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NOTE

Fine-scale analysis of genetic structure in the brooding coral *Seriatopora hystrix* from the Red Sea

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Abstract The dispersal of gametes and larvae plays a key role in the population dynamics of sessile marine invertebrates. Species with internal fertilisation are often associated with very localised larval dispersal, which may cause small-scale patterns of neutral genetic variation. This study on the brooding coral *Seriatopora hystrix* from the Red Sea focused on the smallest possible scale: Two *S. hystrix* stands (~100 colonies each) near Dahab were completely sampled, mapped and analysed at five microsatellite markers. The sexual mode of reproduction, the likely occurrence of selfing and the level of immigration were in agreement with previous studies on this species. Contrary to previous findings, both stands were in Hardy–Weinberg proportions. Also, no evidence for spatially restricted larval dispersal within the sampled areas was found. Differences between this and previous studies on *S. hystrix* could reflect variation in life history or varying environmental conditions, which opens intriguing questions for future research.

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Introduction

In sessile marine invertebrates, scales of larval dispersal play a fundamental role in determining population dynamics. Small-scale dispersal increases the chance of inbreeding and promotes genetic divergence among populations due to selection and/or genetic drift. Species with internal fertilisation have often been associated with the occurrence of localised larval dispersal and pronounced neutral genetic variation on surprisingly small spatial scales. Few population genetic studies, however, focused on scales of decimetres to metres (e.g., Calderón et al. 2007).

Seriatopora hystrix is a hermaphroditic, brooding coral distributed from the Red Sea to the Western Pacific (Veron 2000). It reproduces largely sexually but can pursue a mixed mating strategy of outcrossing and selfing (Sherman 2008). Large heterozygote deficits within local stands of colonies (Ayre and Dufty 1994; Ayre and Hughes 2000; Underwood et al. 2007; Sherman 2008) and isolation by distance effects on limited spatial scales of ≤20 km (Maier et al. 2005) or even below tens of metres (Underwood et al. 2007) imply very localised larval dispersal. Nevertheless, at least some of the well-provisioned larvae are expected to travel far, and assignment tests have successfully identified immigrants (Underwood et al. 2007). Taken together, these findings suggest that the population genetic structure of *S. hystrix* is shaped by processes that act on a broad range of spatial scales. Identifying these processes should shed light on a coral life history and its variability across a vast distribution range. Previous studies on *S. hystrix* focused on scales from tens of metres to hundreds of kilometres.

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The present study was intended as a first step to understand fine-scale patterns of genetic structure on scales from decimetres to metres.

Two *S. hystrix* stands from the Red Sea (~100 colonies each) were analysed using five microsatellites in order to (1) determine whether these sites are internally structured, and (2) examine the role of four processes that could cause such structure, i.e., immigration, clonal reproduction, selfing and small-scale larval dispersal.

Materials and methods

Sample collection

Seriatopora hystrix was sampled in 2002 from two locations in the Dahab region, Egypt, separated by ~8.0 km (Fig. 1). House Reef 2 (*HR2*, 28°29′24.4″ N, 34°30′58.1″ E) and Golden Blocks (*GB*, 28°26′18.9″ N, 34°27′46.6″ E) were situated on the reef slope at 21 and 13 m depth, respectively. The sampled areas were ~600 m^2 (*HR2*) and ~200 m^2 (*GB*) in size. From all colonies, branch tips of approximately 1 cm length were collected (*HR2*: $n = 107$; *GB*: $n = 110$) and the position of each colony was mapped via *x*- and *y*-coordinates. DNA was preserved as described in Maier et al. (2005).

Genotyping

DNA was extracted following Maier et al. (2001). Genotypes were scored at five species-specific microsatellite loci: Sh2-002, Sh3-004 and Sh4-001 (Underwood et al. 2006, 2007) as well as Sh2.15 and Sh4.24 (Maier et al. 2005). Allelic variation was analysed on a MegaBACE 1000, scored with MegaBACE Genetic Profiler v2.2 (GE Healthcare) and checked manually.

Statistical analysis

Allele frequencies, observed (H_o) and expected (H_e) heterozygosities as well as F_{IS} (*f*, Weir and Cockerham 1984) were calculated in *FSTAT* version 2.9.3 (Goudet 2001) and GENEPOP web version 3.4 (Raymond and Rousset 1995). Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibria were performed in *FSTAT*, based on 1,000 and 600 permutations, respectively. *FSTAT* was also used to calculate F_{ST} (θ, Weir and Cockerham 1984) and R_{ST} (ρ, Goodman 1997). Significance of F_{ST} was estimated using Fisher's exact test in GENEPOP (1,000 dememorizations, 100 batches, 1,000 iterations per batch). The software FREENA (Chapuis and Estoup 2007) was used to check the robustness of results in the light of potential null alleles.

In order to identify immigrants, the population exclusion method implemented in GeneClass2 (Piry et al. 2004) was applied. For each colony, the likelihood that it originated within its sampling site was calculated using the partial Bayesian criterion of Rannala and Mountain (1997) and compared to the distribution of likelihoods of 10^4 simulated genotypes, generated using a Monte Carlo algorithm (Paetkau et al. 2004). In order to compensate for the limited power of this data set to identify migrants (unknown source populations, small number of loci), the low stringency criterion of $\alpha = 0.05$ (95% exclusion probability) was applied. From the resulting estimate of *Nm*, the expected Type I error ($N^*\alpha$) was subtracted to obtain a reliable minimum, as suggested by Paetkau et al. (2004).

As a descriptive statistic, the ratio of the observed number of distinct genotypes (N_g) over the sample size (N) was computed. In order to test for asexual reproduction, the probability of finding the observed number of identical multilocus genotypes (MLG) under the null hypothesis of purely sexual, biparental reproduction was assessed using a simulation approach described in Calderón et al. (2007),

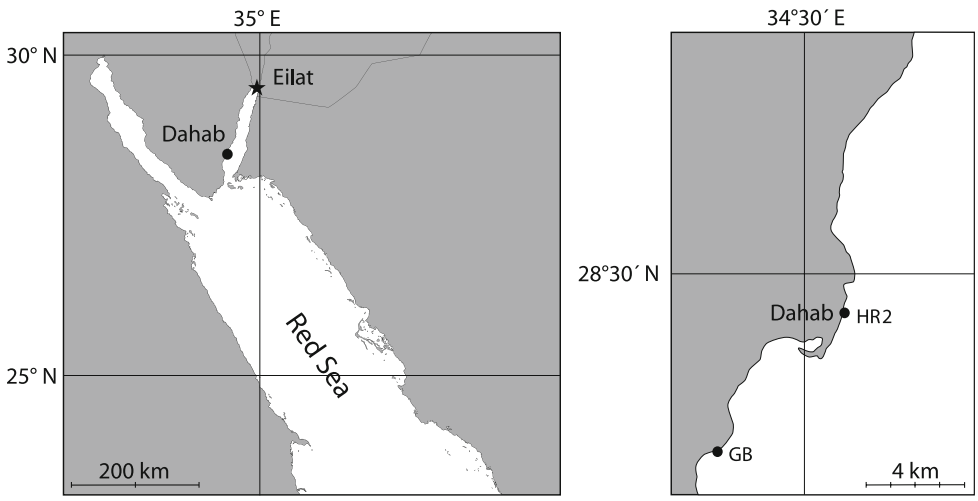

Fig. 1 Location of *Seriatopora hystrix* sampling sites, House Reef 2 (*HR2*) and Golden Blocks (*GB*)

based on 100,000 replicates, keeping sample size, allele frequencies and heterozygosities as observed.

In order to test whether colonies were more closely related than expected under panmixia, pairwise relatedness (*R*, Queller and Goodnight 1989) was calculated in IDENTIX (Belkhir et al. 2002). The mean and variance of *R* were compared to the expected distribution under the null hypothesis of panmixia, based on 1,000 permutations of single-locus genotypes. Autocorrelation analyses in SPAGEDI version 1.2 (Hardy and Vekemans 2002) were used to test whether *R* decreased with increasing distance. In order to avoid bias from potential clonal replicates, identical MLG were removed from analyses of relatedness (randomly keeping one per group).

Results and discussion

A total of 217 *S. hystrix* colonies were analysed (Table 1). Tests for linkage disequilibria were consistent with independent segregation of marker loci. The null-corrected data set generated almost identical differentiation estimates (F_{ST}: 0.0346 vs. 0.0349) and supported all of the conclusions of this study. The mean deviations from HWE per site were small ($F_{IS} = 0.034$ and 0.041, Table 1) and non-significant. This is in contrast to all previous studies on *S. hystrix* which reported large heterozygote deficits based on allozymes (Ayre and Dufty 1994; Ayre and Hughes 2000; Sherman 2008) and microsatellites (Maier et al. 2005; Underwood et al. 2007). These were attributed to factors such as restricted dispersal of sperm and larvae, inbreeding (including selfing) and Wahlund effects. In contrast, the present findings were consistent with free mixing of gametes and larvae within the sampled areas. In the only other study on *S. hystrix* from the Red Sea (Maier et al. 2005), null alleles presumably contributed to the observed large heterozygote deficits. Thus, it is presently unknown whether (approximate) Hardy–Weinberg proportions are common in this region.

Assignment tests in GeneClass2 identified 13 (*HR2*) and 16 (*GB*) colonies as immigrants. After subtracting the expected Type I error (cf. Materials and Methods), the minimum estimate of *Nm* was seven (6.5%) for *HR2* and 10 (9.1%) for *GB*. The true values may well be twice as large as the inferred minimum (Paetkau et al. 2004). Note that between one and five *S. hystrix* colonies per site were identified as immigrants (6% overall, uncorrected, $P \leq 0.05$) at the Scotts Reef in northern Western Australia (Underwood et al. 2007). Especially given the reduced power of immigrant detection in this study (five loci vs. eight used by Underwood et al. 2007), the comparison implies a relatively greater influx of immigrants into *HR2* and *GB*. This may be due to more numerous and larger source populations along the Dahab coast. The small Scott Reef is in a remote location 270 km from the mainland, and most of its *S. hystrix* populations had not yet recovered from a catastrophic bleaching event six years prior to genetic sampling. Moreover, the comparatively simpler shape of the Dahab shoreline may facilitate the transport of larvae through coastal waters (Johnson and Black 2006).

While the source populations of the immigrants remain unknown, data from a previous study help to determine plausible minimum dispersal distances. For seven sites in the Dahab region (max. distance between sites: 20 km),

Table 1 Genetic variation at five microsatellite loci in *Seriatopora hystrix*

	Locus					Overall
	Sh2-002	Sh3-004	Sh4-001	Sh2.15	Sh4.24	
HR2 ($n = 107$)						
N_A	5	4	9	14	31	
N_{PA}	1	0	3	3	11	
H_o	0.514	0.514	0.570	0.757	0.858	
H_e	0.507	0.497	0.605	0.805	0.911	
F_{IS}	−0.015	−0.033	0.058	0.060	0.058	0.034
GB ($n = 110$)						
N_A	5	5	9	16	31	
N_{PA}	1	1	3	5	11	
H_o	0.509	0.361	0.536	0.806	0.798	
H_e	0.446	0.359	0.555	0.834	0.945	
F_{IS}	−0.142	−0.007	0.033	0.034	0.155*	0.041
Total						
N_A	6	5	12	19	42	
Repeat motiv[a]	$(CA)_{18}$	$(AAC)_{12}$	$(CAAT)_{10}$	$(CA)_{18}$	$(CA)_{37}$	
Allele size (bp)	128–155	154–166	130–160	215–331	178–298	

n Sample sizes, N_A number of alleles, N_{PA} number of private alleles, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient (Weir and Cockerham 1984)

Significant values were determined after sequential Bonferroni corrections

* Indicates significance at a nominal α-level of 0.05, $P \leq 0.0042$

[a] Number of repeats are given for the cloned allele

Table 2 Estimates of F_{ST} (θ. Weir and Cockerham 1984) and R_{ST} (ρ, normalised across loci according to Goodman 1997)

Locus	F_{ST}	R_{ST}
Sh2-002	0.015	−0.003
Sh3-004	0.108	0.311
Sh4-001	0.012	0.031
Sh2.15	0.027	−0.004
Sh4.24	0.029	0.167
Total	0.035* (0.012)	0.109

Standard error (s.e.) for θ is based on jackknifing over loci

* $P < 0.0001$ according to Fisher's exact test in GENEPOP. No test for significance was carried out on R_{ST}

Maier et al. (2005) found a mean F_{ST} of 0.094. Eight out of 21 pairwise comparisons yielded F_{ST} estimates greater than 0.1 (min. pairwise distance within this subset: 6.3 km). At that level of differentiation, the power of assignment tests to detect immigrants approaches one for the set of markers used here (Paetkau et al. 2004). Immigrants that are detectable by the present approach may therefore have originated within the Dahab region. However, it is unlikely that any exchange of migrants between *HR2* and *GB* would have been picked up: differentiation between both sites was low if highly significant ($F_{ST} = 0.035$, $P < 0.0001$). The corresponding R_{ST} value was 0.109 (Table 2). While the inferred immigrants appear to be randomly located within site *HR2*, those at site *GB* lie mostly on a line that runs diagonally through the study site (Fig. 2). This curious pattern is presently unexplained. It does not match any features of the substrate such as a ridge or a trough.

At *HR2*, no repetition of MLG occurred ($N_g/N = 1$). At *GB*, five groups of colonies with identical MLG were found, each group consisting of two colonies. No identical MLG were shared between both sites. Thus, genotypic structure at *HR2* was consistent with purely sexual, biparental reproduction. Under that assumption, the observed repetition of MLG at *GB* was highly improbable ($P < 10^{-5}$), but can be explained by selfing (see below). Members of a given putative clone did not occupy closely neighbouring locations but were scattered across the study site (Fig. 2b). In agreement with all previous studies on *S. hystrix* (Ayre and Resing 1986; Ayre and Dufty 1994; Ayre and Hughes 2000; Maier et al. 2005; Underwood et al. 2007; Sherman 2008), the analysis shows that reproduction was largely if not entirely sexual.

Neither mean nor variance of pairwise relatedness (R) was significantly higher than expected under panmixia at *HR2* (Table 3). At *GB*, the mean of R was consistent with panmixia whereas the variance of R was significantly increased. The latter could be due to immigrants that are only distantly related to local colonies. However, removal of presumptive immigrants did not change the results

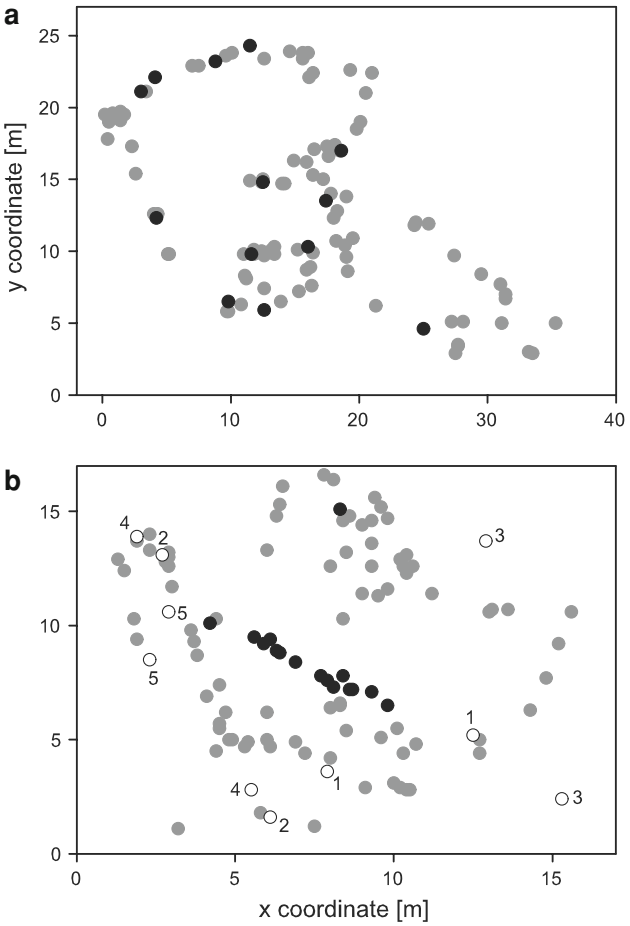

Fig. 2 Location of colonies within the sampled areas, **a** *HR2* and **b** *GB*. Inferred immigrants are marked with black symbols whereas putative clonal replicates (*open symbols*) are denoted with identical numbers. Note that each set of immigrants contains more colonies than the estimated minimum number referred to in the text

Table 3 Mean (R_{mean}) and variance (R_{var}) of pairwise relatedness (R, Queller and Goodnight 1989)

	All colonies		Without immigrants	
	R_{mean}	R_{var}	R_{mean}	R_{var}
Site				
HR2	−0.006	0.071	−0.007	0.075
GB	−0.013	0.071*	−0.019	0.077*

* Indicates significance at a nominal α-level of 0.05, $P \leq 0.013$

(Table 3). In fact, the distribution was skewed to the right, i.e., there was an excess of high relatedness values. This suggests selfing as an alternative explanation for the increased variance. A simulation of selfing among observed *GB* genotypes showed that the largest observed R values ($R > 0.5$) may represent pairs of selfed offspring and their parent. All of these observations also hold for *HR2*, albeit with no greater than expected variance in

R. However, the small F_{IS} estimates suggest selfing rates (*S*) under 0.1 (assuming equilibrium conditions with each individual producing a proportion *S* of selfed offspring, Pollak 1987). In contrast, Sherman (2008) inferred on average almost equal levels of outcrossing and selfing at One Tree Island (GBR) based on the direct comparison of adult and offspring genotypes.

Autocorrelation analyses did not reveal any association between pairwise relatedness and spatial distance. At each site, none of the correlation coefficients was significant, while sample sizes were large (min. $N = 225$ pairs in the seven smallest distance classes). An existing spatial autocorrelation may be obscured by (a) thinning processes after recruitment that weaken allelic aggregations, (b) overlapping dispersal shadows of different maternal colonies that reduce the relatedness among adjacent colonies (reviewed in Asuka et al. 2005) or (c) the random settlement of immigrants within a site. The latter point can be ruled out, as the same results were obtained after removal of presumptive immigrants. As it stands, the data are consistent with unrestricted larval dispersal (cf. Underwood et al. 2007) and random mating within sites, as shown by this analysis and the prevailing Hardy–Weinberg proportions. Thus, in *S. hystrix*, the smallest documented scale for spatially restricted dispersal in the Dahab area is 20 km (Maier et al. 2005).

Approximate panmixia as reported here is the exception rather than the rule in *S. hystrix* populations and, more generally, in marine invertebrates. The examination of four underlying processes revealed both differences and similarities in comparison with previous studies on *S. hystrix*. While there was no detectable tendency of larvae to settle near their parents, such a tendency may still exist yet be obscured by external forces such as water currents. These may also generate well-mixed samples of immigrants from various sites that appear to arrive in a steady stream without causing admixture effects. In keeping with previous studies, asexual reproduction played at most a marginal role in either population. Finally, the data hint at a mixed mating strategy of selfing and outcrossing but with a much lower selfing rate than was found elsewhere (Sherman 2008).

Among the potential causes for the divergent genetic structures of *S. hystrix* populations, there are a few on which we can comment here. Approximate Hardy–Weinberg proportions are not restricted to the Red Sea but have also been reported for several populations on the Great Barrier Reef (Ayre and Dufty 1994; van Oppen et al. 2008). The present study contradicts the notion that panmixia in *S. hystrix* is only found in lagoon habitats (Ayre and Dufty 1994; van Oppen et al. 2008). Instead, *HR2* and *GB* are situated at the reef slope. Both sites may have been populated for some time so that any admixture effects in the colonising cohort would have been eroded by subsequent random mating. If population turnover is generally common in *S. hystrix*, then 'mature' populations will simply not be sampled very often. Life history differences among populations, e.g., with regard to selfing rates, would not be unexpected. Yet, the spatial scale of such variation is presently unknown. Apportioning the observed population differences to variation in either life history or abiotic forces presents a challenge for future studies.

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