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Genetic differentiation among populations of a broadcast spawning soft coral, *Sinularia flexibilis*, on the Great Barrier Reef

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Abstract The genetic structure of 12 reef populations of the soft coral *Sinularia flexibilis* (Octocorallia, Alcyoniidae) was studied along the Great Barrier Reef (GBR) at a maximum separation of 1,300 km to investigate the relative importance of sexual and asexual reproduction, genetic differentiation and gene flow among these populations. *S. flexibilis* is a widely distributed Indo-Pacific species and a gamete broadcaster that can form large aggregations of colonies on near-shore reefs of the GBR. Up to 60 individuals per reef were collected at a minimum sampling scale of 5 m at two sites per reef, from December 1998 to February 2000. Electrophoretic analyses of nine polymorphic allozymes indicated that genotypic frequencies in most populations and loci did not differ significantly from those expected from Hardy–Weinberg predictions. Analysis of multi-locus genotypes indicated a high number of unique genotypes (N_{go}) relative to the number of individuals sampled (N) in each reef population (range of 0.69–0.95). The maximum number of individuals likely to have been produced sexually (N^*) was similar to the number of individuals sampled (i.e. $N^*:N \sim 1$), suggesting that even repeated genotypes may have been produced sexually. These results demonstrated a dominant role of sexual reproduction in these populations at the scale sampled. Significant genetic differentiation between some populations indicated that gene flow is restricted between some reefs ($F_{ST} = 0.026$, 95% CI = 0.011 – 0.045) and even between sites within reefs ($F_{ST} = 0.041$, 95% CI = 0.027 – 0.055). Nevertheless, there was no relationship between geographic separation and genetic differentiation. Analyses comparing groups of populations showed no significant differentiation on a north-south gradient in the GBR. The pattern in the number of significant differences in gene frequencies in pairwise population comparisons, however, suggested that gene flow may be more restricted among inner-shelf reef populations near to the coast than among mid/outer-shelf populations further from the coast.

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Introduction

Soft corals (Cnidaria: Octocorallia: Alcyonacea) are a highly diverse (> 300 spp.) and abundant taxon in Indo-Pacific coral reefs. On the Great Barrier Reef (GBR), they may cover up to 37% of the reef area (Sweatman et al. 1998). Despite their diversity and ubiquity in coral reefs, studies on their ecology are still sparse (e.g. Benayahu and Loya 1977; Dinesen 1983; Fabricius 1995, 1997; Karlson et al. 1996), and the understanding of their functional role in coral reef communities is still poor. For example, concerns that soft corals may outcompete hard corals following disturbances (e.g. Endean 1976; Benayahu and Loya 1987; Endean et al. 1988) have not been supported by long-term or large-scale studies in the GBR (Fabricius 1997; Sweatman et al. 1998).

Soft corals also show a wide variety of life history characteristics, including sexual and asexual reproductive modes or combinations of these (e.g. Dinesen 1985; Lasker 1990; Benayahu 1997). Yet the relative importance and specific roles of sexual and asexual reproduction as factors affecting their distribution and abundance are still unknown for most species of soft coral. This is the case for *Sinularia flexibilis*, a member

of one of the most prolific soft coral genera in the Indo-Pacific (110 species: Verseveldt 1980). It is also a common species in the GBR, where it varies in abundance across the continental shelf, from high abundances on inner-shelf reefs to low or absent on mid- and outer-shelf reefs (Fabricius 1998). *S. flexibilis* can also form monospecific stands or may occur as a dominant member of species-poor stands that can cover extensive areas (hundreds of square metres) on inner-shelf reefs (Fabricius 1998).

Although no studies have been published on the reproductive biology of *S. flexibilis*, other species of *Sinularia* have been shown to reproduce sexually by broadcast spawning of male and female azooxanthellate gametes (review in Benayahu 1997). Eggs and sperm are usually produced in different colonies, although some hermaphroditic species have been recorded (Benayahu 1997). On the GBR, species participate in the synchronised mass spawning event at the end of spring together with scleractinian corals and other invertebrates (Alino and Coll 1989).

Colonies of *Sinularia* spp. are also known to be capable of fission, but Fabricius (1995) reported this process was extremely slow and that it most often resulted from colony injury in populations on mid- and outer-shelf reefs of the GBR. In *S. flexibilis*, however, approximately one-third of colonies observed in the field were dividing by what appears to be an endogenously controlled mechanism (Bastidas, unpublished data). Therefore it is not clear to what extent asexual reproduction contributes to the formation of aggregations dominated by *S. flexibilis*.

The cross-shelf variation in abundance of *S. flexibilis* suggests that propagules do not disperse effectively to outer-shelf reefs, or that these reefs do not have optimal habitats for its growth and/or survival. The broadcast-spawning mode suggests the potential for effective dispersal throughout the GBR. However, the length of larval life for *S. flexibilis* is unknown, and recent genetic studies have demonstrated that even widespread marine species with relatively long larval lives may not meet their dispersal potential (reviewed in Palumbi 1994; Todd 1998; Benzie 1999).

Larval dispersal and the degree of asexual reproduction have major effects on the genetic structure of populations of marine invertebrates (e.g. Neigel 1997). High levels of dispersal among populations usually result in relatively little genetic differentiation among populations, whereas low levels can lead to population differentiation. The occurrence of asexual reproduction can lead to gene frequencies that are skewed from those expected under random mating. If differences in abundance of *S. flexibilis* in the GBR result mainly from differences in asexual rates of reproduction, abundant populations (inner-shelf reefs) would be expected to show less genetic diversity and more departures from Hardy–Weinberg (HW) equilibrium than less abundant reef populations (mid/outer-shelf reefs). The level of dispersal among populations should be detected by the degree and pattern of differences in gene frequencies among populations. To the best of our knowledge this would be the first study of this kind for a tropical soft coral species.

The genetic structure of *S. flexibilis* was investigated to obtain data on the nature of reproduction and dispersal of this ecologically important species, to help understand its patterns of distribution and abundance, and processes leading to local dominance. The aim of this article is to present findings on (1) the spatial pattern of genetic variation of reef populations, ranging from sites within reefs (<2 km apart) to regions (up to 1300 km apart); (2) the degree of connectivity between those populations along the GBR; and (3) the relative importance of sexual versus asexual reproduction.

Materials and methods

Sampling

Samples of *Sinularia flexibilis* (Quoy and Gaimard 1833) from 12 reef populations along the GBR, ranging from the Torres Strait to the Whitsundays Sector (maximum of 1,300 km apart) were sampled from December 1998 to February 2000 (Fig. 1). Two pairs of reefs were sampled in each of four geographic sectors from north to south along the GBR (Table 1). In two of these sectors, pairs of inshore and mid/offshore reefs were sampled to assess cross-shelf

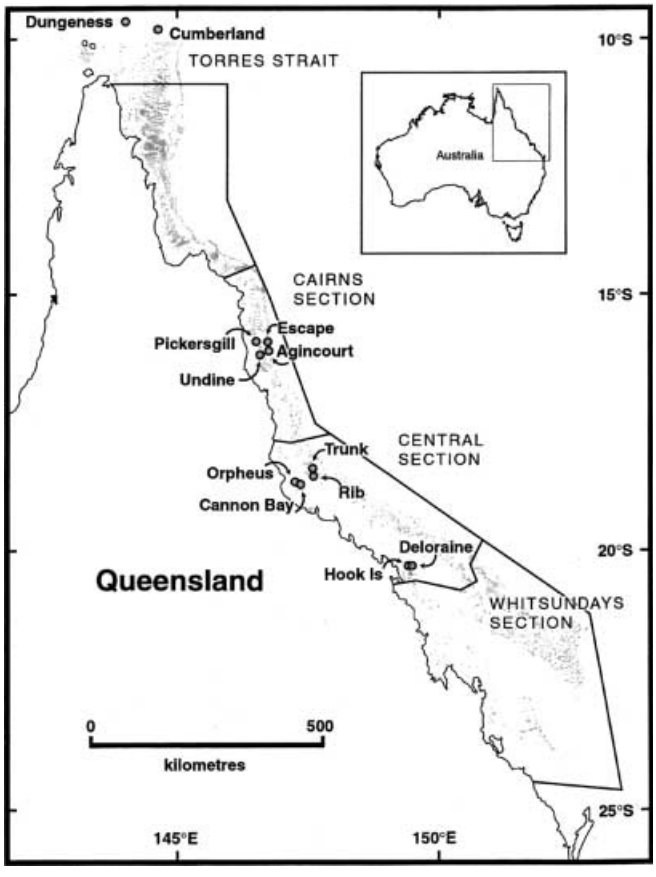

Fig. 1 Map showing the 12 localities of collection of *Sinularia flexibilis* along the Great Barrier Reef, Australia

Table 1 *Sinularia flexibilis*. Descriptive statistics for genetic variability in 12 reef populations along the Great Barrier Reef ordered from north to south. *LOC* Site code used throughout the text; *Date* date of sampling; *I* inner-shelf reefs; *MO* mid- or outer-shelf reefs; *N* mean sample size per locus; *n* mean number of alleles per locus (standard deviation in parentheses); *P* percentage of polymorphic loci when the frequency of the most common allele was less than 95%

LOC	Name	Date	Shelf	Sector	Latitude	Longitude	*N*	*n*	P	Mean Heterozygosity	
										Observed	Expected
CUM	Cumberland	Aug '99	MO	Torres Strait	10°04′S	143°43′E	56.9 (13.5)	2.56 (0.53)	100	0.344	0.336
DUN	Dungeness	Aug '99	MO	Torres Strait	09°53′S	142°55′E	28.3 (3.3)	2.56 (0.73)	100	0.289	0.306
PIC	Pickersgill	Dec '98	I	Cairns	15°52′S	145°35′E	56.4 (6.5)	2.44 (0.53)	100	0.416	0.380
UND	Undine	Dec '98	I	Cairns	16°06′S	145°38′E	44.1 (20.5)	2.44 (0.73)	89	0.284	0.268
AGI	Agincourt	Dec '98	MO	Cairns	15°59′S	145°49′E	46.1 (10.9)	2.56 (0.53)	100	0.265	0.319
ESC	Escape	Dec '98	MO	Cairns	15°49′S	145°48′E	38.1 (3.7)	2.22 (0.67)	89	0.233	0.254
CAN	Cannon Bay	Dec '99	I	Central	18°40′S	146°35′E	45.7 (6.9)	2.67 (0.71)	100	0.355	0.396
ORP	Orpheus	Dec '99	I	Central	18°34′S	146°29′E	46.7 (10.9)	2.67 (0.71)	100	0.318	0.321
RIB	Rib	Mar '99	MO	Central	18°29′ S	146°52′ E	43.6 (9.8)	2.56 (0.53)	89	0.284	0.300
TRU	Trunk	Mar '99	MO	Central	18°21′S	146°46′E	52.8 (6.5)	2.67 (0.71)	89	0.304	0.315
DEL	Deloraine	Feb '00	I	Whitsundays	20°09′S	149°04′E	49.4 (0.7)	2.56 (0.53)	100	0.288	0.311
HOO	Hook Is	Feb '00	I	Whitsundays	20°07′S	148°53′E	49.6 (6.7)	2.67 (0.71)	100	0.309	0.359

variability. Up to 60 individuals or colonies were sampled from two or three replicate sites at each reef. At each site, samples were taken at least 5 m apart along the swimming trajectory to avoid repeated collections. Tissue samples were cut from the top branches of the colony using scissors, transported in plastic bags numbered in the sequence of collection, and frozen in liquid nitrogen within a few hours after collection. Tissue homogenates were prepared by grinding approximately 0.3 g of tissue wet weight with 0.2 ml of 0.05 M Tris-HCl buffer pH 8. Spicules, mucus, and zooxanthellae were removed by centrifugation.

Electrophoresis

An initial screening for activity of 30 enzymes using different electrophoretic conditions showed that 9 enzymes could be used for routine screening based on their resolution, polymorphism, and reliability of scoring: GPI (glucose-6-phosphate isomerase E.C. 5.3.1.9), HK (hexokinase E.C. 2.7.1.1), FBP (fructose biphosphatase E.C. 3.1.3.11), TPI (triose-phosphate isomerase E.C. 5.3.1.1), ME (malate dehydrogenase, oxaloacetate-decarboxilating E.C. 1.1.1.40), MDH (malate dehydrogenase E.C. 1.1.1.37), VL (peptidase valyl-leucine substrate E.C. 3.4.11/13), LGG (peptidase leucylglycylglycine substrate E.C. 3.4.11/13), and FLE (fluorescent esterase with methylumbelliferyl acetate as substrate E.C. 3.1.1.1). Allozyme electrophoresis was carried out using cellulose acetate gels (Cellogel) for FLE in Tris-maleate buffer pH 7.8 (Richardson et al. 1986) and 12% horizontal starch gels for the remaining enzymes. GPI, HK, FBP, and TPI were scored from HC 6.5 buffer (histidine-citric acid) and ME, MDH and peptidases from TG 8.4 buffer (Tris-glycine), following the general procedures of Ballment et al. (1997).

Data analyses

Departures of genotypic frequencies from those expected under conditions of HW equilibrium were evaluated for each locus with the conventional Monte Carlo method using TFPGA software (Miller 1997) and a level of significance of $\alpha = 0.05$ corrected for multiple comparisons (Weir 1980). Independence of loci was evaluated by an estimation of the exact probability of linkage disequilibrium for all possible pairs of loci in each population (Lewis and Zaykin 2000).

Genetic diversity and sexual versus asexual contribution to populations were further evaluated for multi-locus genotypes using (1) observed and expected genotypic diversity ratio (G_o:G_e) calculated as in Stoddart and Taylor (1988); (2) the ratio of the maximum number of individuals to be generated by sexual reproduction summed for all genotypes found (N*) to the number of individuals (N) (N* was calculated using the allelic frequency for each reef and for the pooled allelic frequency over the whole data set); and (3) number of genotypes observed (N_{go}) over N, following reported procedures (e.g. Johnson and Threlfall 1987; Uthicke et al. 1998). Spatial autocorrelation of individual genotypes within reefs was investigated to detect spatial patterns of repeated genotypes within populations (e.g. Miller 1998; Ruckelhaus 1998). Each genotype was given a number and the relative position of the colony was assigned using its collection sequence within a site. Moran's I and Geary's c were calculated allocating an equal number of point pairs (pairwise distance between colonies) to five distance classes (SAAP software: Wartenberg 1989). Because both indexes resulted in similar estimates, only Moran's I is shown. Analyses using multi-locus genotypes were based on seven loci (*GPI** and *LGG**, for which data were missing for many individuals, were excluded, e.g. Table 2).

The degree of genetic differentiation or population subdivision at different spatial scales was evaluated by Wright's F-statistics (F_{ST}: standardized genetic variance) calculated as described in Weir and Cockerham (1984) using TFPGA software (Miller 1997). Significant departures from H_0: $F_{ST} = 0$ were evaluated by χ^2 test for each single locus and for all loci using 95% confidence intervals generated by bootstrapping over each locus; this was evaluated at four spatial scales: sector, cross-shelf position (inner or middle/outer), reefs, and sites within reefs. Genetic differentiation among

reefs was also evaluated from differences in allelic frequencies for each pair of reef populations based on a Fisher's combined probability test, using a Monte Carlo simulation to obtain an approximation for the exact probability of differences (Miller 1997). Also, pairwise F_{ST} between reef populations were plotted against their geographic distance. The average number of migrants per generation between populations (N_em) was calculated as $N_em = [(1/F_{ST}) - 1]/4$.

Results

Electrophoretic analyses of nine polymorphic allozyme loci showed consistent levels of genetic diversity in each population of *S. flexibilis*, with the average number of alleles per locus ranging from 2.2 to 2.7, and observed heterozygosity ranging from 0.23 to 0.42 (Table 1). The percentage of polymorphic loci was high, as expected from a priori selection of enzymes that maximised the information to evaluate genetic differentiation (Table 1). The number of alleles per locus was relatively low in all populations, ranging from two to a maximum of four (Table 2).

In most cases genotypic frequencies for each population and locus did not differ significantly from those expected from HW predictions (Table 3). Genotypic frequencies for loci *MDH**, *FBP**, *LGG**, and *TPI**

Table 2 *Sinularia flexibilis*. Allelic frequencies and number of samples for nine loci and 12 localities along the Great Barrier Reef. Alleles at each locus were labelled based on the mobility of the most common allele (= 100). *N* is the number of individuals assayed for each locus

Locus	Allele	Torres Strait		Cairns				Central				Whitsundays	
		CUM	DUN	PIC	UND	AGI	ESC	CAN	ORP	RIB	TRU	DEL	HOO
*GPI**													
N		21	23	45	17	18	3	30	22	18	41	48	32
	109	0.048	0.152	0.056	0.441	0.139	–	0.167	0.046	0.056	0.049	0.021	0.172
	100	0.810	0.804	0.944	0.529	0.806	1.000	0.683	0.864	0.861	0.927	0.760	0.734
	91	0.143	0.044	–	0.029	0.056	–	0.150	0.091	0.083	0.024	0.219	0.094
*HK**													
N		62	31	64	56	49	45	48	54	44	58	50	53
	107	0.040	0.097	0.141	0.027	0.020	0.011	0.000	0.009	0.034	0.078	0.010	0.066
	100	0.710	0.790	0.578	0.679	0.888	0.867	0.688	0.685	0.750	0.707	0.930	0.745
	95	0.250	0.113	0.281	0.295	0.092	0.122	0.292	0.296	0.216	0.207	0.060	0.170
	90	–	–	–	–	–	–	0.021	0.009	–	0.009	–	0.019
*FBP**													
N		62	31	63	58	50	42	53	55	47	57	50	53
	105	0.202	0.258	0.230	0.078	0.170	0.179	0.151	0.200	0.181	0.254	0.090	0.264
	100	0.597	0.645	0.476	0.750	0.730	0.667	0.679	0.664	0.670	0.561	0.830	0.547
	92	0.202	0.097	0.294	0.172	0.100	0.155	0.170	0.136	0.149	0.184	0.080	0.189
*TPI**													
N		61	29	58	49	43	34	50	44	48	52	49	50
	110	0.041	0.052	0.129	0.041	0.186	0.029	0.350	0.091	0.052	0.058	0.235	0.190
	100	0.861	0.948	0.802	0.939	0.767	0.941	0.550	0.818	0.906	0.846	0.735	0.730
	95	0.098	–	0.069	0.020	0.047	0.029	0.100	0.091	0.042	0.096	0.031	0.080
*ME**													
N		62	28	55	53	52	42	48	54	50	57	50	53
	100	0.879	0.911	0.755	0.868	0.837	0.857	0.865	0.870	0.870	0.825	0.920	0.925
	95	0.121	0.089	0.246	0.132	0.164	0.143	0.135	0.130	0.130	0.175	0.080	0.076
*MDH**													
N		61	28	57	55	51	43	48	52	47	57	49	51
	105	0.074	0.071	0.132	0.100	0.177	0.128	0.177	0.087	0.075	0.193	0.112	0.118
	100	0.926	0.929	0.868	0.900	0.824	0.872	0.823	0.914	0.926	0.807	0.888	0.882
*VL**													
N		61	23	54	51	52	45	43	47	44	57	50	51
	100	0.902	0.913	0.870	0.941	0.914	0.900	0.826	0.819	0.966	0.974	0.770	0.853
	92	0.098	0.087	0.130	0.059	0.087	0.100	0.174	0.181	0.034	0.026	0.230	0.147
*LGG**													
N		61	31	49	1	50	44	41	38	47	43	49	51
	100	0.713	0.597	0.694	1.000	0.620	0.580	0.720	0.763	0.575	0.616	0.663	0.755
	90	0.287	0.403	0.306	–	0.380	0.421	0.281	0.237	0.426	0.384	0.337	0.245
*FLE**													
N		61	31	63	57	50	45	50	54	47	53	50	52
	112	0.271	0.403	0.175	0.123	0.190	0.189	0.060	0.185	0.245	0.104	0.300	0.202
	100	0.623	0.548	0.635	0.754	0.790	0.811	0.780	0.787	0.681	0.849	0.680	0.740
	90	0.107	0.032	0.191	0.123	0.020	0.000	0.130	0.028	0.075	0.047	0.020	0.058
	80	–	0.016	–	–	–	–	0.030	–	–	–	–	–

were not in HW equilibrium in 10, 5, 4, and 1 population, respectively (Table 3). For these loci there was a deficit of heterozygotes, with the exception of *LGG**. On a single-locus basis, all these loci showed a significant inbreeding coefficient consistent with the results that indicated deviation from HW expectations (F_{IS}, Table 4). When *MDH**, *FBP**, and *LGG** were excluded from *F*-statistic analyses (Table 4), the inbreeding coefficient (F_{IS}) over all loci was still significant, but barely.

Analysis of independence of loci showed that from 432 pairwise comparisons, 76 were significantly non-independent and 55 of these involved an association between *MDH** and another locus (data not shown). Further data exploration on the type of association between loci (using principal coordinate analysis) did not indicate that those associations were likely to have resulted from sampling different species. As exclusion of *MDH** from *F*-statistics did not result in a change in the main results, *MDH** was included in further analysis.

Analysis of multi-locus genotypes also indicated a high diversity with a total of 241 unique genotypes in 459 individuals, and an observed to expected genotypic diversity ratio G_o:G_e ranging between 0.72 and 1.37 (Table 5). The maximum expected number of individuals produced sexually belonging to the observed genotypes (*N**) was always equal to, or slightly smaller than, the number of individuals sampled (Table 5). Thus, all individuals may have been produced by sexual reproduction, whether calculations were performed using allelic frequencies for each reef population or for the population of all reefs pooled (Table 5). For each population, the number of individuals per multi-locus genotype indicated few prolific repeated genotypes, that is, most multi-locus genotypes appeared once or twice, with few exceptions (Table 5). No population had a particularly high number of repeated genotypes. The relative spatial distribution of genotypes within reefs, however, indicated that in 4 out of 12 populations some repeated genotypes were closer than expected from a random distribution (Table 6). In three

Table 3 *Sinularia flexibilis*. D-values [(Ho–He)/He] indicating heterozygote deficit (negative number) or excess (positive number) for each locus and population. *LOC* Site code. Bold numbers indicate genotypic frequencies deviating significantly from Hardy–Weinberg expectations after correction for multiple tests. Empty cells indicate no data available

LOC	*GPI**	*HK**	*FBP**	*TPI**	*ME**	*MDH**	*VL**	*LGG**	*FLE**	All loci
CUM	−0.113	0.343	**−0.025**	−0.140	−0.014	**−0.640**	0.109	0.162	0.026	0.024
DUN	−0.337	−0.178	−0.111	−0.648	0.098	−1.000	0.095	**0.676**	−0.157	−0.057
PIC	0.059	0.213	**0.402**	**−0.384**	0.129	**−0.770**	−0.179	0.345	0.168	0.094
UND	0.234	0.225	−0.013	0.050	−0.013	**−0.697**	0.062		0.095	0.058
AGI	0.183	0.106	−0.393	−0.441	0.055	**−1.000**	0.095	0.273	−0.293	−0.169
ESC		−0.240	**−0.285**	−0.477	−0.028	−0.687	−0.629	**0.726**	−0.057	−0.083
CAN	−0.240	−0.104	**−0.070**	−0.009	−0.199	**−0.786**	0.050	0.148	0.080	−0.105
ORP	0.119	−0.121	−0.056	0.086	−0.016	**−0.879**	0.077	0.310	0.019	−0.009
RIB	−0.106	−0.008	−0.228	−0.402	−0.027	**−0.845**	0.035	**0.654**	−0.277	−0.053
TRU	0.060	−0.236	**−0.162**	0.063	−0.030	**−0.662**	0.027	0.524	0.134	−0.037
DEL	−0.163	−0.543	−0.123	−0.344	0.087	**−0.898**	0.299	**0.508**	−0.150	−0.074
HOO	−0.186	−0.311	−0.303	−0.152	0.081	**−0.811**	0.172	0.325	−0.010	−0.139

Table 4 *Sinularia flexibilis*. Hierarchical *F*-statistics for all localities with significance by chi-square per locus and over all loci by 95% CI. Separate results are shown for eight reefs in Cairns and Central sectors for which *MDH**, *FBP** and *LGG** were excluded. F_{ST} Shelf: degree of differentiation between shelf positions; F_{ST} Reefs: degree of differentiation between reefs; F_{ST} Sites: degree of differentiation between sites of a reef; F_{IS} inbreeding coefficient; F_{IT} overall inbreeding coefficient; *N* number of individuals; *n* number of alleles per locus

	N	*n*	F_{IT}	F_{ST}			*df*	F_{IS}	*df*
				Shelf	Reefs	Sites			
GPI*	299	3	0.1679	−0.0006	0.0574**	0.0720**	22	0.1033	3
HK*	590	4	0.0604	0.0024	0.0324**	0.0440**	33	0.0172	6
FBP*	585	3	0.1248	−0.0040	−0.0019	0.0395**	22	0.0888*	3
TPI*	548	3	0.2496	0.0290**	0.0738**	0.0752**	22	0.1886**	3
ME*	592	2	0.0088	−0.0041	0.0094	0.0112	11	−0.0024	1
MDH*	597	2	0.8169	−0.0034	−0.0064	0.0027	11	0.8164**	1
VL*	559	2	0.0114	0.0305*	0.0356**	0.0495**	11	−0.0401	1
LGG*	454	2	−0.3765	0.0178	0.0123	0.0329*	11	−0.4234**	1
FLE*	578	4	0.0560	−0.0013	0.0288**	0.0270**	33	0.0297	6
All loci			0.0836	0.0065	0.0261*	0.0406*		0.0448	
All localities									
95% CI	Upper		0.2733	0.0153	0.0446	0.0547		0.2454	
	Lower		−0.0862	−0.0012	0.0107	0.0273		−0.1273	
Six loci			0.0871	0.0103	0.0434*	0.0546*		0.0343*	
Cairns and Central sectors									
95% CI	Upper		0.1535	0.0268	0.0622	0.0855		0.0900	
	Lower		0.0391	−0.0050	0.0239	0.0252		0.0005	

*$P < 0.05$; **$P < 0.001$

Table 5 *Sinularia flexibilis*. Analysis of multi-locus genotype (using seven loci). *LOC* Site code; *N* number of individuals; *N** estimated maximum number of individuals produced sexually [calculated from the allelic frequency of each reef ('Per reef') and of the pooled data set ('All reefs')]; N_{go} number of unique multi-locus genotypes, G_o observed genotypic diversity; G_e expected genotypic frequency

LOC	*N*	All reefs *N**/*N*	Per reef *N**/*N*	N_{go}	Frequency of repeated genotypes							N_{go}:*N*	G_o	G_o:G_e
					1	2	3	4	5	6	7			
CUM	52	1.00	1.00	40	31	7	1	1				0.77	32.2	0.87
DUN	20	1.00	1.00	19	18	1						0.95	18.2	1.18
PIC	37	1.00	1.00	35	33	2						0.95	33.4	1.37
UND	41	0.99	1.00	30	26	1	1	1		1		0.73	18.5	0.76
AGI	39	1.00	1.00	31	27	2	1		1			0.79	22.0	0.79
ESC	27	1.00	1.00	19	14	4			1			0.70	13.3	0.78
CAN	33	1.00	1.00	31	29	2						0.94	29.4	1.00
ORP	41	1.00	1.00	38	35	3						0.93	35.8	1.12
RIB	34	1.00	1.00	25	19	4	1	1				0.74	19.3	0.78
TRU	45	1.00	1.00	38	32	5	1					0.84	33.2	1.01
DEL	48	0.99	1.00	33	26	4	1	1			1	0.69	19.9	0.72
HOO	42	1.00	1.00	36	32	3		1				0.86	29.4	0.84
Total	459													

of these four reefs (i.e. Undine, Agincourt, and Cannon Bay) the overall significance of the correlogram was due to the significance of the autocorrelation value between all pairwise distances within the first distance class (i.e. the relatively closer colonies).

Hierarchical F_{ST} analysis (nesting sites within reef and reefs within cross-shelf position) demonstrated no significant differentiation between inner- and mid/outer-shelf reefs ($F_{ST} = 0.0065$, Table 4). No genetic differentiation attributable to shelf position was detected, even excluding loci showing HW disequilibria and a high inbreeding coefficient consistently over most populations (*MDH**, *FBP**, and *LGG**) and/or including only the Cairns and Central sectors that had pairs of reefs in each shelf position (e.g. Table 4).

However, F_{ST} was significantly different from zero among reefs and among sites within reefs (F_{ST} reefs = 0.0261 and F_{ST} sites = 0.0406, Table 4) indicating restriction of gene flow at these scales. Genetic differences between sites within a reef were significant only at Hook Island and Undine Reef populations (F_{ST} sites = 0.0288 and 0.0378, respectively).

In a separate hierarchical analysis – nesting reefs within cross-shelf position and those within sectors (that correspond to a latitudinal gradient) – no genetic differentiation over all loci was found among four sectors ($F_{ST} = -0.0042$, 95% CI −0.0102 to 0.0022, data not shown).

The degree of genetic differentiation between each pair of reefs showed no relationship to the geographic separation of the reefs (Fig. 2). However, pairwise comparisons of allelic frequencies between reefs over all loci indicated more significant differences among inner reefs (14 out of 15 pairs) than among mid/outer-shelf reefs (3 out of 15 pairs) (Table 7). This pattern was also observed for pairwise F_{ST} values between reefs (data not shown). These results indicated more heterogeneity in allelic frequencies among inner-shelf reefs than among mid/outer-shelf reefs (F_{ST} inner reefs = 0.039 95% CI 0.019 to 0.060; F_{ST} mid/outer reefs = 0.015 95%CI 0.005 to 0.026). Also, 27 out of 36 comparisons between inner- and mid/outer-shelf reefs resulted in significant differences in allelic frequency (Table 7).

Table 6 *Sinularia flexibilis*. Autocorrelation analysis of genotypes in each reef population using Moran's *I* index. Values are level of significance for the overall correlogram and for each distance class. *LOC* Site code. Bold indicates significance at $P < 0.05$

LOC	Overall	Distance classes				
		1	2	3	4	5
CUM	0.18	0.32	0.39	**0.04**	0.26	0.04
DUN	0.17	0.19	0.13	0.06	0.03	0.05
PIC	0.41	0.34	0.14	0.08	0.30	0.32
UND	**0.00**	**0.00**	0.36	0.46	0.10	0.01
AGI	**0.00**	**0.00**	0.10	0.12	0.47	0.11
ESC	**0.04**	0.37	**0.02**	**0.01**	0.31	0.37
CAN	**0.00**	**0.00**	0.13	**0.00**	**0.01**	0.19
ORP	1.00	0.47	0.37	0.49	0.31	0.46
RIB	0.52	0.11	0.10	0.35	0.44	0.25
TRU	0.16	0.09	0.24	0.32	0.08	**0.03**
DEL	0.84	0.20	0.33	0.17	0.42	0.44
HOO	1.00	0.32	0.33	0.38	0.41	0.44

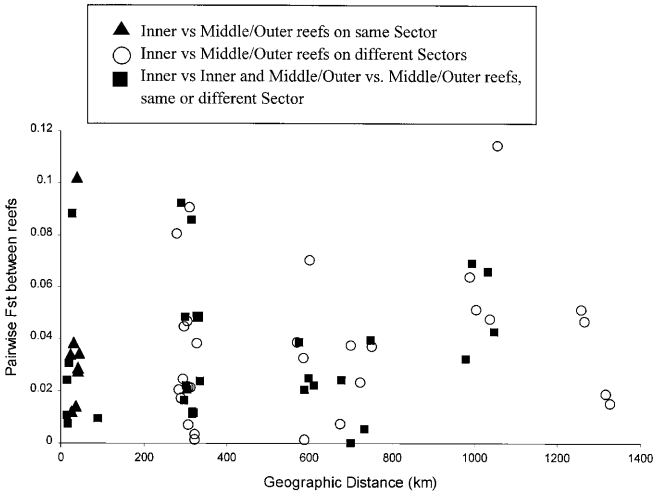

Fig. 2 *Sinularia flexibilis*. Pairwise F_{ST} between reef populations plotted against their geographic distance in kilometres

Table 7 *Sinularia flexibilis*. Matrix of combined probabilities for each pairwise comparison of allelic frequencies between reefs (below diagonal) and geographic distances in kilometres (above diagonal). Bold indicates significance using $P < 0.0008$ after correction for multiple tests

	Inner reefs						Mid/outer reefs					
	PIC	UND	CAN	ORP	DEL	HOO	CUM	DUN	AGI	ESC	RIB	TRU
PIC	–	28	330	316	611	600	676	725	30	26	323	308
UND	**0.0000**	–	304	289	589	575	702	752	24	36	296	280
CAN	**0.0000**	**0.0000**	–	16	320	303	1005	1056	310	328	38	40
ORP	**0.0000**	**0.0000**	**0.0003**	–	336	318	990	1040	295	312	44	40
DEL	**0.0000**	**0.0000**	**0.0000**	**0.0000**	–	20	1266	1328	585	602	307	323
HOO	**0.0000**	**0.0000**	**0.0005**	0.1054	**0.0000**	–	1260	1319	570	589	290	305
CUM	**0.0000**	**0.0001**	**0.0000**	0.2120	**0.0000**	0.0243	–	90	700	677	996	980
DUN	**0.0000**	**0.0000**	**0.0000**	**0.0002**	**0.0000**	0.0032	0.0224	–	750	734	1049	1034
AGI	**0.0000**	**0.0000**	**0.0000**	0.0015	0.0035	0.0144	**0.0000**	0.0109	–	19	298	283
ESC	**0.0000**	**0.0007**	**0.0000**	0.0170	**0.0003**	**0.0001**	0.0028	0.0310	0.0533	–	317	300
RIB	**0.0000**	0.0077	**0.0000**	0.0229	**0.0000**	**0.0004**	0.5527	0.3150	0.0195	0.2115	–	16
TRU	**0.0000**	**0.0000**	**0.0000**	**0.0003**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	0.0015	0.0008	0.0713	–

In consequence, estimations of migrants per generation (N_em) were similar among inshore reefs and between inner- and mid-shelf reefs (average over all estimated pairs: 10 ± 11 and 11 ± 13 SD, respectively), whereas N_em was higher among mid-shelf reefs (43 ± 61). N_em between all pairs of populations ranged from two to infinite, with an average N_em of 17 ± 32 excluding the infinite value.

Discussion and conclusions

Genotypic diversity of 12 populations of *Sinularia flexibilis* in the Great Barrier Reef indicated a predominance of sexual reproduction in all populations. Only one out of nine loci showed a significant deficit of heterozygotes in most populations, whereas 9 out of 12 populations showed a non-significant deficit of heterozygotes over all loci. Based on multi-locus genotypes, all values for the indicators of mode of reproduction in this study (N^*:N, N_{go}:N, G_o:G_e) were higher than those reported for highly clonal organisms (e.g. Stoddart 1984; Ayre and Willis 1988; Coffroth and Lasker 1998; Uthicke et al. 1998, 1999), also indicating a predominance of sexual reproduction. For example, the number of unique genotypes relative to the number of individuals sampled for all reefs (ratio N_{go}:N of 0.53) and for each reef (range of 0.69–0.95) was relatively high. Also, the maximum estimated number of individuals likely to have been produced sexually, based on the genotypic frequencies, was always equal to or slightly less than the number of individuals sampled (N^*:$N \sim 1$), indicating that even repeated genotypes were likely to have been produced by chance from sexual reproduction.

Although this species does reproduce asexually (from colonies observed going through fission in the field), some ecological process, such as low ramet production or high ramet mortality due to disturbances and/or other factors, appears to impede genets from extending considerably over spatial scales greater than 5 m. Spatial autocorrelation analyses indicated that in three reefs repeated genotypes were relatively closer together than expected in a random spatial distribution of the samples. Thus, although the frequency of a repeated genotype was seldom larger than two individuals, it may be possible that some of them were produced asexually. These results also suggested that the effects of asexual reproduction, if any, are likely to be limited to scales less than 5 m (the minimum sampling distance in this study). Because asexual reproduction may produce excesses as well as deficits of heterozygotes, it might be a likely explanation for the few genotypic frequencies deviating significantly from expected under random mating. As asexual reproduction does not make an important contribution in the populations studied, however, some degree of inbreeding may be more important, as discussed below.

There was, therefore, no evidence to suggest that *S. flexibilis* achieves high abundances in inshore reefs (dominance over spatial scales equal to or greater than 20 m^2) by asexual reproduction. On the other hand, it has been suggested that organisms may display a greater degree of asexuality in ecologically marginal habitats (e.g. Peck et al. 1998). There was also no evidence that *S. flexibilis* had more asexually produced individuals in reefs where the species was in low abundance and where populations might be considered marginal within the GBR. There was no evidence that low-abundance populations were genetically different from others on the GBR or that latitude had any effect on the genetic structure of *S. flexibilis* populations on the GBR.

Gene flow seems sufficiently restricted to maintain a significant genetic differentiation between some reef populations (minimum of 16 km apart) and between some sites within reefs (0.8–2 km apart). However, gene flow seems large enough in other instances that allelic frequencies did not differ significantly between some adjacent populations and some populations separated by several hundreds of kilometres.

The level of genetic differentiation of the *Sinularia flexibilis* reef populations ($F_{ST} = 0.026$) was far less than

that of (1) low larval dispersal species such as the viviparous coral *Seriatopora hystrix* ($F_{ST} = 0.43$, Ayre and Duffy 1994); (2) highly asexual species such as *Pocillopora damicornis* ($F_{ST} = 0.21$ Stoddart 1984) and *Actinia tenebrosa* ($F_{ST} = 0.38$, Ayre et al. 1991); and (3) a soft coral such as *Alcyonium rudyi* ($F_{ST} = 0.23 - 0.46$ McFadden 1997) that showed both low larval dispersal and high rate of asexual reproduction.

The level of genetic differentiation of *S. flexibilis* populations on different reefs was similar to those reported for other invertebrate species with a relatively long planktonic larvae phase in the GBR (e.g. Burnett et al. 1994, 1995; Ayre and Hughes 2000). In some of these studies, however, no significant genetic differentiation was found between widely separated populations (e.g. Benzie and Stoddart 1992; Benzie and Williams 1992; Macaranas et al. 1992; Williams and Benzie 1993; Ayre et al. 1997). As these species are subject to the same hydrographic conditions within the GBR, differences in larval behaviour, or larval residency times in the water column, or both may explain their differences in the degree of genetic differentiation. However, details of the larval biology in the field of many of these species remain unknown.

It seems unlikely that natural selection has been responsible for the genetic differentiation found in *S. flexibilis* within and between adjacent reef populations, because results were relatively consistent for all loci, and because this species tends to occur at similar habitat types, typically back reef areas (but see Ayre 1985; Benzie et al. 1995). The lack of relationship between genetic differentiation and geographic distance suggests that *S. flexibilis* populations are not in genetic equilibrium, which is not uncommon (e.g. Ayre et al. 1997). However, the relatively low levels of genetic differentiation found indicate that the populations studied could be considered as highly interconnected along the GBR. The observed pattern of genetic differentiation might mainly have resulted from the effects of genetic drift due to some potential restriction in dispersal at two spatial scales. Firstly, the genetic differentiation detected among some adjacent reefs and sites within a reef may have resulted from greater retention of larvae or gametes at these reefs. Restriction of dispersal at these scales has been shown to occur in species with high dispersal potential, either as a result of local hydrodynamic conditions restricting dispersal (e.g. Sammarco and Andrews 1988), or through behaviour of planktonic larvae (e.g. Knowlton and Keller 1986), or both. Given that *S. flexibilis* tends to occur mainly in the lee of reefs it may increase the proportion of larvae retained in each reef (e.g. Black et al. 1990), but the lack of information on its larval biology prevents any further discussion. Self-seeding has been used to explain genetic differentiation of other species with high dispersal potential (e.g. Burton and Feldman 1982; Hedgecock 1986; Ayre et al. 1997). Because it also leads to a certain degree of inbreeding, it might explain the few deficits of heterozygotes found in this study. Secondly, despite the lack of relationship between genetic differentiation and geographic distance, pairwise comparisons of F_{ST} or allelic frequencies between reefs suggest that there may be more restriction in gene flow among inner reefs, and between inner- and mid/outer-reefs, than among mid/outer-reefs. Although oceanographic and population structure data has indicated that the GBR may be regarded as a highly connected reef system (e.g. Benzie 1994), these differences may be explained by more detailed current patterns. Some limitation has been predicted for the cross-shelf dispersal in the GBR, and the net surface current drift in summer, when this species reproduces, would be more restricted in near-shore waters than in outer-shelf waters (e.g. Dight et al. 1990).

In conclusion, *S. flexibilis* showed genetic diversity consistent with predominance of sexual reproduction at the scales sampled. Although this species has a high dispersal potential, it showed significant genetic differentiation between and within some reefs, which suggests some retention of sexual propagules at those scales. Although genetic differentiation was not related to geographic separation, there was some evidence that gene flow may be less restricted among mid/outer-shelf reefs in the GBR than among inner-shelf reefs.

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