values of d and G_o approached maximum, and the value of G_o was not significantly different from that of G_e (see Table 4).

For the *Ophiocomella ophiactoides* sample, 24 unique five-locus genotypes were detected among the 36 specimens for which data at all five loci were available (Table 3). Values of d and G_o were lower than for the *Ophiocoma pumila* sample, and the value of G_o was significantly lower than that of G_e; the ratio of observed to expected genotypic diversity was 0.68 (Table 4). One explanation for the depressed value of observed genotypic diversity is that the population is clonally structured and that several members of some of these clones were collected (see first subsection of "Discussion"). A plot of genotypic diversity against number of loci provides a way of assessing levels of clone recognition (Hoffmann 1986). This plot began to saturate with the addition of the fifth locus (Fig. 1), suggesting that most clones were recognized, and that the addition of one or two more loci to

Table 2. *Ophiocoma pumila*. Genotypic composition of sample from Discovery Bay, Jamaica. *N*: no. of individuals

	Five-locus genotype					*N*
	Gpi	*Pgm*	*Mdh-1*	*Mdh-2*	*Me*	
1	aa	cc	ac	bb	bc	2
2	aa	cc	ac	bb	cc	2
3	aa	cc	cc	bb	cc	2
4	aa	aa	dd	bb	bb	1
5	aa	bc	cc	bb	cc	1
6	aa	cc	dd	bb	cc	1
7	aa	cd	cc	bb	ac	1
8	aa	cc	ac	bb	aa	1
9	aa	ac	ac	bb	bb	1
10	aa	dd	ac	bb	cd	1
11	aa	bc	ac	bb	cc	1
12	aa	bc	ab	bb	cd	1
13	aa	bc	ab	bb	cc	1
14	ab	cc	bc	bb	bb	1
15	aa	cc	bc	bb	cc	1
16	aa	cc	bb	bb	cc	1
17	aa	bc	bb	bb	cc	1
18	aa	ce	cc	bb	cc	1
19	aa	bb	dd	bb	cd	1
20	aa	cc	ab	bb	bb	1
21	aa	cc	cc	bb	aa	1
22	aa	cc	aa	bb	bc	1
23	aa	cc	dd	bb	cd	1
24	aa	cc	dd	bb	ac	1
25	ab	cc	aa	ab	ac	1
26	aa	cd	ac	bb	cc	1

Table 3. *Ophiocomella ophiactoides*. Genotypic composition of samples from two nearby sites in Discovery Bay, Jamaica. *N*: no. of individuals. –: genotype absent

	Five-locus genotype					Blue Maze Cove (*N*)	Clone Cove (*N*)
	Gpi	*Pgm*	*Mdh-1*	*Mdh-2*	*Me*		
1	ab	ab	bb	aa	ab	1	–
2	aa	aa	aa	ab	ab	1	1
3	aa	bb	aa	bb	aa	1	–
4	aa	aa	aa	ab	aa	1	–
5	aa	ab	bb	aa	aa	1	2
6	aa	ab	aa	bb	bb	4	–
7	aa	aa	aa	ab	bb	1	–
8	aa	ab	bb	bb	bb	1	3
9	aa	bb	bb	bb	ab	1	–
10	aa	aa	bb	bb	ab	1	–
11	aa	aa	bb	ab	bb	1	–
12	aa	ab	aa	ab	aa	1	2
13	aa	aa	aa	bb	bb	1	–
14	aa	ab	bb	bb	ab	–	1
15	aa	ab	bb	ab	aa	–	1
16	ab	ab	bb	bb	bb	–	1
17	ab	ab	ab	bb	bb	–	1
18	aa	ab	cc	bb	bb	–	1
19	aa	aa	cc	ab	bb	–	1
20	aa	aa	bb	aa	aa	–	1
21	ab	ab	aa	aa	aa	–	1
22	ab	ab	bb	aa	aa	–	2
23	aa	ab	aa	ab	ab	–	1
24	aa	bb	aa	bb	ab	–	1

Table 4. *Ophiocoma pumila* and *Ophiocomella ophiactoides*. Five-locus measures of genotypic diversity for samples from Discovery Bay, Jamaica. *N*: no. of individuals; *d*: Hoffmann's (1986) diversity measure; G_o: observed diversity; G_e: expected diversity. Significance of diversity differences from expectation based on Sudent's *t*-tests (two-tailed). *** = $P<0.001$

Species	*N*	*d*	G_o	G_e (±1 SD)	G_o:G_e
Ophiocoma pumila	29	0.90	24.04 NS	23.60 (1.96)	1.02
Ophiocomella ophiactoides	36	0.67	17.03 ***	25.04 (2.26)	0.68

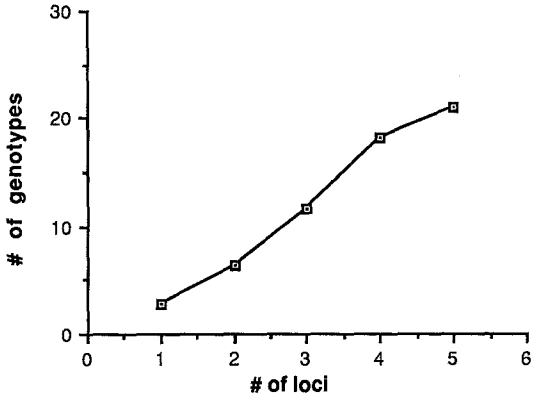

Fig. 1. *Ophiocomella ophiactoides*. Plot of number of unique genotypes against number of loci

the analysis might have been sufficient to recognize all clones in this population. However, at present, clone recognition is probably imperfect.

There was considerable genotypic difference between the *Ophiocomella ophiactoides* samples from Blue Maze Cove and the nearby Clone Cove (Table 3). Nine five-locus genotypes were unique to Blue Maze Cove, and eleven five-locus genotypes were unique to Clone Cove; four five-locus genotypes occurred in both locations.

Locus-by-locus tests for Hardy-Weinberg equilibria revealed significant heterozygote deficiency at three of the five loci studied in *Ophiocoma pumila* (Table 5). Similar tests for Hardy-Weinberg equilibria at *Ophiocomella ophiactoides* loci revealed significant heterozygote deficiency at the *Mdh-1* and *Me* loci (Table 5). Significant heterozygote deficiency persisted at these two loci when the analysis was restricted to unique five-locus genotypes (Table 5).

Genetic structure of *Ophiactis savignyi* populations

Allelic composition differed greatly in sponge- and alga-dwelling populations of *Ophiactis savignyi* (Table 6). *O. savignyi* sampled from algae were monomorphic homozygotes at both the *Gpi* and *Pgm* loci. *O. savignyi* from sponges showed fixed heterozygosity at the *Gpi* locus and fixed homozygosity at the *Pgm* locus (for an allele whose allozyme had a mobility different from that found in the algal sample). *O. savignyi* from sponges and algae were polymorphic at the *Mdh-1*, *Mdh-2* and *Me* loci, but allozymes with differ-

Table 5. *Ophiocoma pumila* and *Ophiocomella ophiactoides*. Analysis of single-locus Hardy-Weinberg equilibria for each population from Discovery Bay, Jamaica. Values in table are H_d (the deviation from expected proportion of heterozygotes), followed in parentheses by χ^2 values for significance of this deviation. * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

Species	Locus				
	Gpi	*Pgm*	*Mdh-1*	*Mdh-2*	*Me*
Ophiocoma pumila	−0.01 [a]	−0.39 (4.6) *	−0.34 (9.0) **	−0.25 [a]	−0.39 (5.6) *
Ophiocomella ophiactoides (using data from all individuals)	0.17 (1.2) NS	0.18 (3.6) NS	−0.95 (72.1) ***	−0.22 (3.1) NS	−0.49 (14.1) ***
Ophiocomella ophiactoides (using unique 5-locus genotypes)	0.03 (0.1) NS	0.06 (0.1) NS	−0.53 (27.3) ***	−0.14 (1.9) NS	−0.42 (4.1) *

[a] No test possible because fewer than five brittle stars expected for one genotypic class

Table 6. *Ophiactis savignyi*. Allele frequencies for sponge- and alga-dwelling populations from Jamaica. N = no. of individuals; –: allele absent

Habitat (site)	*Gpi*		*Pgm*		*Mdh-1*					*Mdh-2*				*Me*			
	a	b	a	b	a	b	c	d	e	a	b	c	d	a	b	c	d
Sponge (Salt Marsh Bay)	0.5	0.5	–	1.0	–	–	0.46	0.47	0.07	0.05	0.95	–	–	0.98	0.22	–	–
N	60		60		60					60				60			
Algae (Discovery Bay)	1.0	–	1.0	–	0.64	0.36	–	–	–	–	–	0.59	0.41	–	–	0.88	0.12
N	34		34		22					29				33			

Table 7. *Ophiactis savignyi*. Genotypic composition (numbers of individuals) of samples from sponges and algae. –: genotype absent

	Five-locus genotype					Sponges		Algae
	Gpi	*Pgm*	*Mdh-1*	*Mdh-2*	*Me*	*Lissodendoryx isodictyalis*	*Tedania ignis*	
1	ab	bb	dd	bb	aa	18	6	–
2	ab	bb	ee	bb	aa	4	–	–
3	ab	bb	cc	bb	aa	4	23	–
4	ab	bb	dd	aa	aa	3	–	–
5	ab	bb	dd	bb	bb	1	–	–
6	ab	bb	cd	bb	aa	–	1	–
7	aa	aa	aa	cd	cc	–	–	5
8	aa	aa	ab	cd	cc	–	–	3
9	aa	aa	bb	cd	cc	–	–	2
10	aa	aa	aa	cc	cc	–	–	2
11	aa	aa	aa	cd	dd	–	–	1
12	aa	aa	ab	dd	cc	–	–	1
13	aa	aa	bb	cd	dd	–	–	1
14	aa	aa	bb	dd	cc	–	–	1
15	aa	aa	ab	cc	cc	–	–	1
16	aa	aa	aa	cc	cd	–	–	1

ent mobilities were involved in each case. These two populations thus appear to be genetically distinct.

For the *Ophiactis savignyi* sample obtained from sponges, six unique five-locus genotypes were detected among the 60 specimens analyzed (Table 7). Values of d and G_o were very low and the value of G_o was significantly lower than that of G_e; the ratio of $G_o : G_e$ was 0.19 (Table 8). A plot of genotypic diversity against number of loci began to saturate with the addition of the fifth locus (Fig. 2), suggesting that most clones present in the sponges were recognized.

Genotypic differences were evident between the *Ophiactis savignyi* aggregations in each of the two individual sponges studied despite their close proximity (Table 7). One five-locus genotype was unique to one sponge (*Tedania ignis*) and three five-locus genotypes were unique to the other sponge (*Lissodendoryx isodictyalis*); two five-locus

genotypes were shared by the brittle stars in each sponge. In *L. isodictyalis*, 18 of 30 specimens analyzed possessed the same five-locus genotype; the remaining specimens were distributed among four other five-locus genotypes. In *T. ignis*, 23 of 30 specimens analyzed possessed the same five-locus genotype; the remaining specimens were distributed among two other five-locus genotypes. The dominant genotypes were different in each sponge.

For the *Ophiactis savignyi* sample obtained from algae, ten unique five-locus genotypes were detected among the 18 specimens analyzed (Table 7). None of the genotypes observed in the algal population were present in the sponge samples. In spite of the small sample size, more five-locus genotypes were present in the algal sample than the sponge sample and there was a more even distribution of these genotypes. This is reflected in the values of d and G_o, which were higher than those for *O. savignyi* from sponges (Table 8). Nonetheless, the value of G_o was significantly lower than that of G_e and the ratio of G_o: G_e was 0.60 (Table 8).

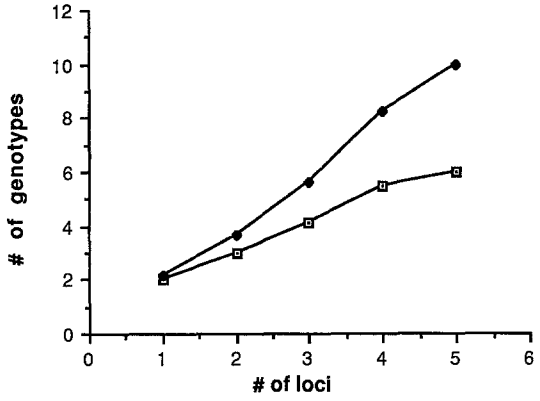

Fig. 2. *Ophiactis savignyi.* Plot of number of unique genotypes against number of loci for samples from sponges (□) and algae (♦)

Table 8. *Ophiactis savignyi.* Five-locus measures of genotypic diversity for samples from sponges and algae. N: no. of individuals; d: Hoffmann's (1986) diversity measure; G_o: observed diversity; G_e: expected diversity. Significance of diversity differences from expectation based on Student's t-tests (two-tailed). ** $P<0.01$, *** $P<0.001$

Habitat (site)	N	d	G_o	G_e (±1 SD)	G_o:G_e
Sponge (Salt Marsh Bay)	60	0.10	2.70***	14.51 (1.37)	0.19
Algae (Discovery Bay)	18	0.56	6.75**	11.23 (1.57)	0.60

A plot of genotypic diversity against number of loci failed to reach an asymptote (Fig. 2), so some clones were undoubtedly combined in this sample.

Locus-by-locus tests for Hardy-Weinberg equilibria revealed large, significant deviations from expectations at all polymorphic loci for sponge-dwelling *Ophiactis savignyi* (Table 9). These were in the direction of heterozygote deficiency at three loci and heterozygote excess at a fourth locus. In the case of alga-dwelling *O. savignyi*, one of three polymorphic loci showed significant deviation (in the direction of heterozygote deficiency) from expectation (Table 9). An analysis using unique genotypes was not carried out because of the small number of unique genotypes encountered in the *O. savignyi* samples.

Discussion

Genetic structure of populations of two closely related brittle star species with contrasting sexual and asexual life histories

On the basis of morphological criteria, *Ophiocoma pumila* and *Ophiocomella ophiactoides* comprise a pair of sibling species with contrasting life histories, the former obligately sexual, the latter fissiparous (Parslow and Clark 1963, Devaney 1970, Mladenov et al. 1983). Results from the present study provide information on the genetic consequences of these differing life histories, as well as a genetic perspective on the taxonomic relationship between these two species.

The observed genotypic diversity of a population of *Ophiocoma pumila* from Discovery Bay, Jamaica, conformed to expectations of genotypic diversity for a sexually reproducing population. In contrast, observed genotypic diversity in a nearby population of *Ophiocomella ophiactoides* was significantly lower than expected for a sexually reproducing population. Since significant heterozygote deficiency was present at two of five loci studied in *O. ophiactoides*, it is possible that the Wahlund effect (i.e., mixing of zygotes from populations with differing allele frequencies leading to heterozygote deficiency and thus decreases in observed genotypic diversity) or a degree of inbreeding (due, perhaps, to packets of related larvae travelling in a water mass together, settling together and fertilizing each others gametes; Nash et al. 1988) was a causal or contributing factor to depression of genotypic diversity. However, significant heterozygote deficiency was present at three of five loci in the *Ophiocoma pumila* sample but did not result in a significant depression in observed genotypic diversity in this population. Thus, although the Wahlund effect or inbreeding may

Table 9. *Ophiactis savignyi.* Analysis of single-locus Hardy-Weinberg equilibria for sponge- and alga-dwelling populations from Jamaica (M: monomorphic locus). Values are H_d (the deviation from expected proportion of heterozygotes) followed in parentheses by χ^2 values for significance of this deviation. * $P<0.05$; *** $P<0.001$

Habitat (site)	*Gpi*	*Pgm*	*Mdh-1*	*Mdh-2*	*Me*
Sponge (Salt Marsh Bay)	M	M	−0.97 (72.3)***	−1.00 (6.3)*	−1.00 (4.8)*
Algae (Discovery Bay)	M	M	−0.21 (0.8) NS	+0.14 (0.5) NS	−0.71 (4.5)*

be operative in the *Ophiocomella ophiactoides* population studied, it is unlikely that it is a major factor contributing to the observed lowering of genotypic diversity. Linkage disequilibrium and selection against heterozygotes also could possibly produce a reduction in genotypic diversity, but it is unlikely that these factors would be detectable within the small samples used in this study. The predominance of clonal proliferation by fission over larval recruitment is thus the most likely explanation for lowered genotypic diversity in the *O. ophiactoides* population at Discovery Bay. This finding is consistent with previous observations of low levels of larval recruitment at this site (Mladenov et al. 1983). It is also consistent with the predicted high rate of clonal proliferation in this population based on arm configuration and regeneration rate data collected by Mladenov et al. (1983). These authors deduced that the interval between fissions was of the order of 89 d (i.e., about four fissions per year). A single specimen could thus potentially produce about 15 (2^4-1) clonemates in a year (i.e., the outcome of four successive divisions less the original individual), or about 4 095 (2^{12}-1) clonemates over a 3 yr period.

The large differences in genotypic composition between the *Ophiocomella ophiactoides* samples from the two nearby (about 50 m separation) coves in Discovery Bay indicate that the spatial scale of clonal mixing is very local in this species. The overlap of a few genotypes between the two coves may reflect the inability of an analysis at the level of five-locus genotypes to perfectly distinguish clones, or it may be the result of limited dispersal of members of some clones between the coves, perhaps by rafting on algal clumps dislodged from the substratum during storms.

The morphological similarity (apart from differences in body size and symmetry) between *Ophiocoma pumila* and *Ophiocomella ophiactoides* has generated confusion over their taxonomic relationship (Parslow and Clark, 1963, Devaney 1970, Mladenov et al. 1983). Early workers often considered *Ophiocomella ophiactoides* to be the juvenile phase of *Ophiocoma pumila,* although more recent morphological, microdistributional, life-history and parasitological data suggest the presence of two closely related species (Parslow and Clark 1963, Devaney 1970, Mladenov et al. 1983, Emson and Mladenov 1987). In this study, we found the value of Nei's genetic identity for the two populations in Discovery Bay to be 0.517, which is of the order expected for sibling or distinct species of invertebrates (Ferguson 1980; p. 98). Our estimate of genetic identity should, however, be viewed with caution because it is based on a small number of variable loci. Inclusion of a larger and less variant sample of loci would probably yield a larger estimate of genetic identity which would indicate a close sibling-species relationship. In any case, a generic distinction between the two species does not seem justified on the basis of our genetic information. It is possible that *Ophiocomella ophiactoides* represents a relatively recently derived, miniaturized version of *Ophiocoma pumila*, the result, perhaps, of heterochrony caused by an acceleration of attainment of sexual maturity. This form may be selected for its capability to exploit both fission as a means of clonal growth (see "Adaptive significance of fissiparity" below) and sex as a means of genome shuffling and long-range dispersal.

Genetic structure of *Ophiactis savignyi* populations in sponges and alga clumps

Observed genotypic diversity was significantly lower than expectation for both sponge-dwelling and alga-dwelling populations of *Ophiactis savignyi.* As in *Ophiocomella ophiactoides,* depression in genotypic diversity in these populations is probably the result of the predominance of asexual proliferation by fission over larval recruitment. Although estimates of clonal growth based on measurements of rates of regeneration are not available for this species, it is known that the incidence of fission is very high in both sponge- and alga-dwelling populations (Mladenov and Emson 1988), which underscores the potential for clonal growth. Furthermore, the presence of only one sex in many sponge-dwelling aggregations, together with extremely high densities, is also consistent with high levels of clonal growth (Mladenov and Emson 1988).

A very small number of genotypes (putative clones) was present in each of the two sponges sampled and each sponge was dominated by a single genotype. Individual sponges may thus represent small, and hence infrequently colonized, targets for sexually produced larvae of *Ophiactis savignyi.* In this case, the first larva to colonize the sponge may establish the dominant clone. Alternatively, sponges may not be recruitment-limited, and the small number of clones present may be the result of the elimination of genotypes through clonal competition. The domination of each sponge by one genotype may reflect the outcome of such competition. In any case, the very high densities (up to 281 brittle stars dl^{-1} of sponge) and total numbers (up to 855 individuals per sponge; Mladenov and Emson 1988) attest to the proficiency with which small localized habitats can be utilized by *O. savignyi.* Assuming similar rates of clonal growth in this species as reported for *Ophiocomella ophiactoides* in the foregoing subsection (i.e., one fission every 89 d), it can be calculated that a single larval recruit could potentially fill a sponge with more than 1 000 clonemates in about 2.5 yr.

Despite the smaller sample size, many more five-locus genotypes were present in the sample of alga-dwelling *Ophiactis savignyi* compared to the sample of sponge-dwelling *O. savignyi.* Measures of observed genotypic diversity were correspondingly much higher. Rates of larval recruitment into these comparatively large expanses of algal turf may thus be higher than into sponges, or the rate of elimination of genotypes through clonal competition may be lower. The more contiguous nature of algal turf habitats might promote the dispersal of adult individuals, and thus the intermixing of clonal genotypes. On the other hand, the disjunct nature of the sponge habitats would probably discourage movement of brittle stars between sponges and encourage the formation of high-density aggregations. The large differences in genotypic composition between the two adjacent sponges studied may be indicative of the small scale of clonal dispersion in this habitat.

Sponge-dwelling populations of *Ophiactis savignyi* are characterized by large body size, high density and elevated levels of sexual reproduction; in contrast, alga-dwelling populations are characterized by small body size, low density and very low levels of sexual reproduction (Mladenov and Emson 1988). Results of this study show that the allelic composition of sponge- and alga-dwelling *O. savignyi* is also strikingly different. Only one of the 17 alleles at the five loci studied was common to both populations. It thus seems possible that sponge- and alga-dwelling *O. savignyi* represent sibling species. Alternatively, there may be intense selection for certain groups of alleles at the loci under consideration in these very different habitats.

It should be emphasized that observations on the population genetic structure of *Ophiactis savignyi* are based on samples taken from only two sponges and one algal site. A comparative study involving more sites and larger samples should be performed to confirm and extend our findings.

Adaptive significance of fissiparity

We proposed earlier (Mladenov and Emson 1984) that fissiparity in brittle stars may have evolved in association with small body size, concomitant low fecundity and planktotrophic larval development as a means of ensuring recruitment. In the light of the information reported here on the population genetic structure of fissiparous brittle stars, it may be more profitable to view fissiparity as a mechanism of clonal growth rather than recruitment, because fission can create a large number of genetically identical modules (ramets) forming a dispersed clone (genet). As pointed out in Hendler and Littman (1986) and by Pearse (1988), such "replicate copy growth" might increase the fitness of genets by increasing genotype specific biomass and, hence, fecundity. A similar argument might be applicable to some other marine invertebrates known to generate clones of locally dispersed individuals, such as fissiparous sea stars (Johnson and Threlfall 1987) and asexually proliferating sea anemones (Hoffmann 1976, 1986, Ayre 1983, 1984).

The sibling brittle stars *Ophiocoma pumila* and *Ophiocomella ophiactoides* may provide a model for critically examining the consequences of replicate copy growth by comparing total biomass and clutch size of individuals of *Ophiocoma pumila* with electrophoretically distinguished genets of *Ophiocomella ophiactoides*. A rough estimate based on existing data provides some notion of how large an *O. ophiactoides* genet might have to be to compare to an *Ophiocoma pumila* individual in terms of clutch size. Maximum clutch size of *O. pumila* is probably in the order of one million eggs per individual (Hendler 1975, Mladenov and Emson 1984), whereas maximum clutch size per ramet of *Ophiocomella ophiactoides* is about 7 400 eggs (Mladenov and Emson 1984). Thus, in terms of fecundity, a single *Ophiocoma pumila* individual would be roughly equivalent to that of a genet of about 135 sexual *Ophiocomella ophiactoides* ramets. Given the high potential for clonal growth in *O. ophiactoides*, genet size is probably often much larger than this. Furthermore, since genets may be long-lived, one should also consider dead ramets, that would have contributed to egg production in the past, in any calculation of total genet size and fecundity. It is thus possible that replicate copy growth dramatically increases genotype specific fitness under certain circumstances in nature. The fact that only about 2% of extant brittle star species have exploited fissiparity (Emson and Wilkie 1980, Mladenov et al. in press) suggests, however, that some important barriers to the evolution of fissiparity are present. One such barrier might involve body size. As discussed in Mladenov and Emson (1984), large-bodied brittle stars may be barred from exploiting fissiparity because of mechanical or energetic constraints that are not shared by small-bodied species.

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