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Local scale genetic structure in coral populations: A signature of selection

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Abstract

Coastal marine reserves in general, and coral reef reserves in particular, are typically composed of scattered patches separated by uninhabited areas. Due to the sessile mode of life of adult corals, larval connectivity is often the only agent of gene flow between reef localities. In this study we examined the connectivity between populations of the common scleratinian coral *Stylophora pistillata* at the northern tip of the Gulf of Aqaba (Red Sea), using the rDNA ITS (internal transcribed spacer) as a molecular marker. Sequence comparisons among recruits indicated very similar, equally-diverse, assemblages of recruits in both the northern (highly affected by anthropogenic disturbances) and southern (less affected) study sites, implying a high larval connectivity or common sources of larval supply. By contrast, sequence diversity observed among adults declined sharply from southern to northern sites, accompanied by genetic differentiation of the respective populations. Based on Fu's *Fs*-test of selective neutrality, it may be suggested that various post-settlement selective regimes, presumably more intense in the northern sites, provide a reasonable explanation for the observed patterns of genetic diversity. The suggested hypothesis is supported by the sharper decline in sequence diversity found between recruits and adults in the northern sites. This study exemplifies the necessity to consider local selective factors, in addition to larval connectivity, when managing marine reserves.

Keywords: Larval connectivity; Anthropogenic disturbance; Genetic diversity; Gene flow; ITS; Scleractinia

1. Introduction

The life history of most marine organisms includes a planktonic stage during which larvae disperse to distances that range between several meters to hundreds of kilometers from their location of release (Kinlan and Gaines, 2003). How far propagules go and what their specific sources are, remain poorly understood for most species (Warner and Cowen, 2002). The dispersal of larvae is influenced by the physical characteristics of the marine environment (e.g., ambient flow regimes, geomorphology; Denny and Shibeta, 1989) and the biological characteristics of the species in question (e.g., duration of larval life, larval behavior, lecithotrophy vs. planktotrophy; Richmond, 1987, 1988; Kingsford et al., 2002). Transport of larval stages may connect apparently distinct populations of a species, and this interconnection can play a major role in driving local demography (e.g., Sale, 1999). Understanding connectivity by means of larval dispersion has become a high priority in the conservation of marine ecosystems, notably coral reefs. Coral reef ecosystems are deteriorating worldwide – an estimated 30% of the world's coral reefs are already severely damaged, and ca. 60% may be lost by 2030 if the same trends continue (Wilkinson, 2002; Hughes et al., 2003).

The coral reefs at the northern tip of the Gulf of Aqaba (Red Sea) have been exposed for years to diverse anthropogenic disturbances, such as municipal sewage

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spills, intensive mariculture, recreational diving pressure, increased sedimentation due to marine construction, and diverse maritime activities (Fishelson, 1995; Ben-Tzvi et al., 2004; Loya, 2004). In recent years a significant deterioration has occurred in the state of these reefs and raised alarming questions about their survival (Abelson et al., 1999; Loya, 2004).

As part of the countermeasures being taken against the decline of coral reefs, there is an international conservation effort focusing on the establishment of interconnecting marine reserve networks to protect threatened populations (Barber et al., 2002). For most sessile marine invertebrates (e.g., corals), larval connectivity represents the only mode of linking between distinct protected areas. Knowledge of the extent of larval connectivity and gene flow between neighboring populations is important for understanding how coral reefs might fare under disturbance, and is of concern for authorities managing local reef ecosystems (Hoegh-Guldberg, 1999; Roberts, 1997). Design of marine reserve systems requires an understanding of larval transport in and out of the reserves, whether the reserves are likely to be self-seeding or to accumulate recruits from surrounding exploited areas, and whether reserve networks can exchange recruits (Palumbi, 2003). Consequently, in most cases, operation and effectiveness of marine reserves should take into account that nature reserves are part of an ecosystem that is much larger than the reserve itself (Agardy, 1994).

Larval connectivity among populations implies gene flow, and local genetic variability among adults may, therefore, provide an indirect estimate of dispersal in marine systems. In general, in many marine species, including corals, low genetic differentiation among populations is associated with high dispersal potential, and high genetic differentiation is associated with larvae that disperse poorly (Palumbi, 1996). Genetic analysis of population differentiation may provide a powerful tool for investigating connectivity (Ayre and Hughes, 2000), despite some important exceptions to the above suggested rules (reviewed by Palumbi, 1996; see 'Discussion'). One example of such an exception is that of selection on early life stages caused by local environmental factors. In practice, most patterns of geographic genetic variation can be explained by some scenario involving selection acting either directly on the markers in question or on genetically linked sites (Hellberg et al., 2002). In some cases, the selection may be strong enough to overcome even high rates of migration, and those populations experiencing selective pressure may exhibit relatively high genetic homogeneity. In the blue mussel, *Mytilus edulis*, for example, an 'oceanic' allele of the leucine aminopeptidase (Lap) locus is very common among newly recruited individuals. This allele is advantageous in high salinity environments (Koehn and Siebenaller, 1981) but, on the other hand, is disadvantageous in low salinity environments. As a result, the frequency of this allele is observed to be very low in adult populations in low salinity environments (Koehn et al., 1980; Hilbish, 1985). Since selection may be strong enough to even blur the genetic signature of connectivity, genetic insights into present-day population connectivity are most readily accomplished with sampling programs that include new recruits (Thorrold et al., 2002).

In this study, we explored the genetic variability within and among populations of the widely distributed scleractinian coral *Stylophora pistillata* from the northern tip of the Gulf of Aqaba. This is the first local scale survey of population genetic structure of any coral from the Gulf of Aqaba. The objective was to assess the level of larval connectivity among local populations and examine the local factors that may affect their genetic structure. Knowledge of the genetic structure of local populations, and the levels of larval connectivity, is essential for establishing a sound conservation program. For example, under conditions of high connectivity among locations, damaged reefs may be quickly re-seeded if the original cause of the damage is removed. By contrast, investment in environmental remediation may not result in re-establishment of damaged local populations under conditions of low connectivity.

2. Materials and methods

2.1. Choice of species and collection of specimens

S. pistillata is a brooding scleractinian coral. It was chosen for this study because of its ubiquitous distribution and relatively high abundance in the Gulf of Aqaba. Takabayashi et al. (1998a, 2003) showed relatively high levels of intra-specific variability of the ITS (internal transcribed spacer of the nuclear rDNA) region in *S. pistillata*. Therefore, ITS sequence analysis may be useful for genetic comparisons among local populations of this species.

Adult colonies of the coral *S. pistillata* were collected from 2 to 4 m depth, by snorkeling or SCUBA-diving at six sites situated at a distance of up to 10 km from each other (Fig. 1): the artificial substrates (pipes and construction debris) between the fish cages and the pier of the 'Ardag' fish farm (FC); the breakwater of the artificial Peace-Lagoon (PL); the knolls in front of the Galei-Elat Hotel (GE); the breakwater of the glass-bottom boat anchorage Tur-Yam (TY); the reef in front of the Marine Biology Laboratory (MBL); and the reef next to the Taba border-crossing station (T). Shorelines at the southern sites (TY, MBL, T) are typically steeper than those at the north beach, and offer a higher abundance of hard substrate. In an attempt to sample only corals that had experienced a similar time period of development, only corals of the same size-class (approximately 30 cm in diameter) were sampled. The sampled corals are thus assumed to be of similar age classes, and to have settled within a limited time duration.

Small nubbins (1 per colony; ca. 2 cm in length) were collected from *S. pistillata* colonies at each site. Sampled colonies were always at least 10 m away from each other in order to reduce the likelihood of sampling products of asexual fragmentation from the same parent colony. Addi-

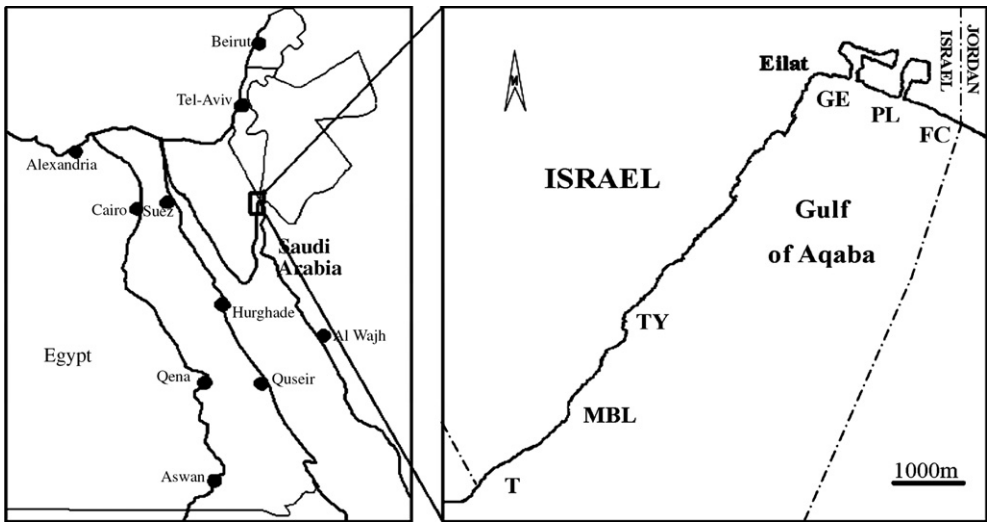

Fig. 1. Map of the northern tip of the Gulf of Aqaba showing locations of *Stylophora pistillata* populations studied. See text for site acronyms. Adult colonies were collected at three sites from the north beach of Eilat (FC, PL, GE) and at three southern sites (TY, MBL, T). Recruits were collected at one site from the north beach (PL) and at the southernmost site (T).

tionally, in order to probe post-settlement selection, juveniles (recently-recruited individuals; $\leqslant$5 mm in diameter) from the Peace-Lagoon and Taba were sampled. Juveniles were located at night, using a UV torch (NightSea), whose light excites fluorescence of green fluorescent proteins (GFPs; for similar phenomena in other corals, see e.g., Gurskaya et al., 2001) in the recruits' tissue. Coral recruits thus located were examined for typical features of *S. pistillata* before being collected. In addition, to ensure that the sampled recruits were, indeed *S. pistillata*, a species-specific primer was designed and used (see below, under '*PCR amplification and sequencing*').

2.2. DNA extraction

Tissues of adult coral tips were ablated from the skeletons using a scalpel and stored in ethanol. DNA of all samples was extracted by suspending approximately 50 µl of tissue (juveniles were suspended as a whole) in 500 µl of extraction buffer (10 mM Tris–HCl [pH = 8.0], 0.1 M EDTA, 0.5% SDS) supplemented with 3 µl of a proteinase K solution (20 mg/ml), and incubating at 65 °C for 3 h. Nucleic acids were purified by two successive phenol:chloroform (1:1) extractions followed by a single chloroform extraction.

2.3. PCR amplification and sequencing

Amplifications were performed in a 25 µl volume of a solution containing approximately 1 ng/µl of DNA, 0.4 mM total dNTP, 0.3 µM of each primer, 2.5 µl of 10× reaction buffer (1.5 mM $MgCl_2$) and 1 unit of Ampli-Taq polymerase (Super-Therm). The coral-specific primer A18S (5′-GATCGAACGGTTTAGTGAGG; Takabayashi et al., 1998b) located in the 18S rDNA and a *S. pistillata*-specific primer Sty-S (5′-CCACCGTCAAAAGTTGTCAC) designed to anneal to a conserved region within the 5.8S rDNA, were used to amplify the ITS-1 region and part of the 5.8S rDNA for all samples. Amplifications consisted of initial denaturation at 94 °C for 4 min, followed by 34 cycles of 0.5 min denaturation at 94 °C, 0.5 min annealing at 57 °C and 1 min elongation at 72 °C. Amplified products were sequenced directly with the primer A18S using the dye terminator method (ABI PRISM® 3100 Genetic Analyzer). Several amplified products were double checked for their nucleotide composition by sequencing them in both directions, also using the *S. pistillata*-specific primer Sty-S.

2.4. Sequence alignment and data analysis

Sequences were aligned using Clustal X software (Version 1.81; Thompson et al., 1997), adjusted by eye, and trimmed to form a 445 bp long data matrix. Sequences were verified to represent ITS regions from *S. pistillata* by a BLAST search.

ITS sequences are present in multiple copies in each genome, and intra-individual variability is, therefore, a potential drawback (e.g., Diekmann et al., 2001; Vollmer and Palumbi, 2004). To address this potential difficulty, as an additional part of this work, the utility of ITS-1 region in the species *S. pistillata* was tested by estimating intra-individual variation. For this purpose, DNA was extracted from seven additional individuals (3 from the north beach and 4 from the southern sites). The ITS-1 region from each sample was amplified separately in 5–6 PCR tubes and the amplification products were sequenced independently from both ends using the primers A18S and Sty-S.

Genetic distance calculations implemented in MEGA version 2.1 (Kumar et al., 2001) were used to estimate sequence divergence, using the method of Tamura and Nei (1993). To test the existence of genetic differentia-

tion between the north beach (FC, PL and GE) and the southern sites (TY, MBL and T), a hierarchical analysis of molecular variance (AMOVA) was applied. This analysis was performed on a matrix of pairwise genetic distances among all the ITS-1 sequences using ARLEQUIN software (Schneider et al., 2000). This matrix of distances was calculated using the Tamura and Nei model and was also used to compare levels of intra-population diversity among different populations, by randomization tests (ANOVA by randomization, RT package; Manly, 1997).

The hypothesis of neutral evolution was tested by Fu's *Fs*-test (Fu, 1997). The population parameter θ and the growth parameter g were estimated in order to assess historical demography. These parameters were estimated with a coalescent approach, using the computer program FLUCTUATE 1.4 (Kuhner et al., 1998).

To explore the genetic differentiation among populations and to construct a spatial representation, a multidimensional scaling approach (MDS; SPSS software, Version 11.0) was used. This multivariate technique is free of assumptions of genetic structure (Jones et al., 2004) and the corrected average pairwise differences between populations (D_A; Nei and Li, 1979) were inserted as input. The corrected average pairwise difference between populations is calculated as

$$D_A = P_{XY} - (P_X + P_Y)/2, \quad (1)$$

where P_{XY} is the average of pairwise differences between populations X and Y, and P_X and P_Y are the averages of pairwise differences among individuals within population X and Y, respectively.

Population pairwise genetic distances (F_{ST}-values) were calculated and tested using ARLEQUIN. The significance level (p-value) of the test is the proportion of permutations leading to an F_{ST}-value equal to or larger than the observed one. Levels of gene flow among populations were estimated, using Wright's (1978) 'island model', where the number of migrants per generation (N_em; i.e., gene flow) is calculated as

$$N_em = (1/F_{ST} - 1)/4. \quad (2)$$

This model assumes that all loci are selectively neutral, that equilibrium exists between gene flow and genetic drift, and that there is constant gene flow in all directions (Wright, 1969). Under these conditions, Wright (1969) showed that when $N_em \leqslant 1$ (i.e., very low levels of gene flow), gene flow is insufficient to prevent fixation of alternative alleles in different subpopulations, whereas high levels of gene flow ($N_em > 20$) are sufficient to prevent marked population differentiation through genetic drift.

3. Results

A total of 45 haplotypes were identified among 81 individuals analyzed from six sampled groups of adults (FC-a, $n = 11$; PL-a, $n = 11$; GE-a, $n = 11$; TY-a, $n = 8$; MBL-a, $n = 9$; T-a, $n = 11$) and two sampled groups of juveniles (PL-j, $n = 11$; T-j, $n = 9$). Only 29 haplotypes were identified among 61 adults, while each of the 20 juveniles was identified as a different haplotype (4 of the sequences were found in both adults and recruits). BLAST searches confirmed that all 81 sequences represent *S. pistillata*. Sequences were deposited at the EBI database, under accession numbers AJ619805–AJ619852. Of the seven samples that were tested for intra-individual variation, only three showed some level of variation or polymorphism. The average sequence divergence within individuals was $0.3 \pm 0.4\%$ compared with $17.1 \pm 16.8\%$ inter-individual variation. Thus, intra-individual variation represents ca. 2% of the total variation observed in this study.

3.1. Intra-population diversity

The average number of pairwise differences among individuals within populations (sampled groups; mean ± SE) is shown in Fig. 2. Statistical comparison of pairwise differences within populations by randomization tests showed that intra-population sequence diversity is similar for both northern and southern juveniles (PL-j vs. T-j; $p \gg 0.05$). By contrast, diversity of adult populations from the north beach (FC-a, PL-a, GE-a) was significantly lower than that of those from the southern sites (TY-a, MBL-a, T-a; $p < 0.01$). Sequence comparisons between juveniles and adults showed that the genetic variability of adults at the southern sites is significantly lower than that of the recruits (Fig. 2). These significant differences are valid both when comparing adults from all three sites (i.e., T-j vs. [TY-a, T-a, MBL-a]; $p < 0.01$), or only the adults from the same site where recruits were collected (i.e., T-j vs. T-a; $p < 0.05$). The apparent difference between the variability of adults and recruits is even larger in the northern sites (Fig. 2), whether all adults are considered (i.e., PL-j vs. [PL-a, FC-a, GE-a]; $p < 0.01$) or only those sampled at the same site where recruits were collected (i.e., PL-j vs. PL-a; $p < 0.01$).

To test whether the reduction in diversity between young recruits and adults is significantly greater in the north beach (PL) than in the south (T), we subtracted, for each site, each pairwise distance among adults (55 distances in PL; 55 in T) from each pairwise distance among recruits (55 in PL; 36 in T). This procedure resulted in 3025 (55×55) and 1980 (55×36) values for PL and T, respectively, representing the reduction in diversity between recruits and adults. A comparison of the differences by randomization showed that reduction in diversity is significantly larger in the Peace-Lagoon ($p \ll 0.01$; Rundom Projects shareware, Version 1.1; Manly, 1997; available at http://pjadw.tripod.com/soft.htm).

Fu's *Fs*-test, population parameter θ and growth parameter g results are presented in Table 1. The null hypothesis of neutrality was rejected specifically for adult samples collected in the northern sites, indicating either selection or exponential population growth. Population parameters calculated for these adults showed that growth was negative in

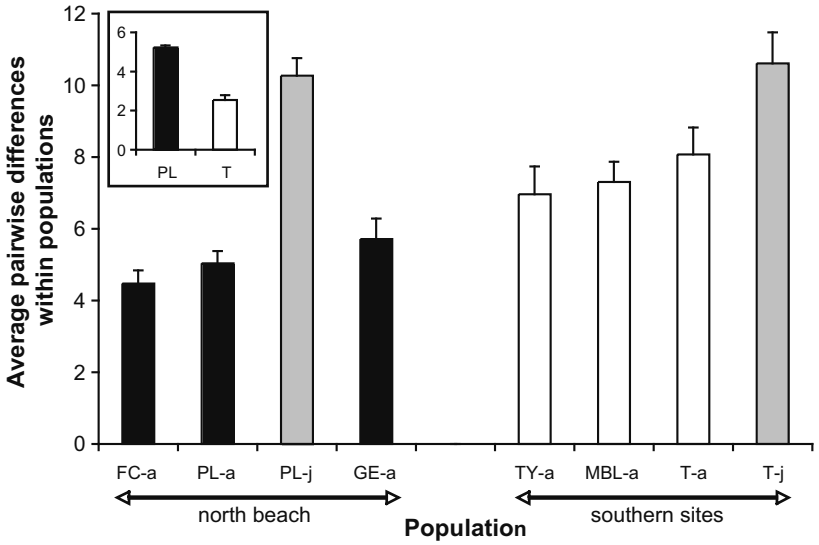

Fig. 2. Average pairwise differences (Tamura and Nei, 1993) within populations (±SE) of adults from the north beach (black bars), southern sites (white bars) and juveniles (gray bars). The insert illustrate the differences between juveniles and adults from the north (PL) and the south (T).

FC-a ($\theta = 0.058$, $g = -6.22$) and positive in PL-a ($\theta = 0.025$, $g = 48.61$) and GE-a ($\theta = 0.017$, $g = 50.19$) but not exponential, indicating that Fu's *Fs* values were most likely due to selection. Regarding the adult populations of the southern sites, the null hypothesis of neutrality could not be rejected (as Fu (1997) noted that a *Fs* statistic should be considered as significant at the 5% level, if its *p*-value is below 0.02, and not below 0.05).

3.2. Inter-population genetic differentiation and gene flow

In general, a formal way of examining the level of differentiation among populations involves *F*-statistics. In this study, since ITS rDNA introns do not behave as diallelic single-copy loci (Hillis and Dixon, 1991), it was not possible to apply statistic analogues to F_{ST} in order to calculate the degree of gene flow. However, analysis of corrected pairwise differences among populations by MDS (Fig. 3) illustrated that adult populations from the southern sites are all genetically similar to each other (MBL-a, T-a, TY-a). By contrast, adult populations from the north beach (FC-a, PL-a, GE-a) do not form a cluster in the MDS chart and are all relatively far from the southern cluster. A hierarchical analysis of molecular variance (AMOVA) among adult populations showed a significant difference between the north beach and the southern sites (16.24% of the total variation, $p < 0.0001$; see among regions in Table 2).

Analysis of juveniles showed a contrary pattern to that of the adults; recruits at the northern and southern sites (PL-j and T-j, respectively) are genetically similar when viewed by MDS and are located near the origin, between the adult populations from the north beach and the southern sites. Juvenile groups did not differ significantly from adults collected at the same site (see Table 3).

Table 1
Fu's *Fs*-test, population parameter θ and growth parameter g results calculated for adult populations

Tested group	*Fs*	*Fs* *p*-value	θ	g
FC-a	−7.04	$p < 0.001^{*}$	0.058	−6.22
PL-a	−6.56	$p < 0.001^{*}$	0.025	48.61
GE-a	−6.12	$p = 0.003^{*}$	0.017	50.19
TY-a	−2.74	$p = 0.043$	0.099	134.95
MBL-a	−2.56	$p = 0.032$	0.190	338.75
T-a	−2.81	$p = 0.026$	0.087	131.56

See text for site acronyms. Notice that a *Fs* statistic should be considered as significant at the 5% level, if its *p*-value is below 0.02, and not below 0.05 (Fu, 1997).
* Significant at the 5% level.

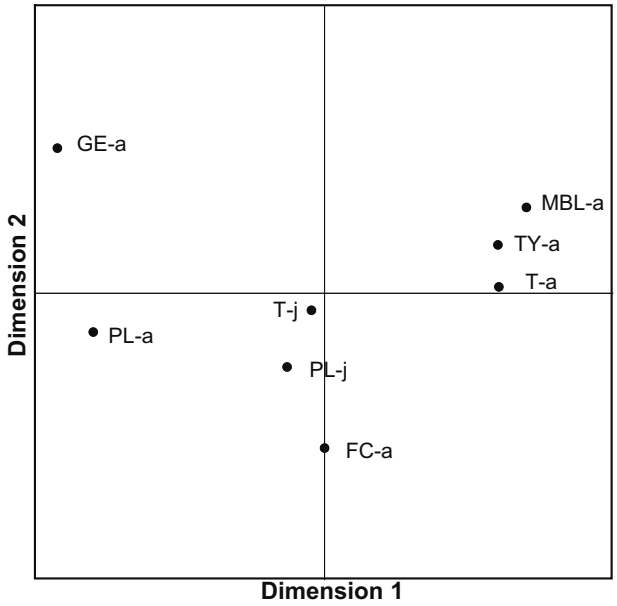

Fig. 3. Multidimensional scaling analysis of corrected pairwise differences among populations of adults and juveniles. Stress value = 0.003.

Table 2
Analysis of molecular variance (AMOVA) among adult populations of *Stylophora pistillata* using the ITS-1 region and part of the 5.8S rDNA data

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation	Fixation indices	*p*
Among regions	1	27.862	0.72053	16.24	0.162	<0.0001
Among populations within regions	4	24.068	0.25220	5.68	0.068	0.023
Within populations	55	190.496	3.46357	78.07	0.219	<0.0001

Two hierarchical levels (regions and populations) were implemented. Region 1 includes adult populations at the north beach of the Gulf of Aqaba (FC-a, PL-a and GE-a). Region 2 includes the southern sites (TY-a, MBL-a and T-a; see Fig. 1). The significance tests were based on 1023 permutations.

Table 3
Comparison of pairs of populations

Population	FC-a	PL-a	PL-j	GE-a	TY-a	MBL-a	T-a	T-j
FC-a	**4.471**	5.237	7.380	6.547	6.192	6.790	6.790	7.705
PL-a	0.045*	**5.037**	7.968	5.517	7.604	8.371	8.371	8.460
PL-j	0.387	0.117	**10.270**	8.980	9.311	9.630	9.630	10.333
GE-a	<0.001*	0.047*	0.018*	**5.716**	8.993	9.756	9.756	9.455
TY-a	0.099	<0.001*	0.036*	<0.001*	**6.963**	6.943	6.943	9.343
MBL-a	0.027*	<0.001*	0.025*	<0.001*	0.712	**7.309**	7.309	9.773
T-a	0.027*	<0.001*	0.018*	<0.001*	0.480	0.378	**8.071**	9.425
T-j	0.252	0.068	0.595	0.009*	0.117	0.063	0.391	**10.610**

Here is shown the average percentage of pairwise differences between populations (above diagonal) and within population (diagonal elements, in bold). Below diagonal, it is shown the *p*-values and the statistical significance of the genetic differences between the populations. The Tamura and Nei parameter distance method was used for the calculations.

* Significant at $p < 0.05$.

Estimates of rates of gene flow among populations were very high among adult populations at the southern sites ($N_em \rightarrow \infty$). By contrast, gene flow was substantially lower among adult populations at the north beach (N_em ranged from 0.87 for FC-a and GE-a to 5.55 for PL-a and GE-a).

4. Discussion

4.1. The utility of ITS-1 region in S. pistillata

The utility of ITS (and other rDNA) sequences as genetic markers was recently challenged by Vollmer and Palumbi, 2004 in a study focused on the scleractinian coral *Acropora*, due to relatively high intra-individual variation. For example, in *A. cervicornis*, intra-individual variation (constituting 'noise' in terms of population genetic analysis) was as high as 11.4% in ITS-1, whereas inter-individual variation (the 'signal') reached a maximum of 13.2%. In the case of *Acropora*, however, high intra-individual variation may be related to the high occurrence of inter-specific hybridization events, which occur exceptionally frequently in this group (Hatta et al., 1999; van Oppen et al., 2001). Consequently, in ITS1, rDNA variation within individual coral colonies constituted up to 86% of the variation in *A. cervicornis*, casting severe doubts as to the utility of these sequences in population-level studies. By contrast, in this study a different pattern of intra/inter-individual variation in *S. pistillata* was found. Similarly to Rodriguez-Lanetty and Hoegh-Guldberg (2002), who examined inter-individual variation in *Plesiastrea versipora* from the Western Pacific, the results in this study show that most of the variation of ITS-1 sequences in *S. pistillata* is accounted for by inter-individual variation. In fact, only ca. 2% of the observed variation is accounted for by intra-individual variation. Thus, these data suggest that the ITS-1 region is a genetically variable and useful marker for analyzing genetic structure in populations of the coral *S. pistillata*.

4.2. Gene flow and connectivity

The genetic composition and diversity of adult populations differed between northern (north beach) and southern sites, as reflected by AMOVA, MDS analysis and the magnitude of the haplotypic diversity. One potential explanation for the different genetic patterns is that of a lack of connectivity between the localities. However, the similar genetic composition of recruits across sites suggests either a high level of connectivity between the north and the south, or a common source of recruits. This pattern is nicely illustrated by the MDS; adult populations from the north beach are all relatively far from the cluster of the southern adult populations (Fig. 3). On the other hand, both the northern and southern juvenile populations are located very close to each other and also close to the MDS origin, which represents the 'average population'.

The significant genetic differences among populations over the very small geographic scale of this study ($\leqslant$10 km) are at odds with the findings of Takabayashi et al. (2003), who found panmixia among populations of the coral *S. pistillata* separated by large distances (thousands of kilometers) across the western Pacific Ocean.

Interestingly, Takabayashi's study also employed the ITS as a molecular marker, and similar sample sizes (6–14 samples per site). The paradox of little differentiation over large geographic scales and extensive differentiation over microgeographic scales can potentially be reconciled by local effects (e.g., local selective factors, local current regimes) that influence gene flow in local populations but may become insignificant over extensive geographic scales (Hellberg, 1994; Yu et al., 1999). Additionally, with respect to the results of Takabayashi et al. (2003), the differentiation among the extant populations of marine invertebrates may reflect a remnant gene flow pattern created by historic oceanography (Williams and Benzie, 1998; Benzie, 1999; Yu et al., 1999), which can lead to overestimation of gene flow over large geographic scales (Palumbi, 1994).

4.3. Local selection on recruits

Although, we tested for selection of a molecule (not a population), when a locus is under selection it is usually indicative of selection also occurring at additional loci, and therefore it offers a representative selection of a population. The results demonstrate a reduction of haplotypic diversity between recruits and adults in both the northern and southern sites. However, the decline of haplotypic diversity in the north is significantly sharper than in the south (see insert in Fig. 2). This sharper decline is consistent with our hypothesis that populations in the northern sites (FC, PL and GE) are under the influence of stronger stressors than those in the south (TY, MBL and T). Evidence in support of the selection processes assumed above is also found in other studies, which have shown that the north beach of the Gulf of Aqaba is exposed to higher levels of stress caused by pollution than the southern sites. Bresler et al. (1999, 2003a,b) compared a range of biotic responses in molluscs from the southern (MBL) and northern (FC) sites, as indicators for the level of environmental stresses (e.g., metabolic performance, integrity of cellular membranes, levels of DNA breakage). All the tested parameters unequivocally indicated the northern sites as more polluted and stressed. The very low recruitment rates at the northern sites, despite the relatively high larval supply, as well as the high mortality rates of recruits (e.g., Ben-Tzvi et al., 2004; Abelson et al., 2005), are also in line with stronger selection taking place at the northern sites.

Additional support from a different direction for the assumed selection processes is given by Fu's *Fs*-test. At the northern sites, this test indicates either selection or exponential population growth for adults. Further analyses of population parameters (i.e., θ population parameter and g growth parameter) suggest that the obtained Fu's *Fs* values are most likely due to selection. Fu's *Fs* tests are known to lack power to detect selection (Simonsen et al., 1995). For example, these tests failed to detect a departure from neutrality for loci for which there is compelling evidence of selective effects (e.g., the *Duffy* blood group locus; Hamblin and Di-Rienzo, 2000). However, in our *S. pistillata* case the *Fs* value is significantly negative, which is an indicator of positive selection (selective sweep; Hartl, 2000). The fact that the Fu's *Fs* tests did detect a departure from neutrality in the ITS region of *S. pistillata* underscores the strength of the signal of selection; coupled with the various indications of stressors in the north beach (e.g., Bresler et al., 1999, 2003a,b; Ben-Tzvi et al., 2004; Loya, 2004; Abelson et al., 2005), purifying selection should be considered as a reasonable explanation of the data.

Selection on early life stages may be strong enough to overcome even high rates of migration (Hellberg et al., 2002). Therefore, the genetic structure of a population under selective pressure depends, among other factors, on the nature of the disturbance factor/s exerting the selection. Populations at the north beach are geographically close to diverse anthropogenic disturbance sources. Moreover, the vary nature of the habitat at the northern sites, where hard substrate (natural or artificial) is more scarce, may also act as a selective agent. The diversity and distribution of stress sources can also explain why adult populations from the north beach (FC-a, PL-a, GE-a), which are exposed to diverse disturbances, do not form a cluster on the MDS (Fig. 3). It is suggested here that each of the populations from the north beach is exposed to a different combination of stress factors.

4.4. Alternative, non-selective explanations

Hypothetically, stochastic factors, such as temporal demographic stochasticity in reproductive success ('Sweepstakes Effects'; Hedgecock, 1994) and/or sampling bias, could also contribute to the observed patterns of haplotypic diversity. Regarding the sampling bias, the randomization tests suggest that the probability of sampling bias as the reason for the strong genetic pattern of the northern adult populations is very low. The number of individuals analyzed from eight sample groups ranged between 8 and 11 only. However, given the multitude of haplotypes encountered in this study (45 haplotypes among adults), the likelihood of obtaining, for example, the sample from FC-a (five different haplotypes with the following frequencies – 5, 2, 2, 1, 1) by chance alone is extremely low. Thus, selection remains the most plausible explanation for these observations. Selection on early life stages may winnow the genetic diversity of larvae and recruits and be strong enough to overcome even high rates of migration (Hellberg et al., 2002). Therefore, differences in genetic composition and variability between recruits and adults from the same location can be explained by life-stage-specific, differential survival.

5. Implications and conclusions

Studying the genetic structure of benthic populations may have several implications for marine conservation. One of the major ones is that of the estimation of gene flow and connectivity among populations. Another possible

implication is that of pinpointing stress-related local selection by indicating differences in diversity between distinct cohorts (e.g., recruits vs. adults). The newly-applied approach used in this study, which examined the earliest stages of recruits (coral spats), in addition to verifying the Fu's *Fs*-test results, assisted in discriminating between lack of connectivity and local selection.

Specifically, studying the genetic composition of populations of *S. pistillata* from the northern tip of the Gulf of Aqaba demonstrated the importance of including adults as well as newly-settled recruits. Considering the genetic composition of adult populations only could have led to the conclusion that the differences in genetic structures are due to lack of connectivity between the localities (north beach and southern sites). However, the similar composition of recruits in both localities refutes this possibility. Furthermore, comparing adults and recruits in each of the localities revealed a reduction in haplotypic diversity between recruits and adults, with a significantly sharper reduction observed in the north beach. This sharper decline suggests that various post-settlement selective regimes, presumably more intense in the north, may offer a reasonable explanation for the observed patterns of genetic diversity.

The data obtained from coral recruits suggest that sites around the northern part of the Gulf of Aqaba are either connected by larval dispersal or have a common sources of larval supply. Hence, we would like to suggest that if the anomalous selective regimes in the north beach can be mitigated, restoration efforts at the north beach may have a high probability of success. Likewise, it is suggested that the spatial geographic scale of this study be expanded to test the level of connectivity between the reefs at the northern tip of the Gulf and healthier reefs at other sites in the Gulf, leading to a joint conservation program by the countries bordering the Gulf of Aqaba.

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