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Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actinidae) with different reproductive strategies

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Abstract Genetic variation and population structure of two tropical sea anemones, *Bunodosoma caissarum* Correa and *Actinia bermudensis* McMurrich were related to their different dispersal capabilities and reproductive modes. *B. caissarum* reproduces sexually and has a long-lived planktotrophic larva; *A. bermudensis* can reproduce both sexually and asexually, supposedly with short-range dispersal. Both species were sampled along 1150 km of Brazilian coastline between 1990 and 1991 and analyzed by horizontal starch gel electrophoresis for 16 enzyme loci in *B. caissarum* and 19 in *A. bermudensis*. *B. caissarum* had higher levels of heterozygosity ($H=0.35$) and lower levels of population structuring ($F_{ST}=0.042$) than *A. bermudensis* ($H=0.17$; $F_{ST}=0.262$). At one locality large genetic differences between two sympatric colour morphs of *A. bermudensis* provide evidence of possible cryptic speciation. Overall levels of genetic variation and heterozygosity in the two species are compatible with their known modes of reproduction.

Introduction

Dispersal is a major factor counteracting the genetic differentiation of geographically separated populations and, therefore, not surprisingly, dispersal capability is considered to strongly influence the genetic structure of marine populations (Olson 1985; Lessa 1990; Hansson 1991; Bertness and Gaines 1993). In marine species, dispersal is related not only to external factors such as water movement, but also to the intrinsic characteristics of the species, like reproductive mode (Jackson 1986), vagility and viability of gametes (Grosberg 1991), and type and mobility of the larvae (Scheltema 1989) and adults (Chadwick and Adams 1991).

Asexual reproduction is usually associated with reduced dispersal (Jackson 1986) since the main dispersal agent is not a planktonic larva, but small individuals or fragments that are likely to attach to the first hard substrate they find (Shaw 1991). Moreover, the sexually produced larvae of facultative asexual organisms generally have a shorter precompetent phase than those produced by strictly sexually reproducing species (Jackson 1986).

We compared the levels of genetic structuring and variability in two related sea anemones (family Actiniidae) with different modes of reproduction and dispersal capabilities. *Bunodosoma caissarum* is common intertidally on rocky shores over much of the coastline of Brazil (Schlenz 1983) and occurs often at high density (>100 ind m^{-2}; authors' unpublished results). Its reproduction was studied by Belém (1987), who suggested that the larva was long-lived, sexually produced and planktotrophic. *B. caissarum* is not known to reproduce asexually. *Actinia bermudensis* is considered to have a wide distribution (Schlenz 1983), occurring intertidally from Southern Brazil northwards to Florida. In Brazil it does not occur in dense aggregations (<5 ind m^{-2}) and is far less abundant than *B. caissarum* (authors' unpublished results). Its mode of sexual reproduction is unclear (Chia 1976; Jennison 1983), although a larva that may be planktonic has been described by Jennison (1983). His description is questionable, not least because he expresses doubt as to the generic identity of the specimens studied. Asexual reproduction occurs through the release of small brooded individuals (Russo 1991), which are unlikely to have significant dispersal capability over any distance.

We hypothesised that, given the greater potential for dispersal in *Bunodosoma caissarum*, there would be higher

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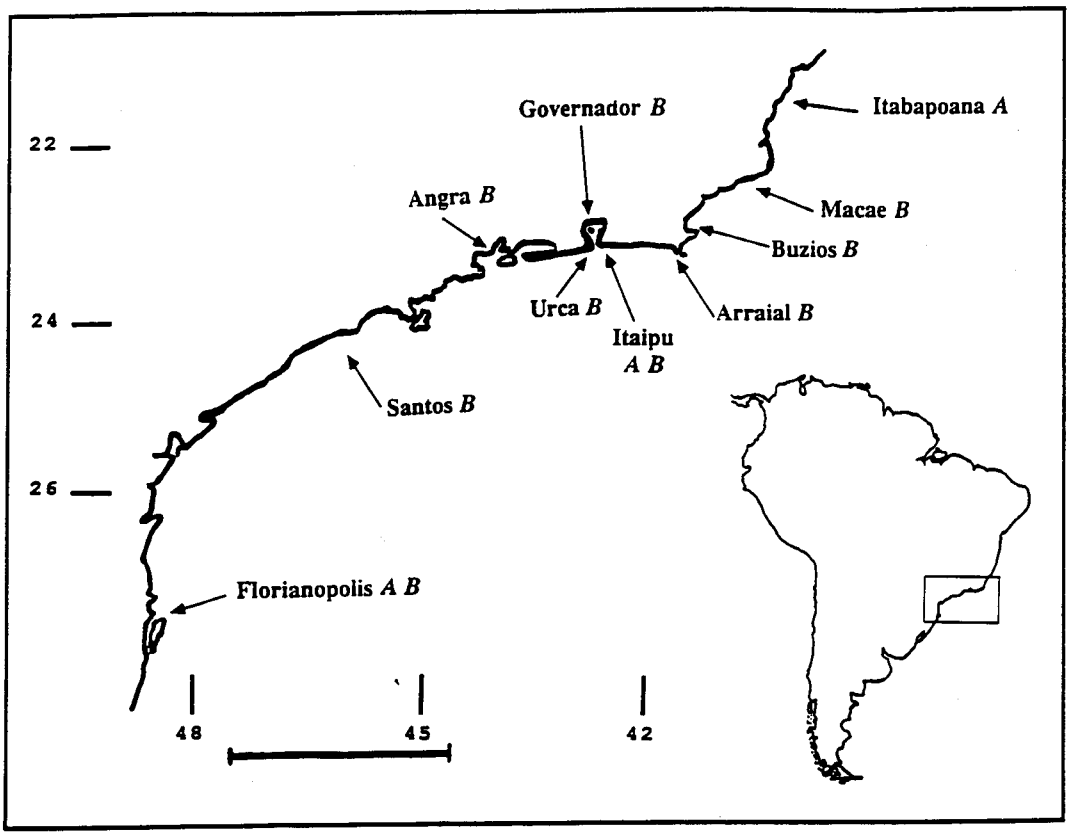

Fig. 1 *Bunodosoma caissarum* and *Actinia bermudensis*. Map showing sites from which sea anemones were collected. *B* (*B. caissarum*) and *A* (*A. bermudensis*) indicate species collected at each site. Scale = 300 km. Inset: map of South America to indicate area covered by larger map

levels of heterozygosity within populations and lower levels of genetic differentiation between populations in that species compared with *Actinia bermudensis*. We used enzyme electrophoresis to study allozyme variation at 16 putative loci in *B. caissarum* and 19 in *A. bermudensis*.

Materials and methods

Ten intertidal sites were sampled from approximately 1150 km of the coastline of Brazil between 1990 and 1991. *Bunodosoma caissarum* was present at nine sites and *Actinia bermudensis* at three (Fig. 1). Total sample sizes were 353 individuals for *B. caissarum* and 193 for *A. bermudensis*. Collections of *A. bermudensis* comprised red and brown morphs at Florianopolis, the most southerly site, red only at Itaipu and red and yellow morphs at Barra de Itabapoana in the north. The colour morphs, although mixed on the shore, were very distinct and were treated as separate samples. To reduce the likelihood of sampling clonemates, individual anemones were collected at least 2 m apart (see Hoffman 1986). Subsequently all genetically identical individuals in any sample were eliminated from the analysis on the assumption that they were from the same clone. This procedure was not necessary in *B. caissarum* because in this species no two individuals sampled were found to be genetically identical over all loci. *A. bermudensis* was not abundant and the numbers of anemones collected were restricted to prevent depletion of the populations. At each site estimates were made (using random quadrats) of the approximate population density of each species. The anemones were transported on ice to the laboratory, where they were kept frozen at −20 °C until required for electrophoresis. No samples were stored for more than 1 mo after collection.

Tissue samples were taken from the oral disc and column, homogenised in not more than an equal volume of distilled water, and analysed by horizontal 13% starch-gel electrophoresis. For details of electrophoretic methods for sea anemones see Solé-Cava et al. (1985). The buffer system used was Tris-citrate pH 8.0 (Ward and Beardmore 1977). Other buffer systems, including Tris-citrate pH 7.0 (Shaw and Prasad 1970) and Tris-citrate/borate (Poulik 1957) were tried but gave at best similar results. Enzyme nomenclature and staining recipes followed Harris and Hopkinson (1978) and Richardson et al. (1986).

A total of 16 putative loci coding for 14 different enzymes were typed for each of the nine populations of *Bunodosoma caissarum* and 19 putative loci coding for 16 different enzymes in each sample of *Actinia bermudensis*. Several other enzymes were stained for, but gave no useful results. After scoring the gels were fixed and preserved in 5% glycerine (Numachi 1981).

Genotype frequency data were analyzed using the Fortran program BIOSYS-1 (Swofford and Selander 1981). Fixation indices F_{IS}, F_{ST} and F_{IT} (Nei 1977) were computed from the genotype data. The significance of F_{IS} [H_0 (mean observed heterozygosity): $F_{IS}=0$] and F_{ST} (H_0: $F_{ST}=0$) were calculated using χ^2 (Waples 1987) for:

$$F_{IS}: \chi^2 = F_{IS}^2 (k-1);\ \mathrm{df} = [k(k-1)]/2 \quad (1)$$

and for

$$F_{ST}: \chi^2 = 2 N F_{ST} (k-1);\ \mathrm{df} = (k-1)(s-1), \quad (2)$$

where N is the total number of individuals sampled, k is the number of alleles and s the number of sub-populations analyzed for each locus. Two separate approaches were used to calculate the number of migrants per generation (mN_e):

$$mN_e = [(1/F_{ST}) - 1]/4 \quad \text{(Wright 1978)} \quad (3)$$

and

$$mN_e = e^{-[\ln p(1) + 2.44]/0.505}/(N/25) \quad \text{(Slatkin 1987)}, \quad (4)$$

where $p(1)$ is the average frequency of all private alleles (alleles present in only one population) and N the average number of individuals sampled per population.

Genetic Identity, I, and Genetic Distance, D, (Nei 1978) were calculated for all possible pairwise comparisons of samples within each species and clusters of pairwise unbiased Genetic Identities (Nei

1978) were produced using UPGMA (unweighted pair group mean analysis) (Sneath and Sokal 1973).

Specimens of each species were deposited in the Cnidarian Collection of the Zoology Department of the Federal University of Rio de Janeiro, under the numbers COL. CNID. DZIBUFRJ 2-867 (*Bunodosoma caissarum*) and DZIBUFRJ 2-896 (*Actinia bermudensis*).

Results

Allele frequencies for all loci studied in each sample of *Bunodosoma caissarum* and *Actinia bermudensis* are given in Tables 1 and 2. Mean numbers of anemones typed per locus for each locality varied from 23.6 to 29.1 in *B. caissarum* and from 10.5 to 30.2 in *A. bermudensis*.

Bunodosoma caissarum showed, within the expected limits of sampling errors, genetic uniformity over all sampling localities, with no locus being significantly (χ^2 test, $P<0.05$) genetically heterogeneous over the sites sampled. *Actinia bermudensis* showed marked structuring between populations, with highly significant (contingency chi-squared; $P<0.001$) differences for all pairwise comparisons between populations of the red morph from each of the three areas sampled. This difference was particularly marked ($P<10^{-6}$) between the sample from Florianopolis and the two other areas. There were also significant differences (contingency χ^2 analysis; $P<0.01$ to $P<0.0001$ in each case) between the red and brown morphs from Florianopolis at six (*Me-1*, *Mpi*, *Gdh*, *Sod*, *Cat*, *Fum*) of the 19 loci. There were smaller differences between the red and yellow morphs from Itabapoana, where only *Pgm* differs significantly ($P<0.05$), and over the three red-morph samples the level of genetic differentiation was very high ($F_{ST}=0.262$). The overall F_{ST} was significantly different from zero ($\chi^2=269.0$, df=32; $P<<0.001$).

Table 1 *Bunodosoma caissarum*. Allele frequencies for 16 enzyme loci. Abbreviations of the locations are – Flo: Florianopolis; San: Santos; Ang: Angra dos Reis; Urc: Urca; Gov: Ilha do Governador; Ita: Itaipu; Arr: Arraial do Cabo; Buz: Buzios; Mac: Macae. F_{IS}: local inbreeding for the locus: F_{ST}: inbreeding due to population subdivision for the locus: N: mean sample size; H_0: mean observed heterozygosity; H_e: mean expected heterozygosity per locus. Levels of significance: * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Locus / Allele	Location									F_{IS}	F_{ST}
	Flo	San	Ang	Urc	Gov	Ita	Arr	Buz	Mac		
Cat											
a	0.28	0.38	0.21	0.32	0.47	0.10	0.03	0.05	0.14	0.0426	0.0690***
b	0.45	0.33	0.40	0.47	0.36	0.63	0.76	0.65	0.56		
c	0.27	0.27	0.27	0.21	0.12	0.20	0.21	0.25	0.26		
d	0.00	0.02	0.12	0.00	0.05	0.07	0.00	0.05	0.04		
D-Est											
a	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	–0.1111	0.0137
b	1.00	1.00	1.00	0.98	1.00	0.98	1.00	1.00	1.00		
c	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00		
Gdh											
a	0.00	0.00	0.02	0.00	0.00	0.04	0.02	0.00	0.00	0.3345***	0.0216
b	0.16	0.13	0.14	0.12	0.17	0.07	0.08	0.09	0.05		
c	0.74	0.72	0.60	0.79	0.67	0.77	0.83	0.81	0.82		
d	0.10	0.10	0.21	0.09	0.11	0.09	0.07	0.10	0.13		
e	0.00	0.05	0.03	0.00	0.05	0.04	0.00	0.00	0.00		
Got											
a	0.00	0.02	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.0627	0.0290*
b	0.21	0.17	0.13	0.09	0.04	0.09	0.15	0.14	0.12		
c	0.62	0.78	0.70	0.69	0.85	0.83	0.73	0.69	0.83		
d	0.17	0.03	0.10	0.22	0.11	0.08	0.12	0.17	0.05		
Hk											
a	0.00	0.02	0.05	0.02	0.00	0.04	0.00	0.00	0.00	0.0141	0.0449***
b	0.21	0.18	0.07	0.17	0.24	0.11	0.22	0.08	0.05		
c	0.47	0.30	0.33	0.26	0.10	0.23	0.25	0.20	0.29		
d	0.32	0.50	0.50	0.55	0.66	0.62	0.53	0.72	0.66		
e	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00		
Lap											
a	0.08	0.02	0.00	0.13	0.18	0.04	0.08	0.09	0.07	0.1511**	0.0270**
b	0.84	0.94	0.76	0.75	0.74	0.83	0.84	0.85	0.87		
c	0.04	0.02	0.12	0.08	0.06	0.09	0.06	0.05	0.04		
d	0.04	0.02	0.12	0.04	0.02	0.04	0.02	0.01	0.02		
Mdh-1											
a	0.00	0.00	0.00	0.01	0.00	0.00	0.03	0.00	0.00	0.0095	0.0187
b	1.00	0.97	0.96	0.99	1.00	0.98	0.92	0.95	0.98		
c	0.00	0.03	0.02	0.00	0.00	0.02	0.05	0.03	0.02		
d	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.02	0.00		

Table 1 (continued)

Locus / Allele	Location									F_{IS}	F_{ST}
	Flo	San	Ang	Urc	Gov	Ita	Arr	Buz	Mac		
Me											
a	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.05	0.00	–0.0279	0.0259*
b	0.92	0.92	0.90	0.94	0.96	0.92	0.84	0.89	0.98		
c	0.08	0.02	0.09	0.02	0.02	0.04	0.14	0.06	0.00		
d	0.00	0.05	0.01	0.02	0.02	0.02	0.02	0.00	0.02		
Mpi											
a	0.59	0.56	0.40	0.36	0.37	0.36	0.50	0.56	0.33	0.3455***	0.0303**
b	0.22	0.19	0.44	0.38	0.32	0.32	0.27	0.29	0.44		
c	0.09	0.15	0.10	0.07	0.23	0.19	0.16	0.11	0.17		
d	0.10	0.10	0.06	0.19	0.08	0.13	0.07	0.04	0.06		
Odh											
a	0.00	0.13	0.13	0.25	0.27	0.08	0.10	0.03	0.10	0.8520***	0.0642***
b	0.22	0.10	0.25	0.42	0.17	0.26	0.30	0.37	0.50		
c	0.02	0.13	0.12	0.03	0.06	0.08	0.05	0.00	0.02		
d	0.76	0.64	0.50	0.30	0.50	0.58	0.55	0.60	0.38		
Pgd											
a	0.00	0.00	0.02	0.00	0.00	0.00	0.09	0.00	0.00	0.3398***	0.0473***
b	0.31	0.38	0.35	0.28	0.12	0.14	0.36	0.31	0.19		
c	0.50	0.40	0.46	0.60	0.53	0.81	0.45	0.55	0.65		
d	0.19	0.22	0.17	0.12	0.30	0.05	0.10	0.14	0.16		
e	0.00	0.00	0.02	0.00	0.05	0.00	0.00	0.00	0.00		
Pgi-1											
a	0.00	0.04	0.07	0.02	0.00	0.00	0.01	0.01	0.02	0.1490**	0.0321**
b	0.50	0.58	0.41	0.54	0.55	0.70	0.50	0.37	0.44		
c	0.50	0.38	0.52	0.44	0.45	0.30	0.49	0.62	0.54		
Pgi-2											
a	0.35	0.44	0.67	0.43	0.57	0.46	0.48	0.43	0.23	0.1621**	0.0552***
b	0.63	0.45	0.30	0.37	0.43	0.48	0.52	0.52	0.68		
c	0.02	0.11	0.03	0.20	0.00	0.06	0.00	0.05	0.09		
Pgm											
a	0.04	0.04	0.03	0.09	0.02	0.04	0.05	0.02	0.00	0.0766	0.0181
b	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
c	0.87	0.89	0.90	0.79	0.87	0.84	0.92	0.96	0.85		
d	0.07	0.07	0.07	0.12	0.11	0.11	0.03	0.02	0.12		
e	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.03		
Sod-1											
a	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	–0.1111	0.0182
b	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00		
Sod-2											
a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	–0.2037***	0.0270
b	1.00	1.00	1.00	0.97	1.00	1.00	1.00	0.98	1.00		
c	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00		
N	23.6	28.4	27.7	29.1	27.4	27.1	27.5	27.6	27.3		
H_0	0.29	0.28	0.29	0.27	0.28	0.25	0.28	0.28	0.26		
H_e	0.35	0.36	0.39	0.39	0.36	0.33	0.35	0.33	0.32		

For the two species fixation indices (Nei 1977) and other estimates of gene flow from Waples (1987) and Slatkin (1987) are given in Table 3. The inbreeding due to population subdivision (Nei 1977) was low in *Bunodosoma caissarum* [$F_{ST}=0.042$; *Nm* (effective number of migrants) $=5.7$ to 9.2 ind generation^{-1}], but very high in *Actinia bermudensis* ($F_{ST}=0.262$; $Nm=0.4$ to 0.7).

Gene identity (Nei 1978) values for all pairwise comparisons between the nine samples of *Bunodosoma caissarum* were all above 0.95 (Table 4). Between the five samples of *Actinia bermudensis* studied, *I*-values (Table 5) ranged down to 0.82. The lower *I*-values (all within the range 0.82 to 0.85) occurred between the two colour morphs from Florianopolis and the other three samples from further north. Fig. 2 shows UPGMA (Sneath and Sokal 1973) dendrograms of identity values between populations of *B. caissarum* and *A. bermudensis*.

Fig. 3 shows pie diagrams of geographical variation in allele frequencies for the two most heterogeneous (highest F_{ST}-value) loci in each of the two species. Our data for

Table 2 *Actinia bermudensis.* Allele frequencies for 19 enzyme loci. Colour morphs and sites are – RF: red colour morph from Florianopolis; BF: brown colour morph from Florianopolis; RI: red colour morph from Itaipu; RB: red colour morph from Itabapoana; YB: yellow colour morph from Itabapoana. F_{IS}: local inbreeding; F_{ST}: inbreeding due to population subdivision (red morph only); N: mean sample size; H_0: mean observed heterozygosity; H_e: mean expected heterozygosity per locus. Levels of significance: * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Locus / Allele	Location					F_{IS}	F_{ST}
	RF	BF	RI	RB	YB		
Cat							
a	0.13	0.05	0.17	0.11	0.27	0.2757	0.0097
b	0.54	0.95	0.46	0.61	0.50		
c	0.33	0.00	0.37	0.28	0.23		
D-Est							
a	1.00	1.00	1.00	1.00	1.00	–	–
Est							
a	0.04	0.00	0.75	0.90	0.75	0.0674	0.5011***
b	0.89	0.72	0.25	0.10	0.25		
c	0.03	0.17	0.00	0.00	0.00		
d	0.04	0.11	0.00	0.00	0.00		
Fum							
a	0.82	1.00	1.00	1.00	1.00	0.1440	0.1289**
b	0.18	0.00	0.00	0.00	0.00		
Gdh							
a	0.12	0.00	0.43	0.10	0.00	0.3300*	0.0930***
b	0.84	1.00	0.50	0.66	0.68		
c	0.04	0.00	0.05	0.16	0.07		
d	0.00	0.00	0.02	0.08	0.11		
e	0.00	0.00	0.00	0.00	0.14		
Got							
a	0.97	0.95	1.00	1.00	1.00	–1.3944***	0.0229
b	0.03	0.05	0.00	0.00	0.00		
Hk							
a	0.95	0.95	1.00	1.00	1.00	–0.4286***	0.0352*
b	0.05	0.05	0.00	0.00	0.00		
Lap							
a	1.00	1.00	1.00	1.00	1.00	–	–
Mdh							
a	1.00	1.00	1.00	1.00	1.00	–	–
Me-1							
a	0.00	0.00	0.07	0.00	0.08	–0.1620	0.0415
b	0.63	1.00	0.79	0.77	0.63		
c	0.37	0.00	0.14	0.18	0.29		
d	0.00	0.00	0.00	0.05	0.00		
Me-2							
a	0.00	0.00	0.05	0.00	0.00	–0.1693	0.0239
b	0.95	1.00	0.95	1.00	1.00		
c	0.05	0.00	0.00	0.00	0.00		
Mpi							
a	0.14	0.61	0.18	0.10	0.00	0.1424	0.1013***
b	0.86	0.39	0.52	0.84	0.96		
c	0.00	0.00	0.30	0.06	0.04		
Odh							
a	0.00	0.05	0.00	0.00	0.00	0.4209***	0.0040
b	0.01	0.05	0.00	0.00	0.00		
c	0.65	0.77	0.58	0.60	0.64		
null	0.34	0.13	0.42	0.40	0.36		
Pgd							
a	0.90	0.97	0.00	0.00	0.00	–0.2167	0.8571***
b	0.10	0.03	1.00	1.00	1.00		
Pgi-1							
a	1.00	1.00	1.00	1.00	1.00	–	–
Pgi-2							
a	0.77	0.70	0.64	0.83	0.65	–0.1111	0.0329
b	0.21	0.30	0.36	0.17	0.35		
c	0.02	0.00	0.00	0.00	0.00		
Pgm							
a	1.00	1.00	0.00	0.15	0.00	0.5305**	0.8164***
b	0.00	0.00	1.00	0.85	1.00		
Sod							
a	0.61	1.00	1.00	1.00	1.00	–0.9686***	0.3017**
b	0.39	0.00	0.00	0.00	0.00		
Xod							
a	1.00	1.00	1.00	1.00	1.00	–	–
N	30.2	19.8	13.8	13.9	10.5		
H_0	0.17	0.14	0.19	0.11	0.15		
H_e	0.21	0.11	0.20	0.16	0.17		

Table 3 *Bunodosoma caissarum* and *Actinia bermudensis.* Fixation indices (Wright 1978) and estimates of gene flow. Only samples from the red morph of *A. bermudensis* were used for this analysis. mN_e (F_{ST}) and mN_e [p (1)] are estimates of gene flow based on the formulae of Waples (1987) and Slatkin (1987), respectively

Species	Fixation index			mN_e (F_{ST})	mN_e [p (1)]
	F_{IS}	F_{IT}	F_{ST}		
B. caissarum	0.234	0.266	0.042	5.7	9.2
A. bermudensis	0.092	0.330	0.262	0.4	0.7

Table 4 *Bunodosoma caissarum.* Pairwise unbiased genetic identities (above diagonal) and distances (below diagonal) (Nei 1978)

Location	Flo	San	Ang	Urc	Gov	Ita	Arr	Buz	Mac
Florianopolis (Flo)	–	0.996	0.983	0.978	0.972	0.978	0.990	0.988	0.976
Santos (San)	0.004	–	0.990	0.982	0.989	0.980	0.985	0.981	0.971
Angra (Ang)	0.017	0.011	–	0.989	0.986	0.981	0.987	0.987	0.976
Urca (Urc)	0.022	0.018	0.011	–	0.989	0.989	0.985	0.985	0.991
I. Governador (Gov)	0.029	0.012	0.014	0.011	–	0.983	0.974	0.975	0.974
Itaipu (Ita)	0.022	0.020	0.019	0.011	0.017	–	0.991	0.988	0.989
Arraial do Cabo (Arr)	0.010	0.015	0.013	0.015	0.026	0.009	–	1.000	0.986
Buzios (Buz)	0.012	0.019	0.013	0.015	0.026	0.012	0.000	–	0.993
Macae (Mac)	0.024	0.029	0.025	0.010	0.027	0.011	0.014	0.007	–

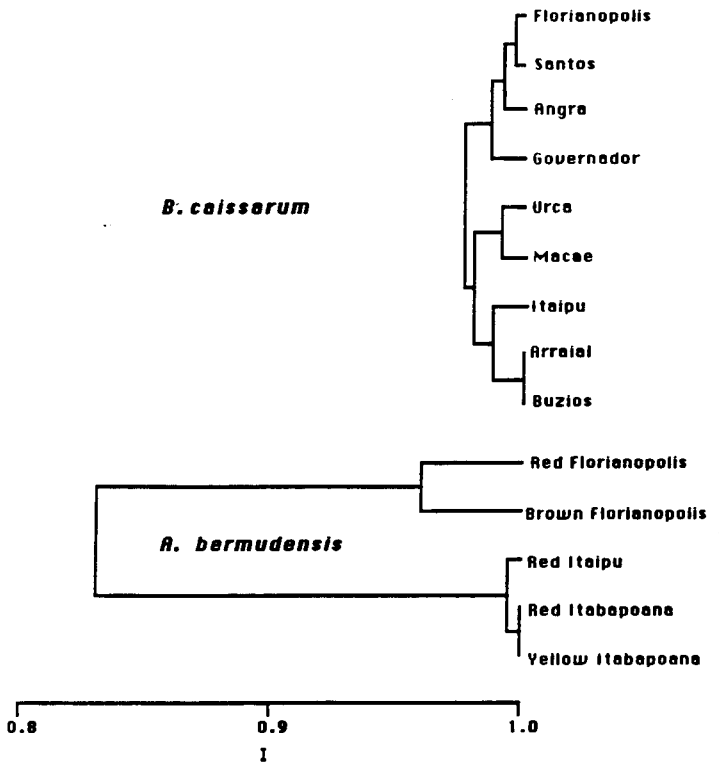

Fig. 2 *Bunodosoma caissarum* and *Actinia bermudensis*. Unweighted pair group mean analysis dendrogram of values for Neil's (1978) genetic identity, *I*, between samples from all sites sampled along the coast of Brazil

Table 5 *Actinia bermudensis*. Pairwise unbiased genetic identities (above diagonal) and distances (below the diagonal) (Nei 1978)

Sample	RF	BF	RI	RB	YB
Red Florianopolis (RF)	–	0.959	0.830	0.848	0.846
Brown Florianopolis (BF)	0.042	–	0.823	0.835	0.821
Red Itaipu (RI)	0.186	0.195	–	0.995	0.995
Red Itabapoana (RB)	0.165	0.180	0.005	–	1.000
Yellow Itabapoana (YB)	0.168	0.197	0.005	0.000	–

Bunodosoma caissarum (Table 4) indicate a positive correlation between geographic distance and the genetic distance between populations ($r=0.33$; $F=4.22$; df=34; $P<0.05$). All nine samples of *B. caissarum* showed an overall heterozygote deficiency. The same was observed in four out of the five *Actinia bermudensis* samples studies. Both the overall F_{IS} (Nei 1977) for *B. caissarum* ($F_{IS}=0.2340$; $\chi^2=943.5$; df=95; $P<<0.001$) and for the red morphotype of *A. bermudensis* ($F_{IS}=0.0920$; $\chi^2=198.4$; df=74; $P<<0.001$) were significantly different from zero.

Bunodosoma caissarum showed significant ($P<0.001$) departures from Hardy-Weinberg equilibrium for the *Odh* (octopine dehydrogenase) locus at all nine collection sites, in each case due to an excess of homozygotes. A notable feature of the results at this locus was that some individuals of the species did not give any enzyme activity for *Odh*, although showing normal activity for the other enzyme systems. Hence it is likely that the heterozygote deficiency is a result of the presence of null alleles (i.e., alleles that code for a non-functional enzyme).

The overall levels of genetic variation found in *Bunodosoma caissarum* were twice as high (mean heterozygosity, $H=0.35$) as those found in *Actinia bermudensis* ($H=0.17$). In *B. caissarum* none of the 16 loci was monomorphic in all populations and in *A. bermudensis* five of the 19 loci were monomorphic across all populations.

Population densities of *Bunodosoma caissarum* were several times higher than those of *Actinia bermudensis*. Mean densities of 35 ind m^{-2} (up to 200 m^{-2} in some places) were observed for *B. caissarum*, whereas *A. bermudensis* were seldom found at densities higher than 5 m^{-2}.

Discussion

The results appear compatible with our initial hypothesis that levels of genetic differentiation between populations are related to dispersal capability. Whereas *Bunodosoma caissarum* showed genetic uniformity over all sampling localities, *Actinia bermudensis* showed marked differences between populations.

The lack of apparent genetic divergence between nine samples of *Bunodosoma caissarum* spread over about 1150 km of coastline is possibly not surprising in a species with a planktotrophic larva, but from various recent studies it is clear that the potential for larval dispersal is not always realised in marine invertebrates. It appears that in *B. caissarum* the larva is an effective mechanism of dispersal. Indeed our data suggest that *B. caissarum* maintains a homogeneous population structure over a greater distance than any other sea anemone. Hunt and Ayre (1991) showed little genetic differentiation ($F_{ST}=0.0295$) between several samples of the sexually reproducing species *Oulactis muscosa* over 735 km of shoreline in southeastern Australia. Unfortunately the larva of *O. muscosa* is not known, but the data for this species appear to be broadly comparable with those for *B. caissarum*. Ayre et al. (1991) studied *Actinia tenebrosa* over about 1000 km in southeastern Australia. This species reproduces both sexually and asexually (by brooded offspring) and shows marked population subdivision over the distance studied. An earlier study of this species (Ayre 1984) showed greater overall genetic uniformity over large distances along the coast of southwestern Australia, but marked discontinuities between this area and populations further east. Within the western region sampled there was local heterogeneity with relatively few clones dominating in any given area.

A notable feature of our results is the clear genetic differentiation between the allopatric populations of what appear to be the same red morph of *Actinia bermudensis*. From other studies comparatively substantial differentiation among samples of particular morphs over short geographical distances appears to be a general feature of the population structure of some *Actinia* species (Ayre et al. 1991; Solé-Cava and Thorpe 1992), as predicted by some

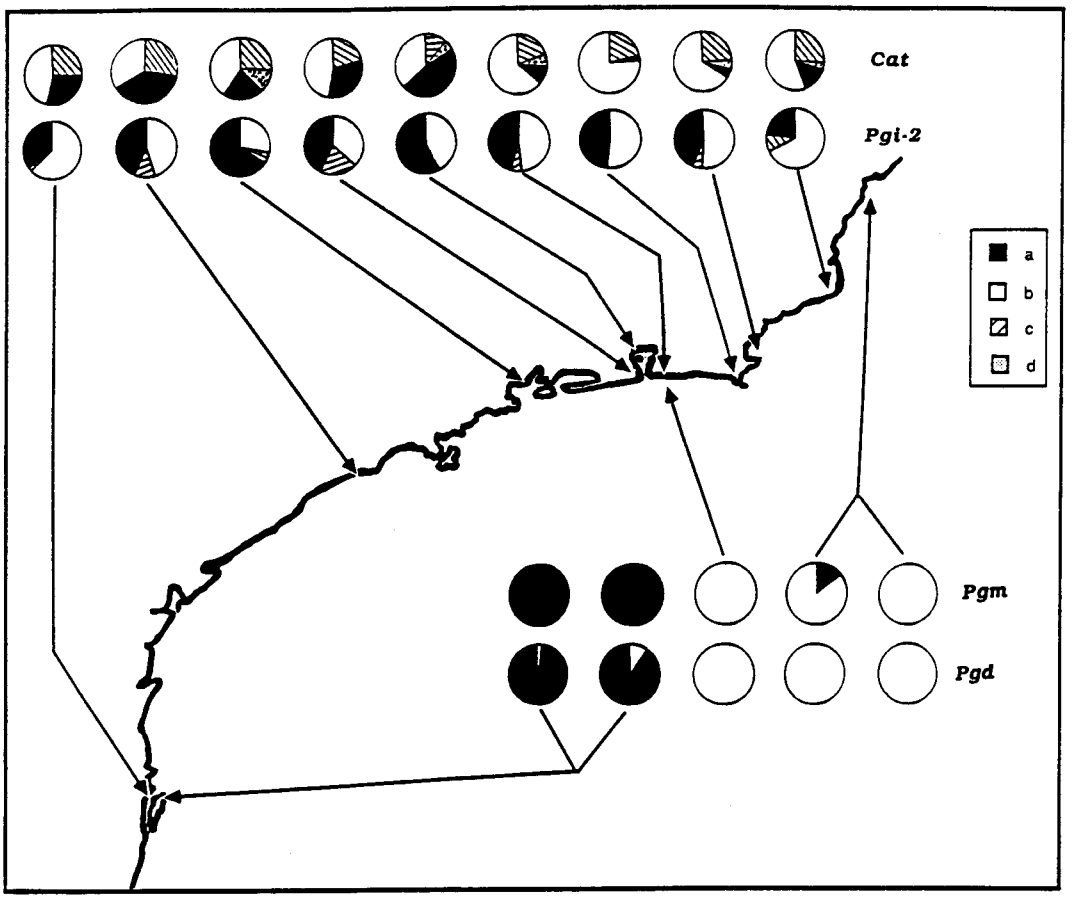

Fig. 3 *Bunodosoma caissarum* (top) and *Actinia bermudensis* (bottom). Pie diagrams illustrating variation in allele frequencies among sampling sites. Diagrams are for the two loci with the highest F_{ST}-values in each species

models of evolutionary strategy for facultative clonal organisms (Williams 1975). Such divergence is likely to be enhanced by the possibility that effective population sizes are small in most *Actinia* species (Solé-Cava and Thorpe 1991) and by the potential for population bottlenecks and founder effects. Thus the divergence found here over longer distances is not entirely unexpected. However, divergence between sympatric morphs is more surprising.

Between sympatric samples of a single species (e.g. conspecific morphs) there should (given certain assumptions) be no significant difference in gene frequency at any locus since, by definition, conspecific individuals should be freely interbreeding and thus part of a single population. Therefore among sympatric populations any significant variation in gene frequency indicates the probability of the existence of a barrier to gene flow and, consequently, that the two populations are likely to be different species (Solé-Cava et al. 1985). Between the red and brown morphs of *Actinia bermudensis* at Florianopolis six (*Me 1*, *Mpi*, *Gdh*, *Sod*, *Cat*, *Fum*) out of the 19 loci analysed differed significantly. This level of difference provides a clear indication of lack of gene flow between populations, thus indicating that the sympatric populations are likely to be reproductively isolated. Assumptions are that the two colour morphs are not subject to heterogeneous selection or recruitment, but it is not plausible that either of these factors could lead to genetic differentiation on the scale observed. Therefore it follows that the two colour morphs are probably not part of the same biological species. The genetic identity found between these two putative species was surprisingly high (0.96), within the range of values usually expected for conspecific populations (for discussion see Thorpe 1982, 1983; Nei 1987). However, in the present work such criteria are not highly relevant to species identification because the numerous significant differences in allele frequencies indicate that the sympatric populations of these morphs are reproductively isolated. Where morphs are sympatric, and allele frequencies show significant differences, high genetic identities should not be taken as evidence that the populations are not reproductively isolated. There are several examples in the literature of pairs of clearly distinct species with surprisingly high identities (e.g. Avise and Aquadro 1982; Solé-Cava et al. 1985).

It should also be noted that in the present work the *I*-values are misleadingly high because they are affected by the high levels of polymorphism. High levels of heterozygosity are common in sea anemone populations (Solé-Cava and Thorpe 1991), and the consequent problem of the interpretation of unexpectedly high *I*-values has been noted and discussed previously (Solé-Cava et al. 1985; Solé-Cava and Thorpe 1992).

Between the red and yellow morphs from Itabapoana there were smaller differences and only *Pgm* differed significantly ($P<0.05$), but this single significant difference (among 19 loci) could be a consequence of sampling error

(see Lessios 1992). Similar divergence and unexpected cryptic speciation have been found in various studies of colour morphs of *Actinia 'equina'* in Britain (Haylor et al. 1984: Solé-Cava and Thorpe 1987, 1992), as well as in a surprisingly high proportion of other sea anemone genera that have been studied (e.g. *Bunodosoma* McCommas and Lester 1980; *Metridium* Bucklin and Hedgecock 1982; *Urticina* Solé-Cava et al. 1985; *Sagartia* Shaw et al. 1987). For the population genetics analysis only anemones from the well characterised and morphologically homogeneous red morph of *Actinia bermudensis* were used.

High genetic divergence between conspecific populations has been reported in other *Actinia* species, even when sampled over short geographical distances. Such localised genetic differentiation indicates the likelihood of low larval dispersal. Unfortunately practically nothing is known of larval dispersal in *Actinia* spp., and the reason that dispersal is apparently so slight could be simply that a dispersive larva may not be produced. Chia (1976) and Carter and Miles (1989) concluded that *A. equina* lacked a dispersive larval stage, although from observed population structure Ayre (1984) considered that the occasional production of dispersed larvae may occur in *A. tenebrosa*. The only offspring known to be produced in most *Actinia* species are the small brooded anemones, which are always genetically identical to the brooding parent (Black and Johnson 1979; Orr et al. 1982; Russo 1991) and, hence, must be asexually reproduced. Sexual reproduction must occur in *Actinia* species because in most populations some individuals can be seen (in histological sections) to have gonads (Carter and Miles 1989; Shick 1991). Also, populations of these species generally show high levels of genetic variability and their allele frequencies, once clones are excluded, usually approximate to Hardy-Weinberg expectations. However, details of mode of sexual reproduction or of larval production or dispersal in most *Actinia* species remain unknown. The few descriptions of alleged sexually produced larvae (e.g. Stephenson 1935) are often clearly wrong and are describing asexual progeny. As mentioned above, Jennison's (1983) description of a planktonic larva in *A. bermudensis* is also questionable. The adults of all *Actinia* species are essentially non-dispersive and, as it is difficult to envisage cross-fertilisation occurring over other than very short distances, it is possible that there may be little or no gene flow between shores.

The much lower levels of genetic differentiation (F_{ST} = 0.042; Nm = 5.7 to 9.2 ind generation^{-1}) found between subpopulations of *Bunodosoma caissarum* than between those of the red morph of *Actinia bermudensis* (F_{ST} = 0.262; Nm = 0.4 to 0.7) could be due to the different modes of reproduction or dispersal of the two species. High levels of heterozygosity and low levels of genetic differentiation are to be expected in species with high dispersal capability (Slatkin 1987; Waples 1987; Hunt and Ayre 1991). From a literature survey of data collected from numerous species of sea anemones we conclude that sexually reproducing species seem to have generally higher identity values between their populations than do facultative asexual species.

The positive correlation between geographic distance and the genetic distance between populations of *Bunodosoma caissarum* suggests that sea anemone populations may follow the "stepping stones" model (see Nei 1987; Maynard Smith 1989) of genetic differentiation. Information on population differentiation with geographical distance is also relevant to current debate on the evolutionary function of the planktonic larvae which occur in the life cycles of many benthic marine invertebrates. Although it has long been assumed as almost being axiomatic that the primary role of these larvae was 'for' dispersal, this claim is questioned (Strathmann 1985; Knowlton and Keller 1986). Some recent genetic studies have indicated that the larval dispersal intuitively expected from a knowledge of the duration of the larval life is often not realised under natural conditions and that species with relatively long-lived larvae can show genetic differentiation on a surprisingly restricted geographical scale (e.g. Hedgecock 1986; Todd et al. 1988). Indeed, Endler (1973) considered that species with high gene flow between geographically distant populations may be uncommon. Our data indicate that there is widespread gene dispersal in *B. caissarum* which is as would be predicted for a species with a long-lived planktotrophic larva. Thus, it may be argued that population genetics data on *B. caissarum* provide support for claims of the dispersive role of marine larvae.

Levels of overall local inbreeding for *Bunodosoma caissarum* were highly significant (F_{IS} = 0.2340; χ^2 = 943.5; df = 95; P<<0.001). However, the main departures from Hardy-Weinberg equilibrium were for the *Odh* locus which showed a significant excess of homozygotes at all nine collection sites. This locus was considered to have a null allele as previously described for *Odh* in the related *Actinia prasina* (Solé-Cava and Thorpe 1992). Null alleles are well known as potential sources of heterozygote deficiences (Foltz 1986). In theory it is simple to calculate the frequencies of the null allele and hence the other alleles from that of the observed null homozygotes, but we avoided this because of potential problems. Such calculations multiply the necessarily large sampling errors in estimates of homozygote genotype frequencies and also assume Hardy-Weinberg equilibrium. The main uses of the gene frequencies would be to estimate divergence (I and D) and fits to Hardy-Weinberg expectations. The use of observed allele frequencies making no allowance for null alleles appears preferable for estimating divergence since frequency errors are not multiplied and fits to Hardy-Weinberg equilibrium cannot be estimated using frequencies themselves estimated by assuming Hardy-Weinberg equilibrium.

The overall levels of genetic variation were high in both anemone species, but twice as high in *Bunodosoma caissarum* (H = 0.35) as in *Actinia bermudensis* (H = 0.17). In the vast majority of anemones studied levels of genetic variation are high with an average heterozygosity of about 0.20 (Shick 1991; Solé-Cava and Thorpe 1991). Anemones with exclusively sexual reproduction exhibit significantly higher (mean H = 0.25; t = 2.54, df = 29; P<0.02) levels of heterozygosity than those with facultative asexual reproduction (mean H = 0.16). With reduced dispersal, the

effective population size is reduced and hence less genetic variation is to be expected (Varvio-Aho 1983).

Bunodosoma caissarum and *Actinia bermudensis* may have similar ecological requirements, since the two species frequently occur interspersed on rocky shores (Traldi and Schlenz 1990). Their different levels of genetic variation could be due, thus, to stochastic phenomena. The neutralist theory of molecular evolution (Kimura 1983, 1991) predicts that the level of genetic variation in a given species will depend principally upon effective population size (assuming no recent bottlenecks, Nei 1987). Based on neutralist estimates (calculated as $4 N_e V = H/(1-H)$, deduced from $H = 4 N_e V/(1+4 N_e V)$, where N_e is the effective population size, and V is the mutation rate (Kimura 1983), and assuming similar mutation rates, populations of *B. caissarum* would be predicted to be approximately 2.6 times larger than those of *A. bermudensis* in order to explain the higher levels of genetic variation. Effective population sizes of any sea anemone species are unknown, but, at least on the 1000+ km of coast covered by our sampling, population densities of *B. caissarum* were several times higher than those of *A. bermudensis*. In *A. bermudensis* asexual reproduction serves to decrease even further the effective population size (N_e) relative to population size (N), since anemones from the same clone count as a single individual for the purpose of estimating N_e. Consequently, it appears that observed levels of heterozygosity in the two species studied here are broadly in line with neutralist expectations based upon relative population sizes in so far as the less polymorphic species probably has a smaller population size.

Overall the results of the present work suggest that in *Bunodosoma caissarum*, an exclusively sexually-reproducing, outbreeding species with a long-lived planktotrophic larva, there is probably widespread dispersal of larvae between populations and heterozygosity is high, as we had predicted. In *Actinia bermudensis*, which reproduces asexually, heterozygosity is lower and dispersal appears much reduced.

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References

Avise JC, Aquadro CF (1982) A comparative summary of genetic distances in the vertebrates: patterns and correlations. Evol Biol 15:151–185

Ayre DJ (1984) The effects of sexual and asexual reproduction on geographic variation in the sea anemone *Actinia tenebrosa*. Oecologia 62:222–229

Ayre DJ, Read J, Wishart J (1991) Genetic subdivision within the eastern Australian population of the sea anemone *Actinia tenebrosa*. Mar Biol 109:379–390

Belém MJC (1987) Aspectos da biologia de *Bunodosoma caissarum* Correa, 1964 (Cnidaria, Anthozoa, Actiniidae) do litoral do Estado do Rio de Janeiro, com ênfase na estimativa do seu comportamento reprodutivo. Unpublished PhD Thesis. Inst Biociências Universidade de São Paulo, São Paulo

Bertness MD, Gaines SD (1993) Larval dispersal and local adaptation in acorn barnacles. Evolution, Lawrence, Kansas 47:316–320

Black R, Johnson MS (1979) Asexual viviparity and population genetics of *Actinia tenebrosa*. Mar Biol 53:27–31

Bucklin A, Hedgecock D (1982) Biochemical genetic evidence for a third species of *Metridium* (Coelenterata: Actiniaria). Mar Biol 66:1–7

Carter MA, Miles J (1989) Gametogenis cycles and reproduction in the beadlet sea anemone *Actinia equina* (Cnidaria: Anthozoa). Biol J Linn Soc 36:129–155

Chadwick NE, Adams C (1991) Locomotion, asexual reproduction and killing of corals by the corallimorpharian *Corynactis californica*. Hydrobiologia 216/217:263–269

Chia FS (1976) Sea anemone reproduction: patterns and adaptative radiation. In: Mackie GO (ed) Coelenterate ecology and behavior. Plenum Press, New York, pp 261–270

Endler JA (1973) Gene flow and population differentiation. Science, NY 179:243–250

Foltz DW (1986) Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica* and other bivalves. Evolution, Lawrence, Kansas 40:869–870

Grosberg RK (1991) Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri*. Evolution, Lawrence, Kansas 45:130–142

Hansson L (1991) Dispersal and connectivity in metapopulations. Biol J Linn Soc 42:89–103

Harris H, Hopkinson DA (1978) Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam

Haylor GS, Thorpe JP, Carter MA (1984) Genetical and ecological differentiation between sympatric colour morphs of the common intertidal sea anemone *Actinia equina*. Mar Ecol Prog Ser 16:281–290

Hedgecock D (1986) Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bull mar Sci 39:550–564

Hoffman RJ (1986) Variation in contributions of asexual reproduction to the genetic structure of populations of the sea anemone *Metridium senile*. Evolution, Lawrence, Kansas 40:357–365

Hunt A, Ayre DJ (1991) Population structure in the sexually reproducing sea anemone *Oulactis muscosa*. Mar Biol 102:537–544

Jackson JBC (1986) Modes of dispersal of clonal benthic invertebrates: consequences for species' distributions and genetic structure of local populations. Bull mar Sci 39:588–606

Jennison BL (1983) Reproductive biology of three species of sea anemones from the central Atlantic Coast of Florida. Fla Scient 46:168–178

Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, London

Kimura M (1991) The neutral theory of molecular evolution: a review of recent evidence. Jap J Genet 66:367–386

Knowlton N, Keller BD (1986) Larvae which fall far short of their potential: highly localized recruitment in an alpheid shrimp with extended larval development. Bull mar Sci 39:213–223

Lessa EP (1990) Multidimensional analysis of geographic genetic structure. Syst Zool 39:242–252

Lessios HA (1992) Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Mar Biol 112:517–523

Maynard Smith J (1989) Evolutionary genetics. Oxford University Press, Oxford

McCommas SA, Lester LJ (1980) Electrophoretic evaluation of the taxonomic status of two species of sea anemone. Biochem Syst Ecol 8:289–292

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 41:225–233

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York
Numachi K (1981) A simple method for preservation and scanning of starch gels. Biochem Genet 19:233–236
Olson RR (1985) The consequences of short-distance dispersal in marine invertebrates. Ecology 66:30–39
Orr J, Thorpe JP, Carter MA (1982) Biochemical genetic confirmation of the asexual reproduction of brooded offspring in the sea anemone *Actinia equina*. Mar Ecol Prog Ser 7:227–229
Poulik MD (1957) Starch gel electrophoresis in a discontinuous system of buffers. Nature, Lond 180:1477–1479
Richardson BJ, Baverstock PR, Adams M (1986) Allozyme electrophoresis. Academic Press, Sydney
Russo CAM (1991) Genetica de Populacoes de anemonas do mar. Unpublished MSc thesis. Departamento de Genética Universidade Federal do Rio de Janeiro, Rio de Janeiro
Scheltema RS (1989) Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographic range of the species. In: Ryland JS, Tyler PA (eds) Reproduction, genetics and distributions of marine organisms. 23rd European Marine Biology Symposium 1988. Olsen & Olsen, Denmark, pp 183–188
Schlenz E (1983) Anêmonas (Cnidaria: Actiniaria) do Brasil. Anals Acad bras Ciênc 55:330–331
Shaw PR, Prasad R (1970) Starch gel electrophoresis of enzymes (a compilation of recipes). Biochem Genet 4:297–320
Shaw PW (1991) Effects of sexual reproduction on population structure of *Sargatia elegans* (Anthozoa: Cnidaria). Hydrobiologia 216/217:519–525
Shaw PW, Beardmore JA, Ryland JS (1987) *Sagartia troglodytes* (Anthozoa: Actiniaria) consists of two species. Mar Ecol Prog Ser 41:21–28
Shick JM (1991) A functional biology of sea anemones. Chapman and Hall, London
Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science, NY 236:787–792
Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman Press, San Francisco, California
Solé-Cava AM, Thorpe JP (1987) Further genetic evidence for the reproductive isolation of the green colour morph of the intertidal sea anemone *Actinia equina*. Mar Ecol Prog Ser 38:225–229
Solé-Cava AM, Thorpe JP (1991) High levels of gene variation in marine invertebrates. Biol J Linn Soc 44:65–80
Solé-Cava AM, Thorpe JP (1992) Genetic evidence of reproductive isolation between sympatric and allopatric populations of the various morphs of the common intertidal sea anemone *Actinia* (Anthozoa: Actiniaria) in Britain. Mar Biol 112:243–252
Solé-Cava AM, Thorpe JP, Kaye JG (1985) Reproductive isolation with little genetic divergence between *Urticina (= Tealia) felina* and *U. eques* (Anthozoa: Actiniaria). Mar Biol 85:279–284
Stephenson TA (1935) The British sea anemones, Vol 2. Ray Society, London
Strathmann RR (1985) Feeding and nonfeeding larval development and life history evolution in marine invertebrates. A Rev Ecol Syst 16:339–361
Swofford L, Selander RB (1981) BIOSYS-1 – A fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Heredity 72:281–283
Thorpe JP (1982) The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. A Rev Ecol Syst 13:139–168
Thorpe JP (1983) Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: Oxford GS, Rollison D (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, pp 131–152
Todd CD, Havenhand JN, Thorpe JP (1988) Genetic differentiation, pelagic larval transport and gene flow between local populations of the intertidal marine mollusc *Adalaria proxima* (Alder and Hancock). Funct Ecol 2:441–451
Traldi ES, Schlenz E (1990) Estratégias de ocupação de um costão rochoso por cinco espécies de anêmonas no litoral do Estado de Sao Paulo. In: Anais do II Simpósio de Ecossistemas da costa Sul e Sudeste do Brasil: estrutura, função e manejo. Academia de Ciências do Estado de São Paulo Press, Sao Paulo, pp 67–74
Varvio-Aho S-L (1983) Genetic variation in relation to dispersal efficiency. In: Oxford GS, Rollinson D (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, pp 325–339
Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution, Lawrence, Kansas 41:385–400
Ward RD, Beardmore JA (1977) Protein variation in the plaice (*Pleuronectes platessa*). Genet Res 30:45–62
Williams GC (1975) Sex and evolution. Princeton University Press, Princeton, New Jersey
Wright S (1978) Evolution and the genetics of populations, Vol 4. Variability within and among natural populations. University of Chicago Press, Chicago