RESEARCH ARTICLE

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Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea

Abstract Pelagic dispersal of larvae in sessile marine invertebrates could in principle lead to a homogeneous gene pool over vast distances, yet there is increasing evidence of surprisingly high levels of genetic differentiation on small spatial scale. To evaluate whether larval dispersal is spatially limited and correlated with distance, we conducted a study on the widely distributed, viviparous reef coral *Seriatopora hystrix* from the Red Sea where we investigated ten populations separated between ∼0.150 km and ∼610 km. We addressed these questions with newly developed, highly variable microsatellite markers. We detected moderate genetic differentiation among populations based on both F_{ST} and R_{ST} (0.089 vs. 0.136, respectively) as well as considerable heterozygote deficits. Mantel tests revealed isolation by distance effects on a small geographic scale (≤ 20 km), indicating limited dispersal of larvae. Our data did not reveal any evidence against strictly sexual reproduction among the studied populations.

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Introduction

Pelagic dispersal of larvae in sessile marine invertebrates could in principle lead to large-scale panmixia and to a homogeneous spatial distribution of genetic variation. This would limit the formation of differentiated clades by either genetic drift or local selection, and most adaptive changes would occur in response to spatially averaged selection pressures. Yet, in contrast to this prediction, numerous studies have demonstrated moderate to high levels of genetic differentiation at genetic marker loci over relatively small spatial scales. Examples of F_{ST} estimates (Wright 1969) greater than 0.1 on relatively limited spatial scales come from sponges (Duran et al. 2004), ascidians (Ayre et al. 1997a; Ben-Shlomo et al. 2001; Paz et al. 2003), gorgonians (Gutiérrez-Rodríguez and Lasker 2004) and scleractinian corals (Stoddart 1984b; Ayre and Dufty 1994; Ayre and Hughes 2000; Nishikawa et al. 2003). Under the assumption of selective neutrality of the genetic markers, these results imply that genetic drift plays an important role in structuring the gene pool of these populations. This could be because gene flow is relatively rare and/or because population processes such as population turn-over or highly skewed reproductive success generate large stochastic fluctuations in local allele frequencies. Disentangling the processes that lead to the observed balance between genetic drift and gene flow is clearly of interest, because it contributes towards a better understanding of the life history strategies of sessile marine invertebrates. Here, we focus on the contribution of spatially limited dispersal to neutral genetic differentiation among populations of the scleractinian coral *Seriatopora hystrix* in the Red Sea. Previously reported estimates of F_{ST} for *S. hystrix* from the Great Barrier Reef are among the highest found in any coral to date (Ayre and Dufty 1994; Ayre and Hughes 2000).

Dispersal distances of planula larvae depend on various parameters such as the pre-competency period, the survival rate of competent larvae, the availability of

suitable substrate and current patterns (Harrison and Wallace 1990; Petersen and Tollrian 2001; Hohenlohe 2004). On the basis of the specific properties of coral larvae, several predictions about dispersal distances have been formulated. For example, brooded larvae typically attain settlement competency earlier than larvae of broadcast spawners (Harrison and Wallace 1990) and could therefore settle relatively closer to their mother colonies. However, as brooded larvae possess symbiotic algae from which they may gain energy resources, they are able to survive long-distance dispersal (Richmond 1987). The above-mentioned predictions would argue for a larger variance in dispersal distances of brooders compared to broadcast spawners, but not necessarily for a difference in the mean. In fact, neither of them is strongly supported by the available evidence. Recent laboratory-based studies have shown that the timing of settlement in brooders versus broadcast spawners is not necessarily all that different (Miller and Mundy 2003) and that at least some broadcast spawners may have longer competency periods than brooders (Nishikawa et al. 2003). Thus, our current knowledge about the biology of coral larvae offers little clues about dispersal distances in nature. Moreover, while a study on *Acropora tenuis* and *Stylophora pistillata* supported the prediction that neutral genetic differentiation among populations is greater in brooders compared to broadcast spawners (Nishikawa et al. 2003), a large comparative study of five brooding and four spawning coral species did not find an effect of larval biology on the levels of neutral genetic differentiation (Ayre and Hughes 2000).

Population genetic analyses offer valuable insight into patterns of sexual and asexual reproduction and larval dispersal, as these traits shape the genetic structure of populations. However, the estimation of gene flow based on the amount of spatial genetic differentiation (viz. F_{ST}) is complicated by the fact that a given pattern of neutral genetic variation could be caused by various combinations of migration between and genetic drift within demes (Whitlock and McCauley 1999). Considering the diverse reproductive strategies of corals and their often erratic local population trajectories (Hughes et al. 1992), it seems likely that highly skewed reproductive success and/or frequent population turnover cause large stochastic fluctuations in local allele frequencies and thus inflate F_{ST} (Whitlock and McCauley 1999). As an alternative, spatially restricted gene flow can be inferred from allele frequency data based on Wright's (1943) isolation by distance model. If the sampling scale is larger than the average dispersal distance, then pairwise estimates of F_{ST} from populations that are separated by some distance x should increase with x (Slatkin 1993). Previous studies have applied this approach to a variety of marine invertebrates including sponges (Duran et al. 2004), bivalves (Launey et al. 2002), cephalopods (Perez-Losada et al. 2002), sea stars (Skold et al. 2003; Perrin et al. 2004), soft corals (Bastidas et al. 2002) as well as scleractinian corals, i.e. the solitary species *Balanophyllia*

elegans (Hellberg 1994, 1995, 1996) and *Paracyathus stearnsii* (Hellberg 1996) as well as the deep-sea coral *Lophelia pertusa* (Le Goff-Vitry et al. 2004).

Seriatopora hystrix is a hermaphroditic coral species commonly found in Indo-Pacific reefs from the Red Sea to the Western Pacific (summarised in Veron 2000). It reproduces sexually via brooded larvae (Ayre and Resing 1986). In the laboratory most larvae have been observed to settle within 24 h after release (Atoda 1951), which suggests that dispersal may be spatially limited. This behaviour may contribute to the very high levels of genetic differentiation: two allozyme studies on the Great Barrier Reef revealed F_{ST} values of 0.3 (Ayre and Dufty 1994) and 0.28 (Ayre and Hughes 2000) for populations within a single reef.

Several asexual modes of reproduction have also been suggested for *S. hystrix*: based on the colonies' delicately branched morphology, reproduction via fragmentation appears likely (Ayre and Dufty 1994; Ayre and Hughes 2000). Moreover, in a laboratory study, single polyps were observed to detach from the underlying skeleton and resettle once they came in contact with an appropriate surface (polyp bail-out; Sammarco 1982). However, allozyme-based population surveys have so far not found any conclusive evidence for a contribution of asexual reproduction to recruitment on the Great Barrier Reef (Ayre and Dufty 1994; Ayre and Hughes 2000). In the present study of populations from the Red Sea, we analysed the patterns of genotypic diversity for a possible signature of asexual reproduction, because life history strategies in corals show enormous intraspecific, geographic variation (e.g. Stoddart 1984a, b; Ayre et al. 1997b) and because we would need to exclude obvious clonal replicates from any analysis of isolation by distance.

The power of the present analyses is aided by the use of highly variable microsatellite loci (Maier et al. 2001). The chances to detect isolation by distance have been shown to increase with the level of allelic variation per locus (Hellberg 1994). The same is true for the analysis of multilocus genotypic diversity in view of detecting asexual reproduction (cf. Discussion). We investigated the genetic structure of ten *S. hystrix* populations within the Red Sea along a distance of ~610 km. We found evidence for spatially restricted dispersal on a scale of ≤ 20 km. There was no evidence that asexual reproduction played an important role in structuring local populations. Given the likely occurrence of null alleles in our data set, we developed a statistical method to test that our inferences about spatial population structure are robust to possible null allele effects.

Materials and methods

Collection and storage of samples

Tissue samples of *S. hystrix* were collected between 1999 and 2002 from ten sites within the Red Sea region, separated between ~0.150 km and ~610 km. The

northernmost population was situated near the city of Eilat, Israel (29°33′40″N, 34°57′06″E) at the dive site Satil (*Sa*). The two southern populations Zabargad (*Za*) and Malahi (*Ma*) were located near Hamata, Egypt (25°05′N, 34°54′E). The remaining seven sites Bells (*Be*), Blue Hole (*Bh*), Eel Garden (*Eg*), House Reef (*Hr*), Islands (*Is*), Lagoon (*La*) and Three Pools (*Tp*) were chosen in the Dahab region, Egypt (28°29′00″N, 34°32′00″E; Fig. 1). Depths of sampling sites ranged from 3 m to 24 m. We sampled all colonies with a diameter of about 20 cm in contiguous stands of *S. hystrix* until the target sample size of about 20 was reached (two exceptions: *n* = 11 and 32). Depending on colony density, the sampled areas ranged from 30 m² to 150 m². Size-selective sampling was done in order to reduce the confounding effects of allele frequency variance among age cohorts (Waples 1987). An exception was the population *Sa* where colonies were sampled irrespective of size. From each colony, a single branch tip of approximately 1 cm length was pruned carefully underwater and, upon return to the shore, immediately blow-dried for the preservation of DNA. Samples were initially stored at ambient temperature and then frozen at −60°C prior to DNA extraction.

Laboratory analysis

DNA was extracted with DNAzol™ and Polyacrylcarrier (Molecular Research Center, Inc.) as described by Maier et al. (2001). PCR reactions were set up in a total volume of 30 μl with 1 μl template DNA, 10 pmol of each primer (one per pair labelled with *Cy5*), 3 μl dNTPs (2 mmol per nucleotide), 50 mM KCl, 1.5–2.5 mM MgCl₂ (cf. Table 1), 10 mM Tris-HCl (pH 9.0 at RT), 0.5 U Taq polymerase (rTaq, Amersham Biosciences) and overlaid with mineral oil. Amplifications were carried out on a Hybaid Touchdown thermocycler. The following cycling protocol was used: an initial denaturation step for 3 min at 95°C was followed by 35 cycles at 94°C for 15 s, 52–54°C for 30 s (cf. Table 1) and 72°C for 30 s. Allelic variation was analysed on an ALFexpress automated sequencer (Amersham Biosciences).

To date, seven microsatellite markers have been developed for *S. hystrix* (this study; Maier et al. 2001). But only four (Sh2.15, Sh3.32, Sh3.39 and Sh4.24) were analysed in the present study, as the other three produced unreliable banding patterns. From our final data set, we also had to exclude locus Sh3.39 because unambiguous scoring of alleles proved impossible, probably due to another microsatellite locus that co-amplified with our primers. In locus Sh3.32, the two most common alleles that differed in lengths by 2 bp were pooled because of confounding stutter bands. Moreover, in 19 isolated sample/locus combinations from seven populations, a clear distinction between two neighbouring alleles was not feasible, also because of stutter bands. In these instances, we adopted a conservative approach of assigning the rarer allele in the particular population to the colony in question, as this tends to bias F_{ST} downward and thus favours the null hypothesis of no spatial structure among populations. In no case did this assignment affect the number of different multilocus genotypes observed within the population.

Statistical analysis

When inferring levels of genetic population subdivision with microsatellite markers, the specifics of their mutation process have to be taken into account: microsatellites mutate to different allele lengths mostly by the addition or loss of single repeat units (e.g. Crozier et al. 1999; Kayser et al. 2000; for exceptions see Di Rienzo et al. 1994; Anderson et al. 2000). The stepwise mutation model (viz. R_{ST}, Slatkin 1995) therefore infers the relatedness between alleles from their similarity in length and derives from this a measure of population divergence. In contrast, the infinite allele model posits that every mutation introduces a novel allele and determines population subdivision solely based on probabilities of identity by descent (viz. F_{ST}, Wright 1969). However, any allelic size homoplasy violates the infinite allele assumption. Considering this concern and the observed preponderance of small mutational steps, it appears that R_{ST} better estimates spatial population structure at mi-

Fig. 1 Map of sampling sites. (**a**) Northern Red Sea including the site near Eilat (*Sa*: Satil) and the two sites near Hamata (*Za*: Zabargad; *Ma*: Malahi). (**b**) Location of sites in the area around Dahab (*Be*: Bells; *Bh*: Blue Hole; *Eg*: Eel Garden; *Hr*: House Reef; *Is*: Islands; *La*: Lagoon; *Tp*: Three Pools)
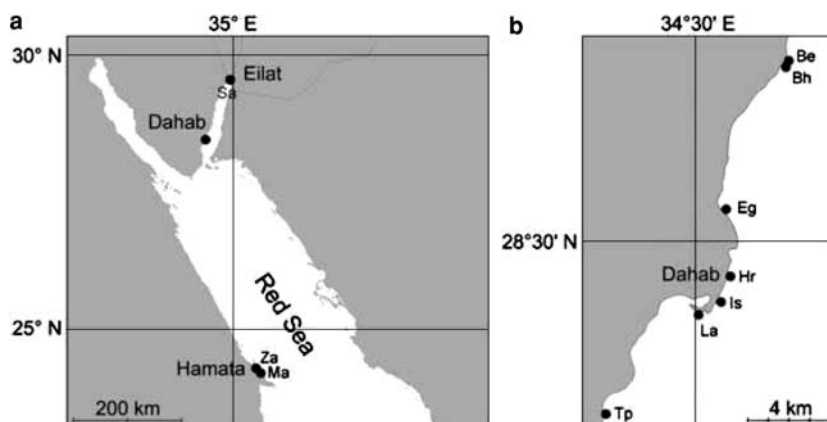
Table 1 Description of three microsatellite loci used in this study on *Seriatopora hystrix*

Locus	Primer sequences [a] (5′ → 3′)	GenBank accession number	Repeat motif	T_a [b] (°C)	No. of alleles	Allele size range
Sh2.15	CGTGCCACTGTGATTTCTTC* AACAAAAACGTCTCCATTACCC	AY604005	$(CA)_{18}$	57 (52)	15	263–315
Sh3.32	CCAAAACCCTGCATTTTGAG* CCCCCTGTAAAAGTGTACCC	AF320766	$(CA)_{20}$	55 (52)	15	219–252
Sh4.24	TCCTCCAGATGAATTTGAACG* TTCAGGGAAGATTTGCCG	AY604006	$(CA)_{37}$	59 (54)	26	178–292

[a]The oligonucleotides marked with * were Cy5-labelled for analysis on an automated sequencer
[b]Annealing temperatures in parentheses apply to amplifications with Cy5-labelled primers. Optimal $MgCl_2$ concentrations were 1.5 mmol for locus Sh2.15 as well as Sh3.32 and 2.5 mmol for locus Sh4.24

crosatellite loci. To check the robustness of our results, we follow the common approach (Gaggiotti et al. 1999; Balloux and Goudet 2002; Reichow and Smith 2001) of using both estimators, F_{ST} as well as R_{ST}, to evaluate genetic differentiation between populations.

Allele frequencies as well as Weir and Cockerham's (1984) estimators of Wright's (1965) F-statistics, f (= F_{IS}, the inbreeding coefficient) and θ (= F_{ST}, the fixation index), were calculated using *FSTAT* Version 2.9.3 (Goudet 2001). R_{ST} was calculated with R_{ST} calc., Version 2.2 (Goodman 1997), with 5,000 iterations performed for the bootstrap and permutation tests. To assess genetic variation, we calculated expected heterozygosity (H_e) for each locality and compared it with the observed heterozygosity (H_o). H_e was calculated as $1 - \Sigma p_i^2$ where p_i is the frequency of the ith multilocus genotype in the population. H_o was calculated using IBD (Bohonak 2002).

Because our results suggested that null alleles were present in the data set (see below), we checked that they did not unduly affect the inference of spatial population structure. Using a maximum-likelihood approach, we computed an alternate, hypothetical data set in which a number of apparent homozygotes were converted into heterozygotes with the observed and a null allele. The alternate data set is a compilation of the resulting maximum-likelihood genotype sets per locus and population based on the assumption of random mating. Details of this method are given in the Appendix. F_{ST} estimates from the alternate and the observed data set were compared in order to check the robustness of our results for the possible occurrence of null alleles.

As a preliminary test to check whether each locus behaved independently, we used *FSTAT* Version 2.9.3 (Goudet 2001) to estimate pairwise genotypic disequilibria based on 60 permutations. Inter-locus associations may result from either physical linkage between loci, which would rule out their use as independent markers, or from population genetic processes such as the mixing of populations and/or asexual reproduction.

To assess the contribution of asexual reproduction to the genotypic composition within populations, we calculated the observed number of distinct genotypes (N_g) over the sample size (N). Observed (G_o) and expected (G_e) genotypic diversity was estimated according to Stoddart and Taylor (1988). To assess the probability of detecting identical multilocus genotypes by chance within a sexually reproducing population, a simulation approach was used as described in Duran et al. (2004). The program generates simulated populations by randomly selecting alleles using the observed frequencies, but maintaining the observed heterozygosity at each locus. The major advantage of this approach is that it does not rely on the assumption of random mating, as this condition is often violated, especially in sessile marine invertebrates with low dispersal capabilities.

To test for a correlation between geographic and genetic distances, we performed a Mantel test (Mantel 1967) using IBD (Bohonak 2002), which compares the matrices of pairwise genetic and geographic distances. We estimated geographic distances as the shortest distance connecting populations by sea. The matrices contained the unscaled (= linear) geographic distance and $F_{ST}/(1 - F_{ST})$ as a measure of genetic distance, respectively. In a one-dimensional habitat, one expects a linear relationship between these two variables (Rousset 1997). A second, analogous set of tests was carried out using $R_{ST}/(1 - R_{ST})$. Each Mantel test was based on 30,000 matrix randomisations. Moreover, slopes and intercepts of the relationships were calculated via reduced major axis (RMA) regression. We considered two spatial scales: the small-scale analysis involved only populations around Dahab (maximum pairwise distance: ∼20 km), whereas the analysis on large scale included all populations (maximum pairwise distance: ∼610 km).

Results

Allelic variation

A total of 207 *S. hystrix* colonies were sampled and genotyped. All three microsatellite loci amplified reliably and were highly polymorphic, revealing 15–26 alleles per locus (mean: 18.66). The longest and shortest alleles per locus differed by 32 bp (Sh3.32), 52 bp (Sh2.15) and 114 bp (Sh4.24), and the frequencies of the most common alleles were 0.212 (Sh4.24), 0.389 (Sh2.15) and

0.519 (Sh3.32), respectively. Averages of expected heterozygosity within sites for the three loci were 0.611 (Sh3.32), 0.747 (Sh2.15) and 0.849 (Sh4.24). The minima and maxima overall were 0.320 (Sh3.32 in population *La*) and 0.933 (Sh4.24 in population *Is*, Table 2), respectively.

Allele frequencies varied markedly among populations, even over short distances (Fig. 2). For example, at locus Sh4.24, allele *232* was the most frequent in population *Eg* (frequency = 0.325). Only 3.5 km to the south, at population *Hr*, its frequency was only 0.053, and it was absent in the next population to the south (*Is*, 4.8 km distance from *Hr*). Several rare alleles were found only in a single population each (alleles *220*, *250* and *252* of locus Sh2.15 as well as alleles *202* and *210* of locus Sh4.24, see Fig. 2). Thus, our data revealed high allelic diversity within and between the ten studied populations.

Genotypic structure within populations

Heterozygote deficits were observed at all three loci. Mean values of observed heterozygosity (H_o) per locus were 0.354 (Sh3.32), 0.581 (Sh2.15), and 0.747 (Sh4.24), which resulted in average F_{IS} estimates of 0.44, 0.21 and 0.11, respectively (Table 2). These relatively high estimates and in particular, the large variance among loci suggested that our samples might include null alleles. This motivates the use of a hypothetical, alternate data set with null alleles being included to check the robustness of our F_{ST}-estimates (see Material and methods, Appendix and below). We evaluate the null allele hypothesis in the light of alternative explanations in the discussion.

Sexual versus asexual reproduction

Preliminary analyses revealed no deviation from linkage equilibrium for any pair of loci ($P < 0.05$, based on 60 permutations). Hence, we considered all loci as statistically independent. In all ten study populations, the expected genotypic diversity (G_e) was greater than 90% of the sample size (Table 3). Thus, our results provide high statistical power for tests of sexual versus asexual reproduction (see Discussion). In eight populations, all colonies were genotypically distinct ($G_o = N$). Somewhat lower genotypic diversity was found in the populations *Eg* and *La*, with the lowest G_o: G_e ratio of 0.85 overall in the latter population ($P < 0.13$, where P corresponds to the proportion of the null distribution with genotypic diversity smaller or equal to G_o). The proportion of simulated populations (based on 100,000 replicates, keeping sample size, allele frequencies and heterozygosity as observed) with at least two colonies with identical multilocus genotypes ranged from 0.09 (location *Tp*) to 0.71 (location *Bh*), with 0.29 for *Eg* and 0.70 for *La*. These values revealed a high probability that the small proportion of identical multilocus genotypes within two of our populations was generated by chance under strictly sexual reproduction. As we did not find any evidence for asexual reproduction, no further adjustment of the data set (viz. removal of clonal replicates) was needed. Non-amplifying alleles do not change these conclusions, since they would tend to increase G_o and we already detected the maximum possible number within most of our populations.

Genetic differentiation among populations

Averaged over all three loci and all populations, our results revealed moderate and highly significant genetic differentiation ($F_{ST} = 0.089$; $R_{ST} = 0.136$, $P < 0.001$ for each locus and overall, Table 4). Considering all population comparisons, the minimum and maximum pairwise estimates for F_{ST} were < 0.001 for the comparison *Hr-Is* (~1.3 km distance) and 0.306 for *La-Bh* (~11 km distance), respectively. Those for R_{ST} were −0.017 for

Table 2 Expected (H_e) and observed (H_o) heterozygosity and F_{IS} estimates for the three loci among different collections

Site	Sh2.15			Sh3.32			Sh4.24		
	H_e	H_o	F_{IS}	H_e	H_o	F_{IS}	H_e	H_o	F_{IS}
Sa	0.778	0.678	0.13	0.718	0.387	0.46[a]	0.892	0.846	0.05
Be	0.721	0.650	0.10	0.729	0.333	0.54[a]	0.796	0.632	0.23
Bh	0.755	0.556	0.26	0.682	0.400	0.41	0.594	0.529	0.11
Eg	0.814	0.550	0.33	0.580	0.316	0.46[a]	0.843	0.950	−0.13
Hr	0.839	0.750	0.11	0.642	0.353	0.45[a]	0.928	0.842	0.09
Is	0.780	0.700	0.10	0.369	0.294	0.20	0.933	0.889	0.05
La	0.322	0.200	0.38	0.320	0.250	0.22	0.908	0.833	0.08
Tp	0.906	0.600	0.34	0.372	0.300	0.19	0.889	0.700	0.21
Za	0.720	0.571	0.21	0.879	0.647	0.26[a]	0.807	0.600	0.26
Ma	0.829	0.550	0.34	0.822	0.263	0.68[a]	0.904	0.647	0.29
Mean	0.747	0.581	0.21 ± 0.04	0.611	0.354	0.44 ± 0.05	0.849	0.747	0.11 ± 0.04

F_{IS} was calculated using *FSTAT*, Version 2.9.3 (Goudet 2001). Mean F_{IS} estimates over populations were estimated according to Weir and Cockerham (1984). Significant values have been determined after sequential Bonferroni corrections. For abbreviations of sampling sites, see Fig. 1
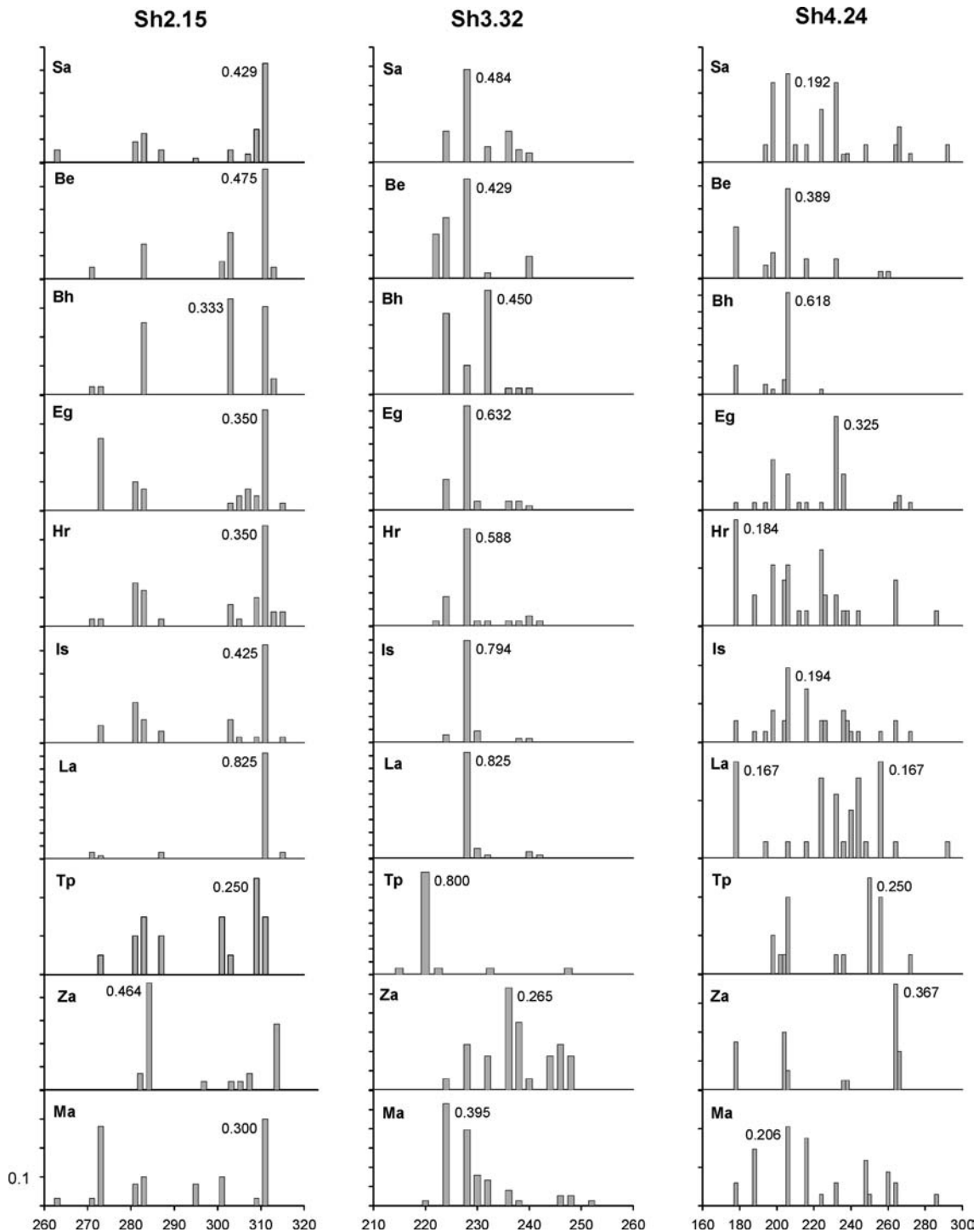[a] Indicate adjusted α-level (5%) ≤ 0.0017

Fig. 2 Allele frequencies at the three loci across all populations (for details see Fig. 1 legend). Alleles were classified according to their sizes (bp). The scales on the y axes vary among graphs. The frequency of the most common allele is given for each population

could not compute R_{ST} for the alternate data set because the length of the inferred null allele is unspecified.

the comparison *Eg-Is* (~4.8 km distance) and 0.453 for *Be-Za* (~475 km distance) (Table 5). There was little change in the overall level of differentiation when we allowed for null alleles: F_{ST} in the alternate data set was 0.097 as opposed to 0.089 (observed). Note that we

Isolation by distance

We considered the relationship between genetic and geographic distance on two spatial scales: (a) populations around Dahab (max. distance ~20 km) and (b) all populations (max. distance ~610 km). To assess differ-

Table 3 Multilocus genotypic diversity per population. Listed are the sample sizes (N), the ratio of the observed number of distinct genotypes (N_g) over the sample size as well as the observed (G_o) and expected (G_e) genotypic diversity estimates

Site	N	N_g/N	G_o	G_e	p	sim
Sa	32	1.00	32.0	31.8	1.00	0.45
Be	21	1.00	21.0	20.6	1.00	0.42
Bh	20	1.00	20.0	18.5	1.00	0.71
Eg	20	0.95	18.2	19.7	0.14	0.29
Hr	20	1.00	20.0	19.9	1.00	0.21
Is	20	1.00	20.0	19.9	1.00	0.15
La	20	0.85	15.4	18.1	0.13	0.70
Tp	11	1.00	11.0	10.9	1.00	0.09
Za	22	1.00	21.0	20.7	1.00	0.40
Ma	21	1.00	22.0	21.9	1.00	0.23

The proportion of the null distribution with genotypic diversity smaller than or equal to G_o is given by p. The proportion of simulated populations with at least one clone of size 2 is given by sim. Abbreviations of sampling sites are as in Fig. 1

Table 4 Estimates of F_{ST} (here: Weir & Cockerham's θ) and R_{ST} (ρ, normalised across loci according to Goodman, 1997)

Locus	F_{ST} (s.e.)	R_{ST}
Sh2.15	0.072 (0.035)	0.063
Sh3.32	0.125 (0.055)	0.255
Sh4.24	0.074 (0.024)	0.118
Total	0.089	0.136

Standard errors (s.e.) for θ are based on jackknifing over populations

entiation based on allelic identity, we computed Mantel correlations between $F_{ST}/(1-F_{ST})$ and geographic distance, given the nearly linear arrangement of habitats (Rousset 1997). The correlation was not significant ($r = 0.4452$, $P = 0.069$). Thus this analysis, based on F_{ST}, does not provide evidence for a distance effect. On the large spatial scale, there is no indication for a distance relationship whatsoever ($r = 0.0647$, $P = 0.4496$). Null alleles had no effect on these results as correlation coefficients of the observed versus the alternate data set were very similar and the patterns of significance were identical. For the analogous quantity, $R_{ST}/(1-R_{ST})$, Mantel tests gave a highly significant correlation be-

tween genetic and geographic distance on the small spatial scale ($r = 0.6314$, $P = 0.018$, Fig. 3). There was also a significant, albeit weaker correlation on the large spatial scale ($r = 0.490$, $P = 0.0408$). The opposing results for R_{ST} versus F_{ST} for the entire data set are illustrated in Fig. 4.

Discussion

This study on the brooding coral species *S. hystrix* from the Red Sea revealed marked genetic differentiation at microsatellite loci on a small spatial scale (≤ 20 km). Thus, our findings are in general agreement with earlier allozyme studies on *S. hystrix* from the Great Barrier Reef, which showed high levels of genetic differentiation within and between reefs (Ayre and Dufty 1994; Ayre and Hughes 2000). Since a given estimate of neutral genetic variation can be translated into measures of gene flow only for populations that conform to Wright's (1969) island model (Whitlock and McCauley 1999), we abstain from a direct translation of F_{ST} estimates into the number of migrants (Nm). Instead, we used the observed increase in pairwise genetic distances with geographic distance to infer spatially limited dispersal in *S. hystrix* on a small scale of ≤ 20 km. Moreover, while several potential mechanisms of asexual reproduction have been discussed for *S. hystrix*, our data are in agreement with strictly sexual reproduction.

Population differentiation

Our inference of spatially limited dispersal is based on the significant regression of pairwise $R_{ST}/(1-R_{ST})$ on geographic distance. Since the mutation process of microsatellites is better approximated by the stepwise as opposed to the infinite allele model (Slatkin 1995), we consider this to be the more appropriate analysis relative to the analogous regression using $F_{ST}/(1-F_{ST})$. The latter is based only on the identity of alleles and ignores information based on similarity of allele lengths. The F_{ST}-based correlation was not significant ($P = 0.069$). Moreover, spatially limited dispersal should lead to ever

Table 5 Pairwise estimates of F_{ST} (above diagonal) and R_{ST} (below diagonal) between populations of *S. hystrix*

Site	Sa	Be	Bh	Eg	Hr	Is	La	Tp	Za	Ma
Sa		0.034	0.125	0.016	0.004	0.030	0.097	0.049	0.096	0.034
Be	0.150		0.070	0.063	0.024	0.054	0.130	0.090	0.125	0.053
Bh	**0.150**	0.030		0.185	0.135	0.191	0.306	0.208	0.155	0.121
Eg	0.011	0.123	0.131		0.017	0.028	0.109	0.048	0.146	0.034
Hr	0.020	0.029	0.026	0.010		< 0.001	0.080	0.029	0.092	0.027
Is	0.009	0.084	0.098	−0.017	−0.014		0.063	0.027	0.155	0.041
La	0.038	**0.191**	**0.259**	0.108	0.085	0.092		0.152	0.252	0.134
Tp	−0.012	0.206	**0.223**	−0.006	0.055	0.016	0.057		0.163	0.057
Za	**0.222**	**0.453**	**0.404**	**0.292**	**0.306**	**0.324**	**0.329**	0.233		0.085
Ma	0.043	**0.212**	0.156	0.039	0.076	0.066	**0.175**	0.035	0.115	

Abbreviations of sampling sites as in Fig.1. Significant R_{ST} values are given in bold. To maintain an experiment-wise error level of 0.05, the critical α-level was 0.001 (Dunn-Šidák method). No significance estimates were provided by IBD (Bohonak 2002) for F_{ST}
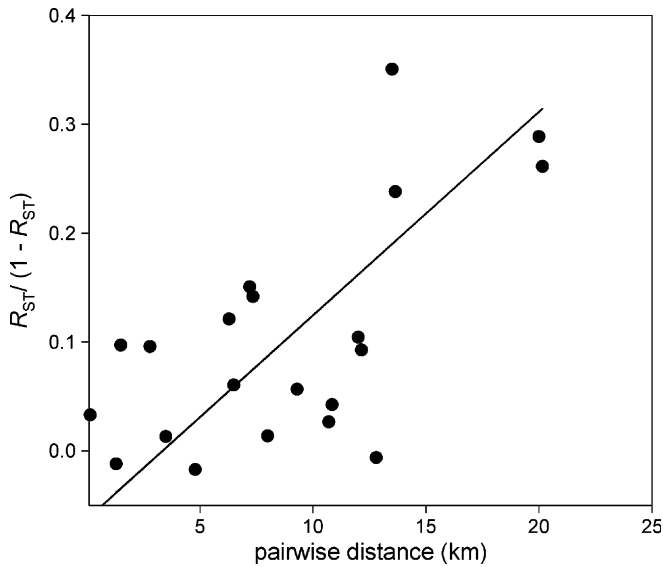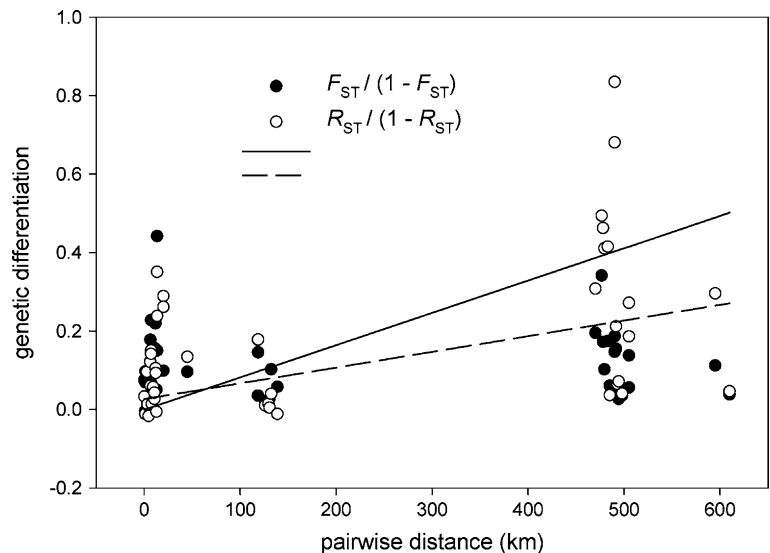
Fig. 3 Isolation by distance within the Dahab area. Plotted is $R_{ST}/(1-R_{ST})$ as a function of geographic distance. The *line* reflects the reduced major axis regression ($P = 0.0181$)

greater levels of differentiation with increasing geographic distances. Indeed, the highest estimates of R_{ST} were found for pairs of populations that were 500 km apart, but overall the regression slope was lower for the entire set of sites than for the small-scale analysis around Dahab, and the correlation was lower as well ($r = 0.4901$ vs. $r = 0.6314$). Given that our data set included only a small number of very distant sites, this difference may simply be a sampling artefact. It could also be due to constraints on allele size at our microsatellite loci, which would cause pairwise R_{ST} values to asymptote at greater geographic distances. Furthermore, historical large-scale population processes may impede the attainment of equilibrium between genetic drift and migration across the Red Sea (Hellberg 1995).

The difference between Mantel test results based on R_{ST} versus F_{ST} might be explained by the different behaviour of these two estimators under the stepwise mutation model. When the difference in average coalescence times of pairs of genes sampled from the same or from different populations is large, new mutations will accumulate and affect pairwise differences in allelic state without causing much divergence in allele frequencies (Slatkin 1995). Given that we observed relatively strong differentiation (max. pairwise $F_{ST} = 0.3$), this effect should play a role in this data set and could explain the observed results. This motivates our choice of R_{ST} in the Mantel tests. We acknowledge, however, that a large sampling variance is often associated with R_{ST} when the number of loci is small (Balloux and Lugon-Moulin 2002). Our findings provide evidence of surprisingly small-scale patterns of larval dispersal in *S. hystrix*. They are consistent with laboratory observations which imply that the majority of larvae in this species settle shortly after release (Atoda 1951). Isolation by distance effects on small and large scales (50 and 3,000 km, respectively) were found in the coral *Balanophyllia elegans* (Hellberg 1995). In this species larvae are typically not pelagic but disperse by crawling over the substrate and usually settle very close to the mother colonies. No isolation by distance was detected in the deep-sea coral *Lophelia pertusa* based on samples from around the rim of the North Sea (LeGoff-Vitry et al. 2004). As pointed out, this does not necessarily imply panmixia but could result from a lack of equilibrium between genetic drift and migration. It also does not preclude distance-dependent genetic differentiation on smaller spatial scales.

In this study, the absolute level of differentiation among populations was lower compared to previous investigations of *S. hystrix*: for the Dahab sites only, F_{ST} was 0.1, whereas within-reef estimates on the Great Barrier Reef ranged from 0.28 to 0.3 (Ayre and Dufty 1994; Ayre and Hughes 2000). This could be because the

Fig. 4 Relationship between pairwise differentiation and geographic distance between all populations. *Solid circles* represent pairwise population comparisons calculated as $F_{ST}/(1-F_{ST})$, whereas *open circles* are used for pairwise population comparisons calculated as $R_{ST}/(1-R_{ST})$. The corresponding reduced major axis regressions are shown as a *broken line* for $F_{ST}/(1-F_{ST})$ ($P = 0.4496$) and a *solid line* for $R_{ST}/(1-R_{ST})$ ($P = 0.0408$), respectively

Red Sea populations are truly less differentiated due to weaker genetic drift effects and/or more frequent gene flow. Alternatively, the difference might stem from the use of differently variable markers (microsatellites versus allozymes). Following the definition of F_{ST} in terms of probabilities of identity of alleles sampled either from the same (f_0) or from different populations (f_1), $F_{ST} = (f_0 - f_1)/(1 - f_1)$, F_{ST} is sensitive to the absolute within-population variation. This effect is most clearly seen in the extreme case when no alleles are shared between populations ($f_1 = 0$). In this case, F_{ST} equals f_0 (Rousset 1997). Thus, for the same set of populations, highly variable microsatellites are expected to yield lower F_{ST} estimates than less variable allozymes (Hedrick 1999). The possible occurrence of homoplasy at microsatellite alleles may further enhance this effect (Viard et al. 1998). The currently available data sets are therefore not suited for a comparison of *S. hystrix* population structures in the Red Sea versus on the Great Barrier Reef. While we could not use as many loci as we intended, high levels of polymorphism of our three microsatellites should serve to reduce the coefficient of variation around our parameter estimates (Kalinowski 2005).

Genotypic structure within populations

We observed considerable heterozygote deficits at all three marker loci. Similar deviations from Hardy-Weinberg expectations have been reported from a variety of marine sessile invertebrates such as sea anemones (Russo et al. 1994), gorgonians (Brazeau and Harvell 1994) and scleractinian corals (Ayre et al. 1997b; Miller 1997, 1998; Yu et al. 1999; Dai et al. 2000; Ridgway et al. 2001; Gilmour 2002), including *S. hystrix* populations from the Great Barrier Reef (Ayre and Dufty 1994; Ayre and Hughes 2000). These findings point towards non-random mating within sites that would result if, for example, dispersal distances of sperm were short relative to the size of the sampling area. Several random-mating subpopulations would then be contained in a given site, and any variance in allele frequency among them would produce a deficit of heterozygotes in the pooled sample (Wahlund 1928). Direct evidence that fertilisation in marine brooders occurs over short distances comes from colonial ascidians and bryozoans (Yund and McCartney 1994). Immigrant recruits from other such demes would further add to the observed pattern.

In microsatellites, null alleles are a common cause for apparent, large heterozygote deficits (e.g. Callen et al. 1993; Pemberton et al. 1995). Since average F_{IS} estimates varied strongly between loci (min: 0.11, max: 0.44), it seems likely that non-amplifying alleles affected our results, especially at locus Sh3.32. The observed levels of heterozygote deficit could thus reflect the combined effect of population processes and null alleles, so that the strength of the former cannot be quantified. Null alleles might also lead to an erroneous assessment of population differentiation, especially if their frequencies vary among populations. However, the close agreement between overall F_{ST} estimates based on the observed and the alternate data sets (0.089 and 0.097, respectively) shows that non-amplifying alleles, if present, introduced little bias in this measure of differentiation. We note that biases due to the presence of more than one null allele remain, but these effects should become progressively weaker as more alleles of this type are involved.

In most of the studied populations, all colonies were genotypically distinct. Simulations suggested that the small proportion of identical multilocus genotypes in two of our study populations may have resulted by chance under strictly sexual reproduction. We have therefore no evidence for clonal reproduction in *S. hystrix* in our samples, despite good statistical resolution afforded by our microsatellite loci. The power to detect a small proportion of clonal replicates in a sample of otherwise sexual origin depends strongly on the allelic diversity (B. Nürnberger, unpublished simulations). In the case of three loci, an effective number of alleles (n_e) per locus of about 3.5 (corresponding to $H_e = 0.72$) is required to insure that a clonal contribution of 10% to a sample of 20 colonies is statistically detectable. This power of detection drops off steeply as allelic diversity declines below this level. Our data set corresponds roughly to the example case just given. Recall, though, that sampling was carried out on a coarse grid, as colonies were sampled according to their size. This involves the possibility that some clonal replicates established close to the mother colony might have been missed.

A highly clonal population structure has been described from a variety of marine invertebrates, including sea anemones (e.g. Ayre 1984; Hoffman 1986), soft corals (e.g. McFadden 1997) and scleractinian corals (e.g. Stoddart 1984a; Adjeroud and Tsuchiya 1999). Indeed, a large proportion of *S. hystrix* populations at the Great Barrier Reef (37 and 44%) showed significantly reduced genotypic diversity (G_o) (Ayre and Dufty 1994; Ayre and Hughes 2000, respectively). But in combination with the observed deficit of heterozygotes, non-random mating within sites presents an alternative explanation as this would also lead to an apparent overrepresentation of certain genotypes and thus reduce G_o (see Duran et al. 2004 for a recently published test that allows for non-random mating). Thus, conclusive evidence for asexual reproduction is lacking at the Great Barrier Reef. In the present study, the observed genotypic diversity was high even though observed heterozygote deficits were strong. Thus, clonal reproduction does not play a detectable role in our studied populations.

In this study, we have shown that spatially restricted larval dispersal contributes to the population structure of *S. hystrix* in the area around Dahab in the Red Sea. Judging by the high heterozygote deficits, it appears likely that also movement of gametes within populations is spatially limited. Any such localised movement opens

up the possibility for adaptation to locally acting selection pressures. The scatter of pairwise F_{ST} estimates around the regression plots (cf. Figs. 3, 4) also shows that genetic drift strongly affects local allele frequencies. The levels of gene flow and genetic drift together set the stage for adaptation and determine the minimal selection strength to which an adaptive response could occur. In order to evaluate these possibilities for any coral, we still need to know more about the actual migration rates (i.e. m rather than Nm) and the patterns of mating within sites. Intriguing insights into the detailed workings of coral populations could be obtained from the identification of migrants and the assessment of parentage of recruits based on multilocus microsatellite profiles.

Appendix

For a given population and locus with n apparent homozygotes, we computed the likelihood of all possible genotype sets in which 1, 2,... n homozygotes had been replaced by heterozygotes involving a null allele. Non-amplifying colony/locus combinations were interpreted as null homozygotes provided that the other two loci of the same colony had amplified, thus controlling for poor DNA quality. The likelihood computations were based on allele frequency estimates from the modified genotype sets under the assumption of random mating. The alternate data set was assembled from the maximum-likelihood genotype sets per population and locus. The estimated maximal local frequencies of null alleles that were inferred by our method were 0.21 (Sh4.24), 0.25 (Sh2.15) and 0.37 (Sh3.32). The minimal local estimates were zero for all three loci. Whenever our method detected the presence of a null allele in a given locus/site combination (ML frequency > 0), its frequency correlated very strongly ($r > 0.75$) with estimates from two published methods (Chakraborty et al. 1992; Brookfield 1996). These approaches compute null allele frequencies solely based on observed heterozygote deficits, either with (Brookfield 1996) or without (Chakraborty et al. 1992) including non-amplifying samples as data (= null homozygotes). It is interesting to note, though, that there were cases in which our ML frequency of the null allele was zero, while the F_{IS}-based methods inferred appreciable non-zero frequencies. In all of these cases,

the likelihood curve was rather flat so that no particular estimate was strongly supported. It appears that the consideration of the specific genotypic constellations by our method (rather than just F_{IS}) tended to favour the hypothesis of no null allele in these cases. In keeping with these observations, F_{IS} estimates based on the alternate data set were not necessarily zero but they were non-significant throughout.

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