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RESEARCH ARTICLE

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Genetic differentiation among populations of the Mediterranean hermaphroditic brooding coral *Balanophyllia europaea* (Scleractinia: Dendrophylliidae)

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Abstract Spatial models of genetic structure and potential gene flow were determined for five populations of *Balanophyllia europaea*, a simultaneous hermaphroditic and brooding coral, endemic to the Mediterranean. Six allozyme loci indicated a genetic structure that departed markedly from Hardy–Weinberg equilibrium, with a significant lack of heterozygotes. The genetic structure observed supports the hypothesis that self-fertilisation characterises the reproductive biology of *B. europaea*. Populations at small spatial scales (8–40 m) are genetically connected, while those at large scales (36–1,941 km) are genetically fragmented; the genetic differentiation of the populations is not correlated to geographic separation. This spatial model of genetic structure is compatible with an inbreeding mating system. Furthermore, it is also consistent with the expected dispersal potential of swimming larvae of brooding corals, i.e. larvae that are able to produce significant gene flows only within limited spatial scales.

Introduction

Evolutionary biology has long been tackled through the study of the adaptive significance of different mating systems and dispersal patterns. Many authors have published their views on this subject (Uyenoyama et al. 1993; Palumbi 1994; Bohonak 1999). Different reproductive strategies have been commonly associated with different dispersal capabilities. In general terms, a high dispersal potential has often been correlated to heterogamous sexual reproduction and to the production of genotypically diverse propagules capable of maintaining genetic homogeneity among interconnected populations (Hedgecock 1986; Palumbi 1992; Ayre et al. 1997a). On the contrary, a low dispersal potential is generally associated with inbreeding, lack of heterozygosity and a greater genetic subdivision among populations due to drift and to site-specific selection (Knowlton and Jackson 1993; Frankham 1995). Exceptions to this emerging paradigm exist. For example, many plants and animals produce clonal propagules with high dispersal capabilities (Jackson 1986; Mogie 1992). On the other hand, the duration of seed and larval dispersal in many species is variable, with some propagules settling immediately after release and others postponing metamorphosis, thereby ensuring a wide geographical range for potential dispersal (Gerrodette 1981; Harper 1977). The distance covered by propagules, their origin and the genetic consequences of past and present dispersal patterns are generally unknown (Ayre and Hughes 2000).

Most of the data available on the evolution of mating systems, and particularly on inbreeding systems, come from botanical studies (Carlon 1999). Marine invertebrates give us the opportunity to test and expand theories on mating systems and on dispersal patterns in a group of organisms living in a different milieu, the sea (Jarne and Charlesworth 1996). Among invertebrates, Anthozoa exhibit an extraordinary diversity of life-cycle traits at the species, population and individual levels (Edmands and Potts 1997; McFadden et al. 2001). Specifically, when referring to the reproductive strategies of scleractinians, there are cases of self-fertilisation (Brazeau et al. 1998), vegetative reproduction by clonal fragments (Highsmith 1982) and asexual production of planulae (Ayre and Resing 1986) and of benthic-crawling larvae (Gerrodette 1981). These reproductive processes generally restrict gene flow and cause a marked subdivision among populations (Ayre and Hughes 2000). However, scleractinians most frequently go through a swimming larval phase

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with pelagic dispersal (Harrison and Wallace 1990). Biogeographic studies (Veron 1995), hydrodynamic models (Wolanski 1994), plankton samplings (Willis and Oliver 1990) and laboratory studies on the physiology and behaviour of larvae (Richmond 1987; Mundy and Babcock 1998; Goffredo and Zaccanti 2004) have supplied data on dispersal range, often leading to contrasting predictions. For example, some hydrodynamic studies of reefs show that water can persist locally long enough to guarantee self-seeding: larvae remain trapped until mature enough to attach to their native reef and begin metamorphosis (see Black et al.1991). According to other models, propagules are transported over great distances, thereby crossing coral reefs and different geographic regions (Wolanski 1994; Veron 1995; Roberts 1998).

Balanophyllia europaea (Risso, 1826) is a zooxanthellate solitary scleractinian endemic to the Mediterranean Sea (Zibrowius 1980). Due to its symbiosis with zooxanthellae, it exhibits a limited depth distribution (maximum 50 m depth, Zibrowius 1980); congeneric azooxanthellate species reach depths >1,000 m (Cairns 1977). Maximum population density of *B. europaea* is reached at depths of 4–8 m, with peaks of 100 individuals m^{-2} (Goffredo 1999; Goffredo et al. 2004). The reproductive biology of this species is characterised by simultaneous hermaphroditism and brooding (Goffredo and Telò 1998). *B. europaea* is the only species in the genus *Balanophyllia*, and one of the few species in the family Dendrophylliidae, to exhibit hermaphroditism (Harrison 1985; Goffredo et al. 2000). Histological observations show that there is neither spatial nor temporal separation between male and female gametogenesis and that encounters occur between mature spermatozoa and oocytes produced by the same individual (Goffredo et al. 2002). These observations suggest that autogamy could be a reproductive strategy in this species. Over the annual reproductive cycle, fertilisation takes place from March to June and planulation between August and September (Goffredo et al. 2002). Planulas have completed ontogenesis at the time they are released and exhibit swimming behaviour (Goffredo and Zaccanti 2004). Evidence of asexual reproduction (either through polyp budding or fission) has not been observed (Goffredo and Telò 1998; Goffredo et al. 2000, 2002, 2004; Goffredo and Zaccanti 2004).

We examine here the genetic structure of populations of *B. europaea* and infer their modes of reproduction and dispersal patterns. In particular, the hypothesised self-fertilisation and the limited dispersal potential, peculiar to brooding organisms (Harrison and Wallace 1990; Ayre and Hughes 2000; McFadden et al. 2001), should lead to strong inbreeding within populations, with a consequently significant departure from Hardy–Weinberg equilibrium for the lower number of heterozygotes, along with low gene flow and marked divergence among populations (Wright 1969; Carlon 1999; Ayre and Hughes 2000).

Materials and methods

Sampling

Samples of *Balanophyllia europaea* were collected from five sites in the Mediterranean Basin (Fig. 1): two in the Ligurian Sea, in the area of Capraia Island and in the area of Calafuria (Leghorn) along the Tuscan coast; one in the northern Tyrrhenian Sea, in the area of Elba Island; one in the Channel of Sicily, in the area of Lampedusa Island; and one in the northern Adriatic Sea, near Pula at the southern tip of the Istra peninsula. These five localities are from 36 to 1,941 km apart. Specimens were collected from August 2000 to August 2001 by SCUBA diving at the depth of maximum population density (4–8 m). Given that the generation time of *B. europaea* is 3.6 years (maximum life span = 20 - years; Goffredo et al. 2004), we do not consider the range in the sampling dates to be significant with respect to differentiation within populations. At each site, polyps were collected from six patches (rectangular areas of 624 cm^2 cordoned off by a plastic frame; 29.7×21.0 cm) 8 m apart (we estimated the distance between patches within localities based on the number of body lengths between them; according to Hellberg 1994). All the

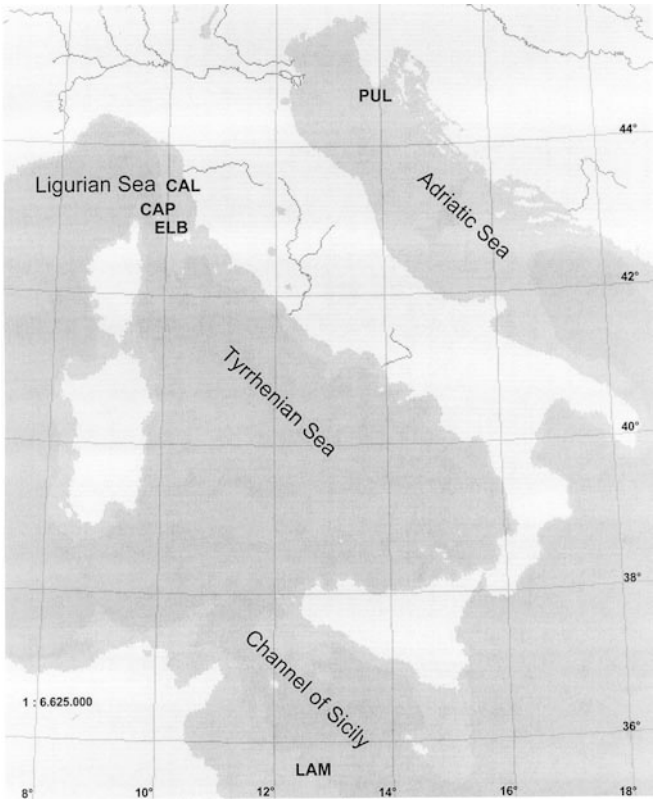

Fig. 1 *Balanophyllia europaea*. Sampled populations (coordinates and abbreviations of populations in decreasing order of latitude: *PUL* Pula, 44°53′N; 13°49′E; *CAL* Calafuria, 43°29′N; 10°19′E; *CAP* Capraia, 43°02′N; 9°48′E; *ELB* Elba, 42°48′N; 10°07′E; *LAM* Lampedusa, 35°30′N; 12°36′E)

polyps found within each patch were collected. Living specimens were transported in refrigerated units and brought to the laboratories of the Department of Experimental Evolutionary Biology of the University of Bologna. Specimens were dissected and then examined under a binocular microscope. All oocytes and embryos were removed to avoid confounding the parental genotype with that of the progeny. We scraped epibionts off the samples and then put each individual into a single tube devoid of seawater, which was stored at −80°C.

Laboratory analysis

Tissue was taken from samples and then homogenised using an equal volume of 0.1 M Tris-HCl buffer pH 7.5 (containing 1 mM Na-EDTA, 0.6 mM NAD, 0.5 mM NADP, 1 mM mercaptoethanol). The homogenates were centrifuged at 10,500 *g* for 10 min at 4°C, and the supernatants were used for electrophoresis. Mucus, zooxanthellae and cell debris were removed by centrifugation (Muscatine and Cernichiari 1969; Stoddart 1983; Ayre et al. 1997b; Dai et al. 2000). Allozyme electrophoresis was carried out under different electrical conditions using cellulose acetate (Diploid dry) as substrate at 4°C. Staining was carried out in an oven at 37°C. Eighteen loci were initially tested using four different buffers: TEC, pH 7.5 (Meera-Khan 1971); Tris-citrate, pH 7.2 (Grunbaum 1981); and TEM, pH 7.4 and 7.8 (Schneppenheim and MacDonald 1984). The latter gave the best resolution. Six loci were polymorphic for all populations and therefore significant for our analysis: phosphoglucose isomerase (*Pgi*, EC 5.3.1.9), phosphoglucomutase (*Pgm*, EC 5.4.2.2), hexokinase (*Hk*, EC 2.7.1.1), adenylate kinase (*Ak*, EC 2.7.4.3), mannosephosphate isomerase (*Mpi*, EC 5.3.1.8) and phosphogluconate dehydrogenase (*Pgd*, EC 1.1.1.44). Alleles were numbered using the most common allele found in the Calafuria population as reference (allele 100).

Statistical analysis

Population genetic analyses were carried out using GENEPOP (version 3.3; Raymond and Rousset 1995) and FSTAT software (version 2.9.3; Goudet 1995, 2001).

Measures used to assess genetic variation were the average number of alleles per locus, the percentage of polymorphic loci (95% criterion) and the comparison between observed (H_o) and expected (H_e) heterozygosity under Hardy–Weinberg equilibrium. Expected heterozygosity corresponds to Nei's (1973) gene diversity and is calculated using Levene's (1949) correction for small samples.

The degree of genetic differentiation among populations was assessed by Wright's *F*-statistics (Wright 1978) as calculated by Weir and Cockerham (1984). This statistic, using departures from expected levels of heterozygosity under complete panmixia, is based on three indexes: F_{IS} (f), an estimate of the deficit of heterozygotes within populations (inbreeding coefficient); F_{ST} (θ), an estimate of the deficit of heterozygotes among populations (indicating the genetic subdivision among populations); and F_{IT} (F), an estimate of the deficit of heterozygotes in the total population.

We used the approximation $N_em = [(1/F_{ST}) - 1]/4$ to calculate the actual number of migrants per generation, i.e. the gene flow (N_em) between pairs of populations (following Hellberg 1996; Miller 1997; Ayre and Hughes 2000). This inference, based on Wright's (1969) "island model" (the assumptions of which are that gene flow is bidirectional and at stable equilibrium, that the rate of migration greatly exceeds that of mutation and that the genetic markers employed are selectively neutral), provides an unbiased estimator of gene flow that is relatively insensitive to moderate levels of selection (Slatkin and Barton 1989; for discussion on the caveats of using F_{ST} to infer N_em, see Whitlock and McCauley 1999; Neigel 2002). Genetic differentiation between pairs of populations was expressed by Nei's measure (D) of unbiased genetic distances (Nei 1978). In order to assess the influence of geographic distance on genetic separation in the populations, we used the model of Slatkin (1993) according to which: $\log_{10}(M) = a + b\log_{10}$ (geographic distance), in which M is equal to N_em between pairs of populations. The geographic distance between pairs of populations is the lowest nautical distance between localities measured on maps based on a scale from 1:24,000 to 1:1,000,000 (Hellberg 1994).

Results

Genetic differentiation at large spatial scales

Table 1 shows the allelic frequencies at the six polymorphic loci in five Mediterranean populations of *Balanophyllia europaea*, ranging from 36 to 1,941 km apart. The total number of alleles per locus ranged from 5 (*Mpi*) to 15 (*Pgm*). Private alleles were rather frequent at all loci. For example, *Mpi*103* and *Mpi*98* were found only in the Elba island population; *Pgm*118*, **111*, **110*, **105* and **104*, in the Calafuria population; and *Pgm*96*, in the Lampedusa sample.

The mean number of alleles per locus within each population ranged from 2.00 to 5.14. The percentage of polymorphic loci varied from 80% to 100%. Observed heterozygosity was lower than the expected heterozygosity under Hardy–Weinberg equilibrium in all populations, ranging from 2.8 times lower in the Pula sample ($H_o = 0.119$, $H_e = 0.329$) to 11.3 times lower in the Capraia one ($H_o = 0.026$, $H_e = 0.294$; Table 2). Departure from Hardy–Weinberg equilibrium was enhanced by the genotypic frequencies at each locus, showing a marked deficit of heterozygotes; the only

Table 1 *Balanophyllia europaea*. Allelic frequencies scored for the five analysed samples (*N* total number of individuals examined for each locus). Population acronyms as in Fig. 1

Locus, allele	CAL	CAP	ELB	LAM	PUL
Pgd					
N	14	13	17	16	14
103	–	0.038	–	–	–
102	–	–	0.265	–	0.214
101	–	–	0.059	–	–
100	0.964	0.385	0.588	0.750	0.786
99	–	0.038	0.088	–	–
98	–	0.538	–	0.250	–
97	0.036	–	–	–	–
Hk					
N	52	21	11	16	14
102	0.038	–	–	–	–
100	0.365	–	–	–	–
97	0.135	–	0.364	–	–
96	0.356	0.286	0.364	0.063	–
95	0.077	0.524	0.227	0.781	0.893
94	0.010	–	–	0.063	–
93	–	0.095	0.045	0.031	–
92	0.019	0.095	–	–	–
91	–	–	–	–	0.107
88	–	–	–	0.063	–
Pgi					
N	66	14	17	12	14
102	0.038	–	–	–	–
101	0.061	–	–	–	–
100	0.447	–	–	–	0.071
99	0.015	0.464	0.294	0.500	0.857
98	0.023	–	0.412	–	0.071
97	0.311	0.429	0.294	0.500	–
96	0.015	0.107	–	–	–
95	0.068	–	–	–	–
94	0.023	–	–	–	–
Pgm					
N	76	18	6	17	14
118	0.007	–	–	–	–
111	0.013	–	–	–	–
110	0.007	–	–	–	–
108	–	–	0.083	0.029	–
107	0.020	0.028	–	0.029	–
105	0.046	–	–	–	–
104	0.046	–	–	–	–
103	0.013	0.028	–	–	0.321
102	0.283	0.111	–	–	–
101	0.046	0.167	–	–	–
100	0.382	0.361	0.917	0.588	0.393
99	0.072	–	–	0.059	0.286
98	0.013	0.222	–	0.235	–
97	0.053	0.083	–	–	–
96	–	–	–	0.059	–
Mpi					
N	15	14	15	13	14
103	–	–	0.133	–	–
101	–	–	0.200	0.077	–
100	1,000	0.857	0.433	0.654	1,000
99	–	0.143	0.133	0.269	–
98	–	–	0.100	–	–
Ak					
N	11	21	12	12	14
102	0.091	–	–	–	–
101	–	0.048	0.333	–	–
100	0.818	0.381	0.208	0.583	–
99	–	0.452	0.083	0.333	0.643
98	0.091	–	0.208	0.083	0.357
97	–	0.119	0.167	–	–

Table 2 *Balanophyllia europaea*. Genetic variability in the five Mediterranean populations (population acronyms as in Fig. 1; *Date* date of sample collection; *N* mean number of individuals examined per locus; *n* mean number of alleles per locus; *P* percent of polymorphic loci in which the most common allelic frequency is $<95\%$; H_o and H_e mean observed and expected heterozygosity, respectively; standard deviations are *in parentheses*)

Population	Date	*N*	*n*	*P*	H_o	H_e
CAL	18 Aug 2000	37.0 (27.1)	5.83 (4.67)	80	0.030 (0.042)	0.150 (0.158)
CAP	30 Dec 2000	17.4 (3.7)	4.00 (1.67)	100	0.026 (0.032)	0.294 (0.173)
ELB	14 Jul 2001	12.1 (4.5)	3.83 (1.17)	100	0.070 (0.121)	0.322 (0.140)
LAM	22 Nov 2001	15.0 (2.7)	3.50 (1.64)	100	0.051 (0.072)	0.284 (0.180)
PUL	15 Aug 2001	14.0 (0.0)	2.17 (0.75)	100	0.119 (0.141)	0.329 (0.138)

exception was the locus *Hk* in the Pula population ($F_{IS}=-0.083$, $P>0.05$; Tables 3, 4). The high rate of inbreeding in the populations was shown by the mean value of F_{IS}, which was >0 at all loci (range 0.768–0.902; $P<0.01$; Table 4).

F_{ST} values departed significantly from zero at all loci; they ranged from 0.087 for the *Pgm* locus to 0.266 for the *Pgi* one (Table 4). The mean F_{ST} value calculated for all six loci was 0.202 ($P<0.01$). These data showed that the populations are genetically subdivided.

Analysis of the genetic subdivision between pairs of populations revealed general genetic fragmentation. F_{ST} values support genetic fragmentation, with a significant departure from zero in all cases except one (the Capraia–Lampedusa pair; Table 5).

Estimated gene flow (N_em) was calculated using F_{ST} values and indicated a mean value of 0.988 migrants per generation among the five populations and a range of 0.423–8.180 migrants per generation for pairs of populations (Table 5). Regression of $\log_{10}$ gene flow (M, individuals per generation) versus $\log_{10}$ geographic distance of separation (km) for all pairwise combinations of five populations examined did not indicate a significant correlation between genetic isolation and geographic separation ($y=-0.0489x+0.2051$, where y is log M and x is log distance; $r^2=0.009$, $P>0.05$). Nei's unbiased genetic distance (D) between population pairs varied from 0.082 to 0.436 (Table 5).

Genetic differentiation at small spatial scales

At Calafuria, the high density of individuals (113 m^{-2} at a depth of 6 m; Goffredo et al. 2004) allowed the collection of a sample large enough to compare the genetic structure between different patches, ranging from 8 to 40 m apart. Our analysis showed a marked deficit of heterozygotes at all loci within all patches, with a mean F_{IS} value of 0.808 ($P<0.01$). For all loci, *F*-statistic analysis yielded F_{ST} values that were not significantly

Table 3 *Balanophyllia europaea*. Heterozygosity at six loci in the five Mediterranean populations. Observed (H_o) and expected (H_e) heterozygosity are shown for each locus (standard deviation *in parentheses*). Population acronyms as in Fig. 1

Population	*Pgd*		*Hk*		*Pgi*		*Pgm*		*Mpi*		*Ak*	
	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
CAL	–	–	0.005 (0.009)	0.206 (0.191)	0.060 (0.061)	0.155 (0.180)	0.024 (0.026)	0.117 (0.150)	–	–	0.000 (0.000)	0.219 (0.080)
CAP	0.039 (0.044)	0.291 (0.247)	0.000 (0.000)	0.321 (0.170)	0.047 (0.041)	0.407 (0.181)	0.032 (0.030)	0.227 (0.156)	0.000 (0.000)	0.254 (0.000)	0.024 (0.028)	0.325 (0.204)
ELB	0.030 (0.034)	0.295 (0.185)	0.046 (0.053)	0.357 (0.186)	0.000 (0.000)	0.452 (0.041)	–	–	0.027 (0.037)	0.301 (0.127)	0.167 (0.228)	0.320 (0.110)
LAM	0.000 (0.000)	0.387 (0.000)	0.075 (0.082)	0.156 (0.113)	0.000 (0.000)	0.522 (0.000)	0.039 (0.048)	0.203 (0.186)	0.154 (0.077)	0.343 (0.171)	0.000 (0.000)	0.377 (0.190)
PUL	0.000 (0.000)	0.349 (0.000)	0.214 (0.000)	0.198 (0.000)	0.095 (0.083)	0.177 (0.067)	0.238 (0.206)	0.457 (0.036)	–	–	0.000 (0.000)	0.476 (0.000)

Table 4 *Balanophyllia europaea*. Estimate of deviation from Hardy–Weinberg equilibrium (F_{IS} estimates per population and locus), and *F*-statistics for the five Mediterranean populations. Statistical significance was determined using permutation tests followed by Bonferroni adjustments. *F*-statistics indicate deviation from Hardy–Weinberg within the total population (F_{IT}), within populations (F_{IS}) and among populations (F_{ST}). Population acronyms as in Fig. 1

Population	*Pgd*	*Hk*	*Pgi*	*Pgm*	*Mpi*	*Ak*
CAL	–	0.974***	0.611***	0.794***	–	1.000*
CAP	0.872***	1.000***	0.887***	0.863***	1.000*	0.928***
ELB	0.903***	0.878***	1.000***	–	0.914***	0.491***
LAM	1.000**	0.526*	1.000**	0.811***	0.561*	1.000***
PUL	1.000**	– 0.083	0.469*	0.488**	–	1.000**
$F_{IT}(F)$	0.920**	0.914**	0.801**	0.788**	0.852**	0.887**
$F_{IS}(f)$	0.902**	0.885**	0.729**	0.768**	0.820**	0.856**
$F_{ST}(\theta)$	0.182**	0.259**	0.266**	0.087**	0.178*	0.216**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 5 *Balanophyllia europaea*. F_{ST} values (*above the diagonal line*), mean number of migrants per generation ($N_e m$, *in parentheses above the diagonal*) and Nei's unbiased genetic distances (*below the diagonal line*) in the five sampled Mediterranean populations. F_{ST} statistical significance was determined using permutation tests followed by Bonferroni adjustments (**$P < 0.01$). Population acronyms as in Fig. 1

	CAL	CAP	ELB	LAM	PUL
CAL		0.213** (0.922)	0.204** (0.978)	0.208** (0.950)	0.371** (0.423)
CAP	0.281		0.142** (1.508)	0.030 (8.180)	0.194** (1.042)
ELB	0.320	0.302		0.134** (1.622)	0.273** (0.667)
LAM	0.249	0.082	0.234		0.189** (1.073)
PUL	0.436	0.208	0.359	0.171	

different from zero (average = 0.038, $P > 0.05$), indicating the existence of genetic connectivity among the patches. The estimate of gene flow ($N_e m$) among patches, based on F_{ST} values, showed an average of 6.329 migrants per generation and, for single pairs of patches, a range of 1.647–15.572 migrants per generation. Among pairs of patches, no significant correlation was found between gene flow and spatial distance of separation ($r^2 = 0.014$, $P > 0.05$). Nei's unbiased genetic distance between pairs of patches varied from 0.076 to 0.248.

Discussion

Hardy–Weinberg equilibrium

We report here for the first time on the genetic structure of a Mediterranean scleractinian coral. The genotypic frequencies revealed a marked departure from Hardy–Weinberg equilibrium, with a considerable deficit of heterozygotes. F_{IS} is significantly positive for all loci, indicating a high rate of inbreeding. These results strongly support the hypothesis that self-fertilisation characterises the reproductive biology of *Balanophyllia europaea* (Goffredo and Telò 1998; Goffredo et al. 2002). More than two-thirds of all scleractinians studied are simultaneous hermaphrodites, and, in most cases, both male and female gametes are produced within the same polyp (Harrison and Wallace 1990). Studies on the mating systems of hermaphroditic scleractinians stress the potential for self-fertilisation in broadcasting and brooding organisms, selfing being common in the latter organisms, but not in the former (reviewed in Carlon 1999). Particularly, the genetic structure of the self-fertilising, brooding coral *Acropora palifera*, which was

studied using allozymatic loci, exhibited a deficit of heterozygotes similar to that found in *B. europaea* (Carlon 1999). Our comparative study now under way on parental and progeny genotypes of *B. europaea*, using highly variable genetic markers (microsatellites), is expected to yield an estimate of the rate of self-fertilisation (*s*) in this brooding coral.

Self-fertilisation is a reproductive strategy common to hermaphroditic terrestrial plants (approximately 65% are at least partly self-fertilising, Jarne and Charlesworth 1993; Barret and Harder 1996). Of the 24 species of simultaneous hermaphroditic marine invertebrates that have been investigated, self-fertilisation has been demonstrated in nine cases (38%; Knowlton and Jackson 1993). There is also well-documented evidence of self-fertilisation in terrestrial pulmonate gastropods (Jarne et al. 1993). Self-fertilisation is associated with poor mobility or a sessile life style, low population density (Tomlinson 1966; Kojis and Quinn 1981), and/or colonisation of disturbed habitats that could limit cross-fertilisation (Bucklin et al. 1984). *B. europaea* is the only species of the genus that is a zooxanthellate, simultaneous hermaphrodite that colonises disturbed shallow waters with low population densities (Goffredo and Telò 1998; Goffredo et al. 2002, 2004). Other species of the genus *Balanophyllia* are azooxanthellate, gonochoric and colonise habitats with high population density (Fadlallah 1983). Given the mode of habitat colonisation of *B. europaea*, we hypothesise that self-fertilisation in this species may represents an adaptive condition (Ghiselin 1969; Knowlton and Jackson 1993).

Biparental inbreeding is another mating system that can generate a deficit of heterozygotes (Edmands and Potts 1997). However, the pelagic swimming behaviour of *B. europaea* larvae (Goffredo and Zaccanti 2004) should favour dispersal and significantly limit the chance for biparental inbreeding (Carlon 1999).

Genetic differentiation at large spatial scales

We found significant genetic differentiation among the populations examined. Differentiation was found at all loci, with F_{ST} values significantly >0 (mean $F_{ST}=0.202$), indicating considerable restrictions to gene flow (mean $N_em=0.988$ migrants per generation). The level of genetic differentiation found in *B. europaea* was similar to the one found in other marine invertebrates characterised by low larval dispersal capability, such as the brooding soft corals *Alcyonium rudyi* ($F_{ST}=0.230$–0.460; McFadden 1997) and *Clavularia koellikeri* ($F_{ST}=0.134$; Bastidas et al. 2002) and the brooding scleractinians *Seriatopora hystrix* ($F_{ST}=0.165$–0.795; Ayre and Dufty 1994) and *Balanophyllia elegans* ($F_{ST}=0.280$; Hellberg 1994). These results are compatible with the theory that dispersal and gene flow are restricted in brooding species (Carlon 1999). Be that as it may, some species of marine invertebrates that brood, or are characterised by reproductive modalities that seemingly limit dispersal capability, do exhibit high gene flows and low levels of genetic differentiation. For example, the black coral with benthic larvae *Anthipathes fiordensis* (Miller 1997) and the brooding scleractinians *Pocillopora daminicornis* (Ayre et al. 1997b), *Acropora cuneata*, *A. palifera* and *Stylophora pistillata* (Ayre and Hughes 2000) exhibit F_{ST} values <0.1, with the majority of these values not departing significantly from zero. These results highlight the fact that an entire range of processes may influence genetic differentiation in populations and may produce strong variations in dispersal capabilities in species that have the same reproductive mode (i.e. brooding or broadcasting).

In *B. europaea*, no relationship was found between the degree of genetic differentiation among populations and their geographic distance of separation. On the other hand, in *B. elegans* (the only congeneric species whose population genetics has been studied), there is an inverse correlation between the extent of gene flow and the geographic distance separating populations (Hellberg 1994). The substantial differences between the two species in relation to larval dispersal could partially explain the differences in their populations' genetic structure (Wright 1943; Kimura and Weiss 1964; Hellberg 1996). *B. europaea* has swimming larvae characterised by neutral buoyancy and pelagic dispersal (Goffredo and Zaccanti 2004). On the contrary, *B. elegans* larvae are non-swimming, with negative buoyancy and benthic dispersal (Gerrodette 1981). Given the behaviour of larvae of *B. elegans*, we would expect gene flow to occur exclusively between adjacent geographic areas, with a consequent decrease in genetic correlation with increasing geographic distance (the "stepping stone" model; Kimura and Weiss 1964). However, the larval behaviour of *B. europaea* does not support the stepping stone model, and, therefore, the geographic distance separating populations does not control gene flow variation among the populations (Hellberg 1996; Goffredo and Zaccanti 2004). A lack of correlation between geographic distance and genetic differentiation has been observed frequently in marine populations (sea urchins, Palumbi and Wilson 1990; antipatharian corals, Miller 1997; sea anemones, Ayre et al. 1991; soft corals, McFadden 1997). In *B. europaea*, genetic divergence between populations could be due to different selective pressures (Hedgecock 1986; Schoen and Brown 1991). Also, the species' reproductive biology could contribute significantly to the genetic structure observed. A mating system characterised by inbreeding through self-fertilisation may lead to co-coordinated genetic complexes, allowing local adaptation and subsequent genetic differentiation among populations (Schoen and Brown 1991; Knowlton and Jackson 1993; Pusey and Wolf 1996). Documented examples of inbreeding systems in marine animals are rare (barnacles, *Balanus improvisus*, Furman 1990; colonial ascidians, *Botryllus schlosseri*, Grosberg 1991; solitary ascidians, *Corella inflata*, Cohen 1992; sea anemones, *Epiactis prolifera*, Bucklin et al. 1984). In plants, more documented cases exist, demon-

strating a correlation between mating systems and intraspecific variation in genetic diversity, as well as in the effective size of the global population (Brown 1978; Schoen and Brown 1991). Particularly, inbreeding systems appear to be associated with both high levels of genetic variation between populations and high levels of variation in effective population size (Schoen and Brown 1991).

Genetic differentiation at small spatial scales

Our results on the genetic differentiation between patches within the Calafuria population are in line with the swimming behaviour of *B. europaea* larvae (Goffredo and Zaccanti 2004). We found a genetic connection among the Calafuria patches (8–40 m apart), while patches of *B. elegans* 4–30 m apart are known to be significantly subdivided (Hellberg 1994, 1995). We conclude that, while the crawling larvae of *B. elegans* are not capable of sustaining significant genetic connectivity among populations (even at small spatial scales), *B. europaea* larvae are able to sustain genetic connectivity among populations that are tens of meters apart.

Conclusions

The genetic structure of *B. europaea* is characterised by a marked departure from Hardy–Weinberg equilibrium, with a notable deficit of heterozygotes and a high rate of inbreeding. The examined populations were fragmented at large spatial scales, but genetically connected at smaller ones. The mating system of *B. europaea* could explain the observed genetic structure, since it is likely characterised by self-fertilisation, with swimming pelagic larvae that appear to guarantee genetic connectivity among populations at small spatial scales.

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