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Genetic variation and population structuring in two brooding coral species (*Siderastrea stellata* and *Siderastrea radians*) from Brazil

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Abstract *Siderastrea stellata* and *S. radians* are scleractinian coral species that present a remarkable overlap of diagnostic characteristics and sympatric distribution. Moreover, both are viviparous with similar reproductive strategies and with a gregarious larval behavior. Samples of both species from the Brazilian coast were analyzed using 18 isozymic loci to quantify their genetic variability and populational structure. Results confirmed species identity, high intrapopulational variability and revealed moderate genetic structuring among all samples (*S. stellata*: F_{ST} = 0.070; *S. radians*: F_{ST} = 0.092). Based on genotypic diversity analysis, there was evidence that local recruitment may have a minor role in the populations (mean, G_o:G_e = 1.00 ± 0.0003 SD for *S. stellata* and 0.99 ± 0.0023 SD for *S. radians*). Deviations towards heterozygote deficiencies found in both *Siderastrea* species could be explained by the Wahlund effect, since there was evidence that populations might be composed of colonies of different ages. In *S. radians* it is also likely that there is some inbreeding occurring in the studied populations. Despite the brooding pattern and the gregarious larval behavior, our data suggest the occurrence of gene flow along the Brazilian coast. This is the first study on population genetics of Brazilian reef corals.

Keywords Brazilian reefs · Gene flow · Genotypic diversity · Population structure · Scleractinian corals · *Siderastrea*

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Introduction

Biological information concerning Brazilian coral fauna is sparse, and somewhat restricted to its reproductive biology (Pires et al. 1999; Calderon et al. 2000; Neves and Pires 2002; Neves and da Silveira 2003). In Brazil, coral reefs are discontinuously distributed along 3,000 km of the northeastern coast, from the South of Bahia State to Maranhão, and represent the only reefal ecosystem found in the South Atlantic Ocean (Maida and Ferreira 1997). So far, approximately 23 species have been recorded along the northeastern and southeastern coasts, six of which are endemic, 16 also found in the West Indies and one in the Indo-Pacific region (Neves et al. 2006). The 'Northeastern Coastal Region' is characterized by the occurrence of reefs and estuaries associated with mangrove vegetation, in which the influence of the warm Brazilian Current and river discharges create optimal temperature and water enrichment for corals (Maida and Ferreira 1997). According to Leão et al. (2003), Brazilian coral reefs show peculiar features, being characterized by an exquisite architecture (pinnacles with mushroom-like forms), very

low diversity, remarkable endemism among reef builders, and species that are predominantly massive.

Siderastrea is a small scleractinian coral genus comprising five colonial zooxanthellate species, two of which occur in the Indo-Pacific region, *S. savignyana* Edwards and Haime 1850 and *S. glynni* (Budd and Guzmán, 1994), while the remainder occur in the Atlantic Ocean, *S. radians* (Pallas, 1766), *S. siderea* (Ellis & Solander, 1786), and *S. stellata* Verrill, 1868 (Laborel 1974). Neves (2004) thoroughly revised the diagnostic characters of the genus, and proposed the sympatric occurrence of *Siderastrea radians*, and *S. stellata* on the Brazilian coast. In the literature, *S. stellata* has been referred to as a synonym of *S. siderea* and *S. radians* and it was previously considered to be the only Brazilian siderastreid (Laborel 1974; Werner 1996; Maida and Ferreira 1997). However, *S. radians* and *S. stellata* undergo intratentacular budding, while *S. siderea* does not. Moreover, *S. radians* and *S. stellata* are known to brood their embryos, whereas *S. siderea* has been reported to shed gametes for external fertilization (Duerden 1904; Neves and da Silveira 2003; Szmant 1986). A recent survey using the nuclear ITS region confirmed the divergence between *S. siderea*, *S. radians* and *S. stellata* (Forsman et al. 2005).

Life history and reproductive behavior may be highly variable among sessile marine invertebrates (Levin and Bridges 1995). Dispersal differences are expected to produce distinct patterns of genetic exchange among populations. In contrast to externally developed planulae, brooded planulae are generally released at an advanced stage and are expected to settle immediately (Harrison and Wallace 1990), supporting local recruitment. *Siderastrea radians* and *S. stellata* undertake internal fertilization and brooding (Duerden 1904; Szmant 1986; Soong 1991). In the laboratory, planulae of *S. radians* remained mostly competent for 24–48 h, while *S. stellata* displayed an extended competency period, being unable to metamorphose before 72 h (Duerden 1904; Neves and da Silveira 2003). Besides larval development (phylopatric versus teleplanic larvae), physical-environmental factors (e.g. ocean currents, wind regime) have been also considered important in the dispersal of coral species (Jokiel 1990; Veron 1995).

The reproductive mode, e.g. gamete spawners or brooded planulae, also may have an important effect on gene flow and population structure (e.g. Ayre et al. 1997; Ayre and Hughes 2000; Nishikawa and Sakai 2005). Limited dispersal is generally associated with inbreeding, loss of heterozygosity and population subdivision due to genetic drift. We investigated if Brazilian *Siderastrea* populations fit these predictions, also using hierarchical surveys of genetic variation to quantify local and regional patterns of genetic variability along the northeastern and southeastern coast of Brazil. We also tested whether the genetic structure of reef communities in Brazil conforms to the "stepping stone" model, in which only adjacent populations exchange genes (Kimura and Weiss 1964). This relationship between gene flow and geographic distance is usually recognized as the mechanism that could explain differentiation of coral species (Hellberg 1994; Palumbi 2003). Samples of *S. radians* and *S. stellata* from Brazilian reef systems and coral communities were subject to populational analysis using isozymes, which seem to be a suitable molecular marker for characterizing and understanding population structure and migration in coral reefs (Ridgway 2005).

Material and methods

Study sites and collection

Nine locations were sampled, six distributed among the reefs of the northeastern coast (NE), and three in the coral communities of the southeastern coast (SE), covering about 2,000 km of the Brazilian littoral (Fig. 1). Respectively, eight and seven populations of *S. stellata* and *S. radians* were sampled; detailed information concerning locations and dates is given in Table 1. In this study, we use the term 'collection' to refer to each sample or population to avoid confusion, as the term 'sample' could also suggest a single coral individual. Due to the low abundance on the reefs, some collections have a very small number of colonies sampled, especially from *Siderastrea stellata*. Colonies with diameter of about 10 cm were collected randomly at each sampling site during low tide, at depths of 2–5 m with a hammer and chisel. In order to reduce the mucus production due to handling during field activities, colonies

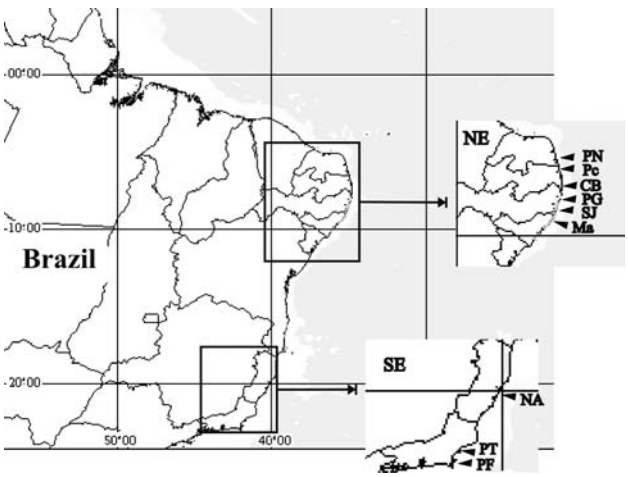

Fig. 1 Sampling sites in the reefal systems and coral communities from the northeastern (NE) and southeastern (SE) regions of the Brazilian coast. See Table 1 for abbreviations of the collections

Table 1 Locations sampled for *Siderastrea stellata* (Ss) and *S. radians* (Sr)

Region	spp.		Collections	Lat./Long.	Date
NE		Sr	Pirangi do Norte (PN)	5°.58′ S/35°.48′ E	28 Mar 01
	Ss	Sr	Picãozinho (Pc)	7°.7′ S/34°.48′ E	05 Apr 00
					26 Mar 01
	Ss	Sr	Cabo Branco/Ponta dos Seixas (CB)	7°.9′ S/34°.47′ E	04 Apr 00
					18 Mar 01
	Ss	Sr	Porto de Galinhas (PG)	8°.30′ S/34°.59′ E	09 Apr 00
					21 Mar 01
	Ss	Sr	São José da Coroa Grande (SJ)	8°.54′ S/35°.9′ E	08 Apr 00
					20 Mar 01
	Ss	Sr	Maragogi (Ma)	9°.10′ S/35°.21′ E	22 Mar 01
SE	Ss	Sr	Nova Almeida (NA)	19°.57′ S/42°.39′ E	23 Aug 01
	Ss		Praia da Tartaruga (PT)	22°.46′ S/42°.55′ E	24 Aug 01
	Ss		Praia do Forno (PF)	22°.96′ S/42°.2′ E	25 Jan 00
					20 Aug 01

Abbreviation of respective collections and locations in parentheses

were kept alive in foam boxes and left to rest for approximately 24 h. Fragments of skeleton with tissue were scraped off and frozen in liquid nitrogen until electrophoresis. Pieces from the same colonies were bleached, and species identification was performed using the corallites structures. Voucher specimens were deposited in the Museu de Zoologia da Universidade de São Paulo, São Paulo (Cabo Branco/Ponta do Seixas: MZUSP476, MZUSP477; São José da Coroa Grande: MZUSP478, MZUSP479; Porto de Galinhas: MZUSP480, MZUSP481; Maragogi: MZUSP482, MZUSP483; Picãozinho: MZUSP484, MZUSP485; Nova AlmeidaS: MZUSP488, MZUSP489; Praia do Forno: MZUSP486, MZUSP487; Praia da Tartaruga: MZUSP490, MZUSP491).

Electrophoresis

A total of 143 colonies of *S. stellata* and 177 of *S. radians* were analyzed. Small tissue pieces (3–5 mm^2) were squashed in extraction buffer (0.1 M Tris pH 8.0, 0.5% β-mercaptoethanol). The extracts were blotted onto Whatman #3 filter paper wicks and loaded on to horizontal 8.5% starch gels (hydrolyzed potato starch, Sigma, St. Louis, MO, USA). Four buffer systems were used: (I) electrode: 0.25 M Tris and 0.057 M citric acid, pH 8.0, and gel: electrode solution diluted 1:25, 50 mA/4 h (Ward and Warwick 1980) (II) electrode: 0.04 M citric acid and N(3 aminopropyl) morpholine, pH 6.1, and gel: electrode solution diluted 1:20, 50 mA/5 h (Clayton and Tetriak 1972); (III) electrode: 0.01 lithium hydroxide, 0.095 boric acid and 0.003 EDTA, pH 8.0, and gel: electrode solution diluted 1:40 50 mA/4 h; (IV) electrode: 0.3 M boric acid, 60 mM NaOH, pH 8.0, and gel: 10 mM Tris, pH 8.5, 180 V/4 h (Shaw and Prasad 1970, with modifications).

Eleven enzyme systems were surveyed: leucine aminopeptidase (EC 3.4.11.1, LAP), L-leucyl-L-glycylglycine peptidase (EC 3.4.11-, PLGG), L-leucyl-L-alanine peptidase (EC 3.4.13.18, PLA), malic enzyme (EC 1.1.1.40, ME), phosphoglucomutase (EC 5.4.2.2, PGM), malate dehydrogenase (EC 1.1.1.37, MDH), isocitric dehydrogenase (EC 1.1.1.42, IDH), esterase (EC 3.1.1.1, EST), fumarase (EC 4.2.1.2, FUM), glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49, 6PGD) and hexokinase (EC 2.7.1.1, HK). Staining procedures were adapted from Shaw and Prasad (1970), and Alfenas et al. (1991).

Data analysis

Alleles were identified by their mobility relative to the most common allele of the Maragogi collection. The BIOSYS-1 program (Swofford and Selander 1981) was used to estimate the genetic variability, namely, the percentage of polymorphic loci (P, 95% criterion), the mean number of alleles per locus (A_p), the gene diversity (H_e) and the observed heterozygosity (H_o) per collection (unbiased estimate, Nei 1978). BIOSYS-1 was also used to calculate identity matrices (Nei 1978).

Departures from Hardy–Weinberg expectations were calculated using the TFPGA program (Miller 1997). An exact test for each locus was performed for all collections using the conventional Monte Carlo method (adapted from Guo and Thompson 1992), performing 10 batches of 1,000 permutations (10,000 permutations). The sequential Bonferroni procedure was applied to correct type 1 errors introduced by multiple tests (Rice 1989).

The F_{IS} coefficient was calculated using Weir and Cockerham's (1984) f estimate. About 95% confidence intervals were determined for the F_{IS} value by

bootstrapping 10,000 times across loci using the GDA program (Lewis and Zaykin 1999). GENETIX v. 4.02 (Belkhir 2001) was used to calculate the F_{IS} coefficient per collection and to make random permutations in the matrix individuals versus genotypes in order to obtain the expected distribution of F_{IS} under the null hypothesis of random mating ($F_{IS} = 0$). The probability of a value equal or larger than the estimated value was calculated by P = (n + 1)/(N + 1), where n is the number of pseudo-values larger than or equal to the estimate, and N is the number of random permutations (Sokal and Rohlf 1995). Sequential Bonferroni correction was also used here.

To verify possible associations among the studied loci, genotypic data were tested for linkage disequilibrium within collections using the GENEPOP program, without collapsing less frequent alleles (Raymond and Rousset 1995). One hundred batches of 5,000 iterations per batch with 10,000 dememorization steps were made. Again, the sequential Bonferroni procedure was applied. Significant associations among loci could denote episodes of inbreeding, asexual reproduction, selection, recent colonization and the Wahlund effect (in which case interloci associations will vary among collections, Crow and Kimura 1970).

The relative frequencies of sexual or asexually derived colonies were inferred using the approach described by Stoddart and Taylor (1988). First, multi-locus genotypes of each colony (N) were obtained and the number of the unique multi-locus genotypes (N_g) was counted. The ratio N_g:N provides the simplest index of the effects of asexual reproduction on genotypic diversity. The ratio of observed multilocus genotypic diversity (G_o) was then obtained and compared to that expected (G_e) under conditions of sexual reproduction with free recombination. Departures of G_o:G_e from unity provide an index of the combined effects of departure from Hardy–Weinberg equilibrium and multilocus linkage equilibrium. We assessed the statistical significance of differences among G_o and G_e using an unpaired t-test (Stoddart and Taylor 1988).

A hierarchical analysis was performed to partition genetic variability within and among major sampling regions (NE and SE) for *S. stellata*: F_{SR}, variation among sites within regions; F_{RT}, total variation among regions; F_{ST}, total variation among all sampling sites. The coefficients were calculated using the Weir and Cockerham (1984) θ parameter. Ninety-five percent confidence intervals were determined for the total F_{ST} value by bootstrapping 10,000 times across the loci using the GDA program (Lewis and Zaykin 1999). Gene flow was inferred for each hierarchical level using Wright's (1969) Island model ($N_em = [(1/\theta) - 1]/4$), where N_e is the effective population size and m is the proportion of migrants per generation.

To test if there is a correlation between geographic distance and F_{ST}, a Mantel test was performed comparing each pair of *S. stellata* and *S. radians* collections. The matrices were compared by the Mantel test using the TFPGA program (Miller 1997). The GENETIX v.4.02 program (Belkhir 2001) uses random permutations on the matrix of individuals versus genotypes to find the expected distribution of F_{ST} under the null hypothesis ($F_{ST} = 0$). The probabilities of each F_{ST} estimated were calculated as presented above for F_{IS}.

Results

Genetic variability within populations

For the 11 enzymes surveyed, 18 loci were scored. None of them were diagnostic, but most loci presented exclusive alleles for each species (Appendix).

A total of 14 and 13 loci were polymorphic (95% criterion) for *S. stellata* and *S. radians*, respectively. The most polymorphic loci were Idh for *S. stellata*, with seven alleles, and Fum and Idh for *S. radians*, with eight alleles. Percentage of polymorphic loci and gene diversity indicate some genetic variability in the collections (*S. stellata* P = 38%, $H_e = 0.120$; *S. radians* P = 45%, $H_e = 0.168$), as seen in Table 2. There is no relationship between the collection size and the percentage of polymorphic loci (*S. stellata* $R^2 = 0.09$, $P = 0.47$; *S. radians* $R^2 = 0.04$, $P = 0.65$). The H_o values were very low and F_{IS} per location were very high (Table 2), the averages for *S. stellata* and *S. radians* were, respectively, $F_{IS} = 0.330$ (95% CI = 0.170–0.592) and 0.580 (95% CI = 0.414–0.720).

Three *S. stellata* collections and six of *S. radians* showed departures from Hardy–Weinberg expectations in several loci; all deviations were due to deficit of heterozygotes. Using the Monte Carlo method, 4 out of 65 tests were significant for *S. stellata* and 18 out of 70 were significant for *S. radians* (Table 3).

Siderastrea stellata presented only four significant loci associations ($P < 0.05$) in 169 tests. When the sequential Bonferroni correction was applied, none of these were significant. The test of linkage disequilibrium revealed that only 15 in 234 tests in *S. radians* are significant ($P < 0.05$). After applying the Bonferroni procedure, one association (Pgm-1/Pgm-2) was significant in two collections, SJ and NA.

In both *Siderastrea* species, each collection contained high genotypic diversity: the majority of colonies displayed unique multi-locus genotypes (N_g:N ranged from 0.87 to 1; Table 4). The collections showed >93% of the multi-locus genotypic diversity (G_o) expected for random mating and free recombination (G_e). For both species, G_o was never significantly different from G_e (mean G_o:$G_e = 1.00 \pm 0.0003$ SD for *S. stellata* and 0.99 ± 0.0023 SD for *S. radians*; unpaired Student's t-tests, $P > 0.1$).

Table 2 Average genetic variability for *Siderastrea* species

Species	Collection	N	P	A_p	H_e	H_o	F_{IS}
S. stellata	Pc	9.8	38	1.6	0.101	0.040	**0.619**
	CB	2.7	27	1.2	0.115	0.056	**0.571**
	PG	10.1	66	1.9	0.210	0.124	**0.427**
	SJ	9.9	22	1.3	0.074	0.060	0.209
	Ma	13.2	38	1.8	0.109	0.092	**0.170**
	NA	3.5	27	1.4	0.163	0.093	**0.675**
	PT	24.9	38	1.9	0.078	0.051	**0.352**
	PF	31.1	44	2.2	0.106	0.087	**0.183**
Mean		13.2	38	1.7	0.120	0.075	
S. radians	PN	27.2	33	2.3	0.139	0.065	**0.538**
	Pc	13.4	27	1.6	0.101	0.056	**0.458**
	CB	23.4	61	2.3	0.160	0.092	**0.434**
	PG	11.7	66	1.9	0.177	0.103	**0.457**
	SJ	15.7	27	1.8	0.136	0.037	**0.735**
	Ma	13.0	44	2.0	0.217	0.050	**0.771**
	NA	26.4	55	2.6	0.243	0.109	**0.563**
Mean		18.7	45	2.1	0.168	0.073	

See Table 1 for abbreviation of the collections

N, mean collection size per locus; P, percentage of polymorphic loci (95% criterion); A_p, mean number of alleles per locus. H_e, gene diversity (unbiased estimate, Nei 1978); H_o, observed heterozygosity; F_{IS}, fixation index (significant values in bold)

Table 3 F_{IS} values of loci with deviations from the expected values in HW equilibrium

Species	Collection	*Mdh*	*Pgm-1*	*Pgm-2*	*Pgm-3*	*Fum*	*Hk-1*	*Idh*	*Me*
S. stellata	Ma	–	−0.142	**0.726**	–	−0.050	–	−0.066	–
	PT	1.00	1.00	0.00	–	−0.055	1.00	**0.745**	−0.100
	PF	–	–	0.00	–	0.662	**0.732**	0.383	−0.154
S. radians	PN	0.663	0.488	**0.868**	0.869	−0.021	**0.650**	0.130	**0.492**
	Pc	0.650	0.622	–	–	**0.814**	–	0.00	0.036
	CB	**1.00**	0.217	**1.00**	–	**0.555**	0.661	−0.055	0.375
	SJ	**1.00**	**0.814**	**1.00**	–	–	0.784	0.00	0.257
	Ma	–	**0.907**	**0.919**	1.00	0.231	1.00	0.00	0.747
	NA	**0.900**	**0.882**	**0.767**	**0.728**	0.365	−0.052	1.00	**0.621**

In bold, loci with significant deviation after sequential Bonferroni correction ($P < 0.001$); – monomorphic loci. See Table 1 for abbreviation of the collections

Population structure and genetic identity

Siderastrea stellata

There was significant genetic differentiation (F_{ST} = 0.070, 95% CI = 0.021–0.100) among all collections and the number of migrants per generation (N_em) was 3.3. The F_{SR} values between NE and SE collections were moderate: F_{SR} = 0.048 and 0.091 (95% CI = 0.002–0.092; 0.009–0.158), respectively. Estimated N_em was 4.9 for NE collections, and 2.5 for SE collections. There was no inter-region differentiation (F_{RT} = −0.006, 95% CI = −0.040–0.045; N_em = 41.9), suggesting no hierarchical structuring.

Geographic distances ranged from 7.56 km to 1748 km among the collections. The F_{ST} parameter ranged from −0.006 (Pc − PF) to 0.404 (NA − PT) in the pairwise estimates. The correlation between geographic distance and genetic structuring was not significant (r = 0.10, P = 0.28). The F_{ST} estimates calculated for each pair of populations are presented in Table 5, and eight out 28 tests were significant when sequential Bonferroni correction was applied.

Table 4 Estimates of contribution of asexual reproduction in each reefal system of *Siderastrea* species

Species	Collection	N_g	N	N_g:N	G_o:G_e	Species	Collection	N_g	N	N_g:N	G_o:G_e
S. stellata	Pc	12	12	1.00	1.000	*S. radians*	PN	34	34	1.00	1.000
	CB	3	3	1.00	1.000		Pc	16	16	1.00	1.000
NE	PG	13	13	1.00	1.000	NE	CB	24	26	0.92	1.000
	SJ	12	12	1.00	1.000		PG	16	16	1.00	0.937
	Ma	18	18	1.00	1.000		SJ	18	18	1.00	1.000
SE	NA	6	6	1.00	1.000		Ma	19	19	1.00	1.000
	PT	32	35	0.91	1.000	SE	NA	42	48	0.87	1.000
	PF	39	44	0.88	1.001						

See Table 1 for abbreviation of the collections

N_g, number of unique multi-locus genotypes; N, number of colonies sampled; G_o, observed multi-locus genotypic diversity; G_e, expected multi-locus genotypic diversity

Table 5 Paired F_{ST} estimates of *Siderastrea stellata*, with 5,000 permutations

Collection	NE				SE		
	CB	PG	SJ	Ma	NA	PT	PF
Pc	−0.003	0.014	0.017	0.064	0.225	0.028	−0.006
CB		0.004	0.098	0.027	0.184	−0.005	0.072
PG			0.042	0.081	0.095	**0.146**	**0.086**
SJ				0.049	**0.331**	0.065	0.041
Ma					**0.372**	0.057	**0.112**
NA						**0.404**	0.216
PF							**0.051**

Significant values in bold. See Table 1 for abbreviation of the collections (NE and SE indicating splitting by region)

The genetic identities (*I*, Nei 1978) ranged from 0.912 (Ma − NA) to 1.00 (CB − PT) in *S. stellata*, with an average of 0.976.

Siderastrea radians

Collections presented significant structuring (θ = 0.092, 95% CI = 0.024–0.15), with 2.5 migrants per generation (N_em). The F_{SR} obtained only among NE collections was moderate, θ = 0.078 (95% CI = 0.017–0.143) and the estimated N_em were 3. Similarly to *S. stellata*, *S. radians* did not present a correlation between geographic distance and genetic structuring (r = 0.22, P = 0.17) in a geographic distance range of 186.10 km to 1,672 km. The F_{ST} pairwise estimates calculated varied from −0.042 (NA − PT) to 0.197 (CB − Ma), and only four out of 21 were significant (Table 6).

The mean genetic identity of *S. radians* collections was also high (I = 0.974), and ranged from 0.935 (Ma − Pc) to 1.00 (PN − PG). The mean genetic identity among *Siderastrea* species was also very high (I = 0.92).

Discussion

Intrapopulational genetic variability

In our study, the percentage of polymorphic loci in both species varied significantly among collections. Some

Table 6 Paired F_{ST} estimates of *Siderastrea radians*, with 5,000 permutations

Collections	NE					SE
	Pc	CB	PG	SJ	Ma	NA
PN	0.030	0.031	−0.042	0.056	0.120	**0.112**
Pc		−0.004	0.050	0.085	0.211	0.144
CB			0.023	**0.075**	**0.197**	**0.137**
PG				0.077	0.038	0.067
SJ					0.157	0.078
Ma						0.057

Significant values in bold. See Table 1 for abbreviation of the collections (NE and SE indicating splitting by region)

collections, as the one from Porto de Galinhas of *S. stellata*, presented a high value (66%), as well as the *S. radians* samples from Cabo Branco/Ponta do Seixas and Nova Almeida (61.1% and 55.5%, respectively). However, most of the collections presented polymorphism values below 40%, which is unlikely to be an effect of the sampling size. Nevertheless, both species also presented a strikingly high diversity of genotypes (Table 4). Some of them, as Porto de Galinhas of *S. stellata*, presented a high value (66%), as well as *S. radians* collection from Cabo Branco/Ponta do Seixas and Nova Almeida (61.1% and 55.5%, respectively). Most of the polymorphism values were below 40%, which might not be an effect from the sampling size. Nevertheless, both species also presented strikingly high diversity of genotypes (Table 4). A model proposed by Bengtsson (2003) showed that even a small frequency of sexual reproduction per generation would be sufficient to make a population highly genotypically variable. In this model, a population started by a number of sexually-derived propagules may retain its initial genotypic variation for a very long period of time. The population might keep a record of its earlier genetic history, a phenomenon that the author designated as 'memory-effect'. Thus, besides the population size, the sexual recruitment and the possible effect of environmental heterogeneity, the longevity of the colonies may play an important role in maintenance of genetic variability in *Siderastrea* collections.

Hardy–Weinberg deviations are commonly found in corals (e.g. Brazeau and Harvell 1994; Márquez et al. 2002; Ng and Morton 2003; Miller and Ayre 2004). In the present study, heterozygote deficiency was observed in both species, with different intensities in many loci and collections. This could reflect complex interactions among a range of factors, including the occurrence of null alleles (Gardner 1992), selective mortality (Zouros and Foltz 1984), the Wahlund effect (Ayre and Dufty 1994), differences in the time of spawning ('genotype-dependent spawning', Zouros and Foltz 1984) and inbreeding (Smith and Potts 1987). There is the possibility of the occurrence of more than one species or sub-species in our collections. If so, preferential intraspecific crosses would generate linkage disequilibrium, but significant or consistent associations among genotypes were very scarce. The occurrence of inbreeding seems to be very likely in our collections for *S. radians*. Indeed, the collections of *S. radians* presented the higher number of loci with deviation and the highest F_{IS} values. The shorter planktonic phase of this species associated with the phylopatric behavior of the larvae can explain the high Hardy–Weinberg deviation observed. However, if inbreeding had a major role in our collections, it would affect all loci simultaneously to a similar degree (Lewontin and Krakauer 1973). Thus, associated with inbreeding events, the Wahlund effect caused by temporal variation of reproductive cohorts would be also a possible explanation for the observed heterozygote deficiency. In this case, the sampled populations of both species would be composed of colonies from different ages (which could also explain the observed genetic variability), generated by the settlement of different cohorts whose parents did not interbreed before. One possible reason for the formation of these populations would be the temporally different sources of larvae, which seems to be a common feature in scleractinians (Jokiel 1990; Veron 1995), due to the variation of the hydrodynamic conditions.

Local recruitment and structuring

Among scleractinians, recent electrophoretic investigations have revealed small values of F_{ST} and genetic distance, indicating that the genetic structure may be independent of geographic distance, breeding system and mode of larval development (Márquez et al. 2002; Ng and Morton 2003). A moderate to low structure has also been reported among broadcast-spawning species (e.g. *Pocillopora verrucosa*, Ridgway, Hoegh-Guldberg & Ayre, 2001), suggesting that local recruitment may have a restricted role in the maintenance of genetic structure of coral populations. Genetic evidence from nine coral species showed that population variability of brooding and broadcasting species could be maintained by a balance between localized settlement of larvae and high gene flow along the Great Barrier Reef caused by long-dispersal larvae (Ayre and Hughes 2000). In that study, the authors found significant genetic variation among local populations within geographic regions for all species, but not among the geographic groups on a larger scale (1,800 km) for two broadcasting and three brooding species. Nishikawa and Sakai (2005) also found evidence of high gene flow along a macrogeographic scale despite of a substantial proportion of local recruitment in the brooding species *Goniastrea aspera*.

Reproductive behavior, phylopatric behavior and early development might explain the moderate structure documented for *S. stellata* ($F_{ST} = 0.07$) and *S. radians* ($F_{ST} = 0.09$). However, our data do not support the prediction that these brooding species have a very restricted dispersal, regardless of the analyzed scale. Indeed, in all evaluated hierarchical levels, the inferred number of migrants per generation is substantially high. *Siderastrea stellata* is likely to be lecitotrophic, depending on yolk reserves and photosynthetic products during the early larval stage (Neves and da Silveira 2003). The long competency period of *S. stellata* (up to 15 days) would be expected to enhance homogenization, reducing the

population differentiation. The competency period of *S. radians* larvae is shorter (up to 48 h, Duerden 1904), which could explain the higher structuring; however the values of F_{ST} for both species are not statistically different. Virtually nothing is known about the dispersal capabilities of *Siderastrea radians* and *S. stellata* under field conditions. Thus, the competency time observed in laboratory conditions may be an underestimation and in their natural environmental, with suitable physical and biotic conditions, larvae could potentially live on plankton for a longer time.

There was no hierarchical structuring in *S. stellata*, but we found significant structuring within regions, especially in region SE. The estimated values of F_{SR} and F_{ST} in *S. stellata* are also not significantly different, suggesting that there is homogeneous gene flow among collections. Additional evidence of extensive gene flow in both species is provided by the shared rare alleles among geographically distant collections in most of the loci (Appendix). The lack of correlation between geographical distance and genetic differentiation (F_{ST}) suggested that the observed genetic differentiation among the populations distributed along the coast cannot be explained solely by geographical isolation.

Considering our results, there are no apparent physical or ecological barriers between the north and southeastern coastal regions. We also did not find any evidence of a "stepping stone" pattern in the collections of either species. *Siderastrea stellata* populations are most likely distributed discontinuously along the north/southeastern coast (Laborel 1974; Neves pers. obs.). There is no such record for *S. radians*, but it is expected that this species generally has a similar distribution, since it usually occurs in the same natural conditions as those in which *S. stellata* populations are found. Thus, despite the discontinuous distribution structure of reef communities in Brazil (Maida and Ferreira 1997), there is strong evidence that *Siderastrea* populations exchange genes along the coast. Some long distance gene flow might also occur via a series of short distance recruitment along generations. If there is local recruitment in the Brazilian siderastreids, the gene flow seems to somewhat override its effect, preventing the development of high geographic structuring at the evaluated scale.

Interspecific genetic identities in Siderastrea

Recognition of interspecific limits among scleractinians can be a difficult task (Laborel 1974; Lang 1984; Veron 1995; Forsman et al. 2005). In this context, the presence of exclusive alleles may provide a relevant support to taxonomical analyses, particularly when the species are closely related and with similar morphological traits. Although diagnostic loci were not found, some alleles were restricted or particularly predominant in one of the studied species (see loci Pgm and Mdh in Appendix). This is evidence of genetic divergence between the two species and confirms the maintenance and acceptance of their specific status.

The mean interspecific genetic identity between the Brazilian *Siderastrea* species was high, and not very different from intraspecific values. Our data showed that the species are distinct although closely related. Indeed, a similar pattern has been reported in other coral species (Weil and Knowlton 1994; McFadden 1999). Contrasting with the southeastern coral communities, where *S. stellata* was the only species identified, *S. radians* and *S. stellata* have a sympatric distribution along the northern shallow-water reefal system and are also very similar morphologically (Neves 2004). Thus, these species could be considered 'pseudo-sibling species', as they are genetically distinct but only recognizable when the appropriate morphological attributes are considered (Knowlton 1994).

This study is the first evaluation of the genetic variability of two reef-building species of scleractinian corals from Brazil. Moreover, the results contribute to the maintenance of the specific status of the two congeners, supporting Neves (2004) morphological analysis and refuting Werner (1996), who suggested *S. stellata* as synonym of *S. radians*. On the other hand, finer-scale investigations would be necessary to confirm whether the spatial and temporal patterns found are consistent. Further studies might determine if local recruitment is the main influence on the structure of the *Sideratrea* populations located at the extremes of species distribution.

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Appendix

Allelic frequencies from loci of *Siderastrea stellata* and *S. radians* (N = total collection size)

Siderastrea stellata									*Siderastrea radians*						
Collection N	Pc 12	CB 3	PG 13	SJ 12	Ma 18	NA 6	PT 35	PF 44	PN 34	Pc 16	CB 26	PG 16	SJ 18	Ma 19	NA 48
Alleles	*Mdh*														
1									0.015						0.014
2	0.091		0.077						0.955	0.900	0.846	0.875	0.824	1.00	0.843
4						0.083	0.033						0.059		0.086
5	0.909	1.00	0.846	1.00	1.00	0.583	0.967	1.00		0.100	0.077	0.063	0.059		
6						0.334			0.030		0.077		0.059		0.057
7			0.077									0.063			
Pgm-1															
1			0.063		0.111	0.167			0.039	0.031	0.063				0.042
2	1.00	0.833	0.750	0.722	0.778	0.667	0.971	1.00	0.731	0.813	0.854	0.900	0.556	0.469	0.292
3			0.188	0.056	0.111				0.115	0.156	0.083	0.100	0.333	0.313	0.333
4				0.222			0.029		0.115				0.111	0.219	0.333
5		0.167				0.167									
Pgm-2															
1					0.031		0.014	0.011	0.024						
2	0.833	1.00	0.667	0.850	0.688	1.00	0.971	0.989	0.786	1.00	0.880	0.727	0.500	0.222	0.263
3	0.083		0.333	0.150	0.125							0.046	0.333	0.111	0.145
4							0.014						0.056	0.056	0.316
5	0.083				0.094				0.191			0.227	0.056	0.528	0.132
6													0.056	0.083	0.132
7					0.063						0.120				0.013
Pgm-3															
1															0.167
2	1.00	1.00	0.800	1.00	1.00	1.00	1.00	1.00	0.765	1.00	1.00	0.750	1.00	0.250	0.583
3			0.200												
4									0.235			0.250		0.500	
5															0.250
6														0.250	
Fum															
1										0.107	0.039	0.046			
2					0.067						0.019			0.053	
3					0.033			0.018			0.115	0.046		0.105	
4						0.167		0.036		0.107	0.039			0.079	0.074
5	1.00	1.00	0.708	1.00	0.900	0.750	0.929	0.946	0.953	0.786	0.750	0.909	1.00	0.763	0.927
6			0.167			0.083	0.071				0.039				
7									0.031						
8			0.125						0.016						
Est-1															
1											0.039				
2											0.019				
3	0.056		0.050	0.046				0.079							
4	0.944	1.00	0.950	0.909	1.00	1.00	1.00	0.921	1.00	1.00	0.942	1.00	1.00	1.00	0.988
5				0.046											0.013

Appendix continued

Siderastrea stellata									*Siderastrea radians*						
Collection N	Pc 12	CB 3	PG 13	SJ 12	Ma 18	NA 6	PT 35	PF 44	PN 34	Pc 16	CB 26	PG 16	SJ 18	Ma 19	NA 48
Est-2															
1								0.030							
2	0.042		0.100												
3	0.958	1.00	0.900	1.00	1.00	1.00	1.00	0.970	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Hk-1															
1			0.050					0.035	0.039		0.022	0.091	0.100		0.071
2	1.00	0.833	0.850	1.00	1.00	1.00	0.913	0.862	0.885	1.00	0.935	0.909	0.833	0.778	0.929
3		0.167					0.087	0.035	0.039				0.067		
4			0.100					0.069			0.044				
5									0.039					0.222	
Hk-2															
1	1.00	1.00	1.00	1.00	0.875	1.00	1.00	0.950	1.00	1.00	0.952	0.938	1.00	1.00	1.00
2					0.125			0.050			0.048				
3												0.062			
6pgd															
1						0.500					0.139			0.143	0.056
2							0.031	0.020							0.056
3		0.333	0.111				0.031	0.040		0.167					0.111
4	1.00	0.667	0.889	1.00	0.917	0.500	0.938	0.880	1.00	0.833	0.833	0.875	1.00	0.786	0.667
5											0.028	0.125			
6								0.060						0.071	0.056
7					0.083										
8															0.056
Pep-lgg															
1	0.042						0.021						0.029		
2							0.042	0.035							
3	0.875	0.667	0.917	1.00	0.941	1.00	0.833	0.931	0.953	1.00	0.923	0.893	0.971	1.00	0.955
4	0.083	0.333	0.083		0.059		0.104	0.035	0.016		0.058	0.107			0.046
5									0.031		0.019				
Idh															
1														0.031	
4			0.083		0.059				0.015	0.033		0.094	0.028		
5									0.030						
6	0.083							0.026							
7	0.917	1.00	0.917	1.00	0.882	0.800	0.929	0.934	0.909	0.967	0.904	0.906	0.972	0.969	0.947
8							0.036								0.026
9									0.015						
10								0.026							
11					0.059	0.200	0.018	0.013			0.039				0.026
12									0.030		0.058				
13							0.018								
Pep-la															
1	0.917	0.833	1.00	1.00	0.971	1.00	0.913	0.978	0.956	1.00	0.885	0.906	1.00	0.933	0.833
2	0.042	0.167			0.029				0.029		0.058	0.063			0.048
3							0.022							0.033	0.071
4	0.042						0.065	0.022	0.015		0.058	0.031		0.033	0.048

Appendix continued

Siderastrea stellata									*Siderastrea radians*						
Collection N	Pc 12	CB 3	PG 13	SJ 12	Ma 18	NA 6	PT 35	PF 44	PN 34	Pc 16	CB 26	PG 16	SJ 18	Ma 19	NA 48
Me															
1								0.074	0.037	0.031	0.048	0.067			0.125
2	0.182		0.273	0.286		1.00	0.109	0.278	0.444	0.250	0.357	0.567	0.281	0.533	0.475
3	0.591	1.00	0.455	0.714	1.00		0.891	0.519	0.482	0.656	0.595	0.367	0.719	0.467	0.350
4	0.227		0.273					0.130	0.037	0.063					0.050

References

Alfenas AC, Peters I, Brune W, Passador GC (1991) Eletroforese de Proteínas e Isoenzimas de Fungos e Essências Florestais. Universidade Federal de Viçosa, Viçosa

Ayre DJ, Dufty SL (1994) Restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. Evolution 48:1183–1201

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54:1590–1605

Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier Reef, Australia. Mar Ecol Prog Ser 159:175–187

Belkhir K (2001) GENETIX, logiciel sous WindowsTM pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060. Université de Montpellier II, Montpellier, France

Bengtsson BO (2003) Genetic variation in organisms with sexual and asexual reproduction. J Evol Biol 16:189–199

Brazeau DA, Harvell CD (1994) Genetic structure of local populations and divergence between growth forms in a clonal invertebrate, the Caribbean octocoral *Briareum asbestinum*. Mar Biol 119:53–60

Budd AF, Guzmán HM (1994) *Siderastrea glynni*, a new species of scleractinian coral (Cnidaria, Anthozoa) from the Eastern Pacific. Proc Biol Soc Wash 107:591–599

Calderon EN, Castro CB, Pires DO (2000) Natação, assentamento e metamorfose de plânulas do coral *Favia gravida* Verrill, 1868 (Cnidaria, Scleractinia). Bol Mus Nac Ns zoo 429:1–12

Clayton J, Tetriak D (1972) Amine-citrate buffers for pH control in starch gel electrophoresis. J Fish Res Bd Can 62:1169–1172

Crow JF, Kimura M (1970) An introduction to population genetic theory. Harper and Row, New York

Duerden JE (1904) The coral *Siderastrea radians* and its postlarval development. Carnegie Institution of Washington, Washington

Forsman ZH, Guzman HM, Chen CA, Fox GE, Wellington GM (2005) An ITS region phylogeny of *Siderastrea* (Cnidaria: Anthozoa): is *S. glynni* endangered or introduced? Coral Reefs 24:343–347

Gardner JPA (1992) Null alleles and heterozygote deficiencies among mussels (*Mytilus edulis* and *M. galloprovincialis*) of two sympatric populations. Malacologia 34:99–106

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48: 361–372

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world 25: coral reefs. Elsevier, New York, pp 133–207

Hellberg ME (1994) Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. Evolution 48:1829–1854

Jokiel PL (1990) Long-distance dispersal by rafting: re-emergence of an old hypothesis. Endeavour 14:66–73

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561–576

Knowlton N (1994) Sibling species in the sea. Annu Rev Ecol Syst 24:189–216

Laborel J (1974) West African reef corals a hypothesis on their origin. In: Proceedings of the 2nd international coral reef symposium, vol 1. Province, Australia, 22 June–2 July 1973, pp 425–443

Lang JC (1984) Whatever works: the variable importance of skeletal and non-skeletal characters in scleractinian taxonomy. Paleontogr Am 54:18–44

Leão ZMAN, Kikuchi RKP, Testa V (2003) Corals and coral reefs of Brazil. In: Cortés J (ed) Latin American coral reefs. Elsevier Science, New York, pp 9–52

Levin LA, Bridges TS (1995) Pattern and diversity in reproduction and development. In: McEdward L (ed) Ecology of marine invertebrate larvae. CRC Press, Florida, pp 1–48

Lewis PO, Zaykin D (1999) Genetic data analysis: computer program for the analysis of allelic data, version 1.0 (d12). Distributed at http://www.chee.unm.edu/gda

Lewontin RC, Krakauer J (1973). Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics 74:175–195

Maida M, Ferreira BP (1997) Coral reefs of Brazil: an overview. In: Proceedings of the 8th international coral reef symposium, vol 1. Panama City, 24–29 June 1996, pp 263–274

Márquez LM, Van Oppen MJH, Willis BL, Miller DJ (2002) Sympatric populations of the highly cross-fertile coral species *Acropora hyacinthus* and *Acropora cytherea* are genetically distinct. Proc R Soc Lond B 269:1289–1294

McFadden CS (1999) Genetic and taxonomic relationships among Northeastern Atlantic and Mediterranean populations of the soft coral *Alcyonum coralloides*. Mar Biol 133:171–184

Miller MP (1997) Tools for population genetic analyses (TFPGA) 1.3: a windows program for the analysis of alloenzyme and molecular population genetic data. Free software

Miller KJ, Ayre DJ (2004) The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity 92:557–568

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

Neves EG (2004) Complexo *Siderastrea*: espécies distintas? Significado da variabilidade do gênero *Siderastrea* de Blainville, 1830 (Anthozoa, Scleractinia) no Brasil. Ph.D. thesis. Universidade de São Paulo, São Paulo

Neves EG, Pires DO (2002) Sexual reproduction of Brazilian *Mussismilia hispida* (Verrill. 1902). Coral Reefs 21:161–168

Neves EG, da Silveira FL (2003) Release of planulae larvae and settlement behavior of *Siderastrea stellata* Verrill, 1868 (Anthozoa, Scleractinia), in Brazil. Hydrobiologia 501:139–147

Neves EG, Johnsson R, Sampaio C, Pichon M (2006) The occurrence of *Scolymia cubensis* in Brazil: revising the problem of the Caribbean solitary mussids. Zootaxa 1366:45–54

Nishikawa A, Sakai K (2005) Genetic connectivity of the scleractinian coral *Goniastrea aspera* around the Okinawa Islands. Coral Reefs 24:318–323

Ng WC, Morton B (2003) Genetic structure of the scleractinian coral *Platygyra sinensis* in Hong Kong. Mar Biol 143:963–968

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13(Suppl):S146–S158

Pires DO, Castro CB, Ratto CC (1999) Reef coral reproduction in the Abrolhos reef complex, Brazil: the endemic genus *Mussismilia*. Mar Biol 135:463–471

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rice WR (1989) Analysing tables of statistical tests. Evolution 43:223–225

Ridgway T (2005) Allozyme electrophoresis still represents a powerful technique in the management of coral reefs. Biodivers Conserv 14:135–149

Ridgway T, Hoegh-Guldberg O, Ayre DJ (2001) Panmixia in *Pocillora verrucosa* from South Africa. Mar Biol 139:175–181

Shaw CR, Prasad R (1970) Starch gel eletrophoresis of enzymes—a compilation of recipes. Biochem Genet 4:297–320

Smith BL, Potts DC (1987) Clonal and solitary anemones (*Anthopleura*) of Western North America: population genetics and systematics. Mar Biol 94:537–546

Sokal RR, Rohlf FJ (1995) Biometry, the principles and practice of statistics in biological research. WH Freeman Company, New York

Soong K (1991) Sexual reproductive patterns of shallow-water reef corals in Panama. Bull Mar Sci 49:832–846

Stoddart JA, Taylor JF (1988) Genotypic diversity: estimation and prediction in samples. Genetics 118:705–711

Swofford DL, Selander RB (1981) Biosys-1: a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematic. J Hered 72:281–283

Szmant AM (1986) Reproductive ecology of Caribbean reef corals. Coral Reefs 5:43–53

Veron JEN (1995) Corals in space and time. The biogeography and evolution of the Scleractinia. University of New South Wales Press, Sydney

Ward RD, Warwick T (1980) Genetic differentiation in the molluscan species *Littorina rudis* and *L.* arcana (Prosobranchia: Littorinidae). Biol J Linn Soc 14:417–428

Weil E, Knowlton N (1994) A multi-character analysis of the Caribbean coral *Montastraea annularis* (Ellis and Solander, 1786) and its two sibling species, *M. faveolata* (Ellis and Solander, 1786) and *M. franksi* (Gregory, 1895). Bull Mar Sci 55:151–175

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Werner TB (1996) Recent zooxanthellate corals (order: Scleractinia) from Ascension and St. Helena Is., South Atlantic, with a summary of their geographic distribution in the Atlantic Ocean. Dissertation. University of Maryland at College Park

Wright S (1969) The evolution and genetics of populations. Vol. 2. The theory of gene frequencies. University of Chicago press, Chicago

Zouros E, Foltz DW (1984) Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25:583–591