

Location and disturbance affect population genetic structure in four coral species of the genus *Acropora* on the Great Barrier Reef

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ABSTRACT: The impact of a mass bleaching event on temporal and spatial population genetic structure in 4 scleractinian coral species in the *Acropora aspera* group was studied around the Palm Islands in the central Great Barrier Reef. Species status of sympatric populations of 2 of the 4 species, *A. millepora* and *A. spathulata*, was confirmed by the population genetic data; these species have recently been separated based on morphological and breeding characters. Spatial analyses of population samples from 2004 detected differences in the level of gene flow among locations. No significant genetic differentiation was inferred between conspecific populations at Orpheus and Pelorus Islands, which are both located in the northern part of the island group and separated by ~1000 m. In contrast, all populations at Fantome Island were genetically differentiated, despite this island being located only 11 km south. Sampling of *A. millepora* and *A. pulchra* in the year prior to the 1998 mass bleaching event enabled a temporal comparison across this event. The genetic composition of these populations changed between 1997 and 2004, but patterns of genetic differentiation among locations were similar in 1997 and 2004. Extensive mortality of these species following the 1998 bleaching event did not cause an apparent reduction in genetic diversity and identical multi-locus genotypes were encountered in both temporal samples, suggesting that re-growth of surviving genotypes contributed to the recovery of these populations. Comparisons among the 4 study species revealed lower genetic diversity in *A. papillare*, consistent with its low abundance throughout its distributional range.

KEY WORDS: *Acropora* · Climate change · Palm Islands · Central Great Barrier Reef · Genetic diversity · Genetic connectivity · Coral bleaching

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INTRODUCTION

Coral reefs are threatened worldwide by anthropogenic disturbances, including destructive fishing, sedimentation, terrestrial run-off and the effects of greenhouse gas emissions (Hoegh-Guldberg 1999, Hughes et al. 2003, van Oppen & Gates 2006, Lough 2008, Wilkinson 2008). Although a range of stressors can cause corals to bleach, manifested as the loss of endosymbiotic dinoflagellates and/or their photosynthetic pigments from host tissues (Weis 2008), mass coral bleaching events

have most commonly occurred during extended periods of unusually warm and still weather, when water temperatures rise and light levels are high. Mass coral bleaching events have occurred on a worldwide scale on a few occasions in the last 2 decades, most notably in 1998 (Oliver et al. 2009), but the frequency of such extreme weather events is predicted to increase with climate change (Donner et al. 2005). Therefore, a thorough understanding of the population and community level responses of corals to bleaching is required to effectively manage coral reefs into the future.

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Severe bleaching is likely to reduce effective population sizes of reef corals, either directly through mortality or indirectly through reduced gene flow among populations due to reduced growth, fecundity and disease resistance of surviving corals (Michalek-Wagner & Willis 2001, Bourne et al. 2009, Mydlarz et al. 2010), all of which would have fitness consequences for populations. Moreover, random genetic drift is likely to exacerbate loss of genetic diversity in small populations, which also reduces population fitness. Hence, smaller and more genetically isolated populations face greater risk of extinction than larger and more genetically diverse populations (Pannell & Charlesworth 1999, 2000). Indeed, population size and degree of isolation are among the most important criteria for listing species as endangered under the IUCN system (www.iucnredlist.org/static/categories_criteria_3_1).

Population genetic data can contribute to conservation efforts through enabling the estimation of effective population sizes, levels of genetic diversity, patterns of gene flow and the identification of conservation units. The identification of such units is particularly valuable if species are hard to distinguish using morphological characters. Many scleractinian corals display considerable morphological plasticity, and overlapping morphologies may occur between some species (Miller & Babcock 1997, Flot et al. 2008). Furthermore, introgressive hybridisation (van Oppen et al. 2000, Willis et al. 2006) and the occurrence of cryptic species (Souter 2010) have the potential to obscure the units relevant for conservation purposes. The genus *Acropora*, the most abundant and species-rich Indo-Pacific scleractinian genus (Veron 2000), has been the subject of numerous genetic, morphological and breeding studies (reviewed in Willis et al. 2006). The genus is presently divided into 20 species groups based on morphology and growth form (Wallace 1999). One such group, the *A. aspera* group, is comprised of 7 morphologically distinct species, 5 of which are found in sympatry on the Great Barrier Reef (GBR). Some species within the group lack discrete breeding barriers, and hybridisation and introgression can occur among them (Willis et al. 1997, van Oppen et al. 2002). However, pre-mating isolation in no-sperm-choice crossing experiments has been demonstrated between 2 species in the group, *A. millepora* and *A. spathulata* (Willis et al. 1997). In combination with detailed morphological investigations (Wallace 1999), this has suggested the species status of *A. spathulata*, which was previously considered a 'thick branch morph' of *A. millepora*.

The 1998 coral bleaching event reduced coral cover in *Acropora*-dominated communities by over 50% in the Palm Islands, but bleaching patterns were patchy over small spatial scales (<10 km) (Marshall & Baird 2000). Prior to the mass bleaching, exposed reef flats at both Pelorus and Orpheus were dominated by *A. pul-*

chra (B. L. Willis pers. obs.; Fig. 1), but this species suffered close to 100% mortality on these reefs in 1998 (Page 1999, Galton 2001); only small patches of live tissue at the bases of a few colonies could be located in 1999, despite extensive searching over many days (B. Willis pers. obs.). Analyses of video transects of the reef flat sites at Pelorus and Orpheus Islands recorded in 1998 and 1999 demonstrated a drop in overall percent cover of corals, from means of approximately 70% cover in 1998 to 5–10% cover in March 1999 (Galton 2001). In sympatric populations of *A. millepora* located on the deeper reef slope, 32% of all colonies suffered bleaching-induced, whole-colony mortality at Pelorus Island following the 1998 bleaching event (Baird & Marshall 2002). By November 2001, percent coral cover had risen by more than 30% on the inner reef flat and slope at the Pelorus site, although this recovery was mostly attributed to encrusting and massive corals (Galton 2001). The GBR experienced another major coral bleaching event in 2002, but the reefs around the Palm Islands remained mostly unaffected by this event (Oliver et al. 2009). While the extent of the 1998 bleaching at a community level indicates the intensity of this event, additional information at the population level is required to fully understand the evolutionary responses of corals to such disturbances (Day et al. 2008). Using high resolution molecular markers (microsatellites), we verified current morphological species boundaries for 4 species in the *A. aspera* group, examined spatial patterns in their population genetic structure at sites located within the Palm Island group, and analysed temporal patterns in their population genetic structure across 2 bleaching events.

MATERIALS AND METHODS

In 2004, a total of 564 colonies from 4 morphologically discrete species within the *Acropora aspera* group (*A. millepora*, *A. spathulata*, *A. pulchra*, and *A. papillare*) (Fig. 2) were sampled from 3 inshore reefs at SE Pelorus, NE Orpheus and NE Fantome Islands in the Palm Island group within the central GBR (Fig. 3). At the time of sampling, the corymbose species *A. millepora* and *A. spathulata* were common on outer reef flats and reef slopes at most locations around the Palm Islands. Although the arborescent species *A. pulchra* dominated inner reef flats at the sites prior to the 1998 bleaching event, it became locally extinct or very rare immediately after the event (B. L. Willis pers. obs.). Similarly, populations of the encrusting, digitate *A. papillare* sustained extensive mortality during the 1998 bleaching event and only occurred in low numbers on the reef crest at Pelorus and Orpheus in 2004. For 2 of the species (*A. millepora* and *A. pulchra*), addi-

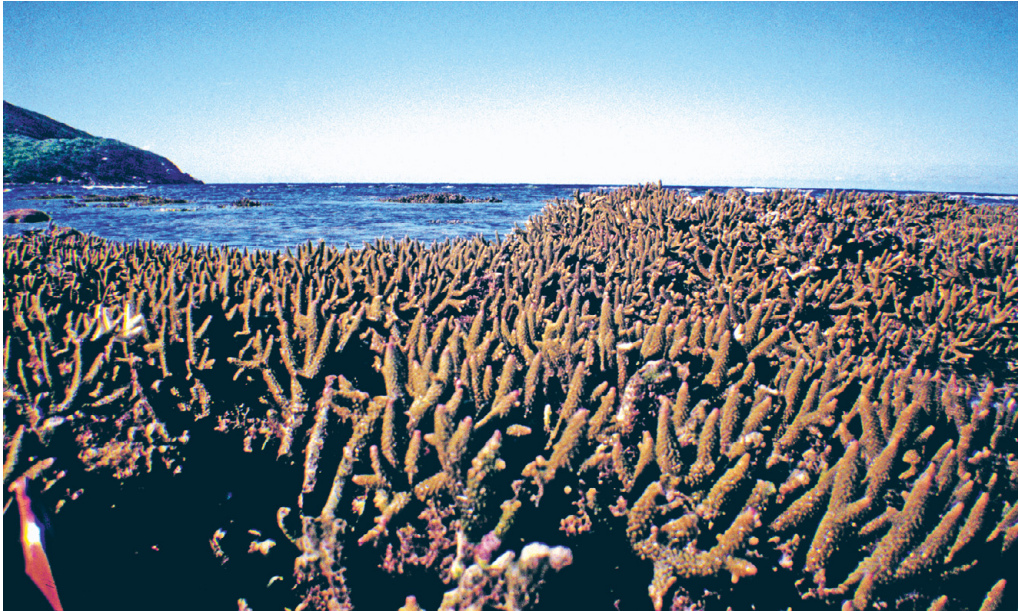


Fig. 1. *Acropora pulchra* at NE Orpheus Island in 1997. Only small patches of remnant tissue were found during extensive surveys in 1999

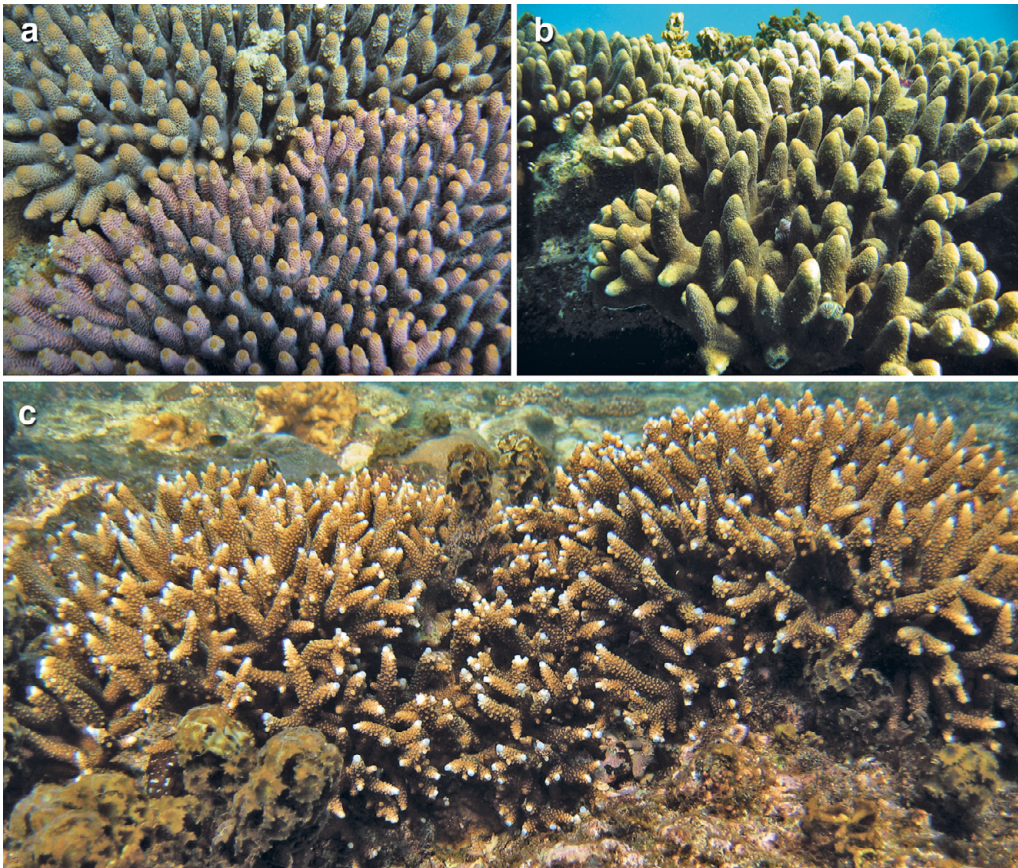


Fig. 2. Species included in the study: (a) *Acropora spathulata* (upper left) and *A. millepora* (lower right) in sympatry, (b) *A. papillare* and (c) *A. pulchra*

tional samples collected from the same locations at Pelorus and Orpheus in 1997 were included in the study (see Table 1 for sample sizes). Samples were stored in 100% EtOH at ambient temperature.

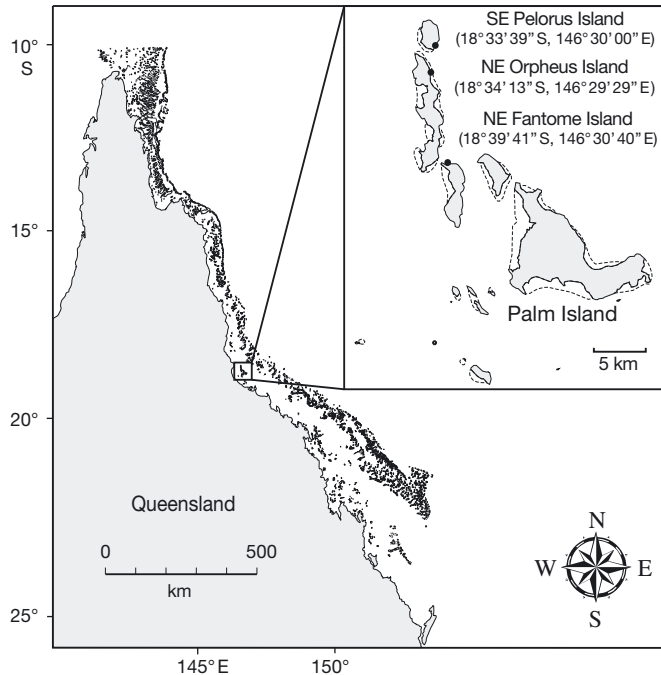


Fig. 3. Sampling locations in the Palm Island group in the central Great Barrier Reef

DNA was extracted following Wilson et al. (2002) and each colony was genotyped at 9 DNA microsatellite loci developed for *Acropora millepora* (van Oppen et al. 2007). PCR was carried out in 3 multiplex reactions as follows: Multiplex 1 comprised primer pairs Amil2_002 (forward HEX-labelled 5'), Amil5_028 (forward TET-labelled 5') and Amil2_006 (forward FAM-labelled 5') at a final concentration of 0.1 μM each primer. Multiplex 2 comprised Amil2_022 (forward TET-labelled 5'), Amil2_023 (forward HEX-labelled 5') and Apam3_166 (forward FAM-labelled 5') at a final concentration of 0.1 μM each primer. Multiplex 3 comprised Amil2_007 (forward HEX-labelled 5'), Amil2_010 (forward FAM-labelled 5') and Amil2_012 (forward TET-labelled 5'). Amil2_007 primers were at 0.1 μM each, the rest at 0.05 μM each. Reactions were carried out in 10 μl reaction comprising 5 μl of 2 \times Master Mix (Qiagen[®] Multiplex PCR), 1 μl of 10 \times Primer Mix, 0.75 μl of template and 3.25 μl of Milli-Q water. Cycling conditions were 95°C for 15 min; 35 cycles of 94°C for 30 s, 50°C for 90 s, 72°C for 60 s; 60°C for 30 min and held at 10°C.

The Excel Microsatellite Toolkit (Park 2001) was used to identify identical multilocus genotypes (MLGs) that were likely to be a result of asexual reproduction within each population. The probability of identical (PID) MLGs being produced by random mating was calculated in GENCLONE (Arnaud-Haond & Belkhir 2007) taking into account the level of inbreeding

Table 1. *Acropora* spp. Sample statistics. Sample labels given as species name, location, collection year (OI: Orpheus Island; FI: Fantome Island; PI: Pelorus Island). Number of colonies sampled (n), number of unique multi-locus genotypes (N_G), expected (H_E) and observed (H_O) heterozygosity, average allelic richness per sample (A) (\pm SD) and Wright's inbreeding coefficient (F_{IS}). **Bold** F_{IS} indicates a significant deficit of heterozygotes ($p < 0.005$). Samples under the solid line are those where $n < 15$ that were excluded from the population genetic statistics. na: not applicable

Sample	n	N_G	H_E	H_O	A	F_{IS}
<i>A. millepora</i> PI 1997	18	18	0.630 (\pm 0.092)	0.493 (\pm 0.041)	7.11 (\pm 3.14)	0.222
<i>A. millepora</i> PI 2004	50	50	0.620 (\pm 0.080)	0.585 (\pm 0.025)	6.43 (\pm 3.14)	0.058
<i>A. millepora</i> OI 1997	17	17	0.672 (\pm 0.096)	0.529 (\pm 0.043)	6.88 (\pm 3.36)	0.218
<i>A. millepora</i> OI 2004	50	50	0.647 (\pm 0.069)	0.580 (\pm 0.023)	6.98 (\pm 3.59)	0.121
<i>A. millepora</i> NF 2004	50	50	0.648 (\pm 0.068)	0.602 (\pm 0.025)	6.47 (\pm 3.47)	0.071
<i>A. spathulata</i> PI 2004	50	49	0.509 (\pm 0.103)	0.185 (\pm 0.025)	5.12 (\pm 2.61)	0.047
<i>A. spathulata</i> OI 2004	50	50	0.516 (\pm 0.105)	0.492 (\pm 0.025)	5.27 (\pm 2.62)	0.046
<i>A. spathulata</i> NF 2004	45	45	0.568 (\pm 0.111)	0.519 (\pm 0.026)	6.08 (\pm 3.28)	0.087
<i>A. pulchra</i> PI 1997	35	30	0.445 (\pm 0.112)	0.367 (\pm 0.031)	4.80 (\pm 3.12)	0.180
<i>A. pulchra</i> PI 2004	49	33	0.438 (\pm 0.100)	0.373 (\pm 0.026)	5.41 (\pm 3.47)	0.149
<i>A. pulchra</i> NF 2004	53	43	0.485 (\pm 0.108)	0.396 (\pm 0.025)	5.73 (\pm 3.20)	0.184
<i>A. papillare</i> PI 2004	50	33	0.289 (\pm 0.107)	0.186 (\pm 0.021)	2.88 (\pm 1.96)	0.359
<i>A. papillare</i> OI 2004	25	21	0.323 (\pm 0.114)	0.154 (\pm 0.027)	2.60 (\pm 1.50)	0.352
<i>A. aspera</i> OI 2004	9	9	0.452 (\pm 0.070)	0.225 (\pm 0.051)	3.38 (\pm 1.06)	na
<i>A. aspera</i> NF 2004	4	4	0.679 (\pm 0.045)	0.313 (\pm 0.082)	3.50 (\pm 0.93)	na
<i>A. aspera</i> PI 1997	6	6	0.162 (\pm 0.082)	0.067 (\pm 0.038)	1.50 (\pm 0.76)	na
<i>A. spathulata</i> PI 1997	11	11	0.563 (\pm 0.120)	0.477 (\pm 0.061)	3.63 (\pm 2.33)	na
<i>A. pulchra</i> OI 2004	6	6	0.424 (\pm 0.104)	0.125 (\pm 0.050)	2.63 (\pm 1.60)	na
<i>A. papillare</i> PI 1997	5	5	0.271 (\pm 0.087)	0.179 (\pm 0.067)	2.00 (\pm 1.00)	na

(Wright's inbreeding coefficient F_{IS}) of the sample. All but one of the identical MLGs within populations that were likely to have been produced asexually ($PID < 0.01$) were removed from the data set. Each population sample was checked for the presence of null alleles and scoring errors using the van Oosterhout algorithm in the software MICROCHECKER (van Oosterhout et al. 2004). This correction adjusts the allele and genotype frequencies in the population based on Hardy-Weinberg Equilibrium expectations. A dataset containing corrected allele frequencies and excluding loci that had null allele frequencies of over 30% was used for all subsequent population genetic analyses.

Despite being considered neutral, microsatellites may be subject to selective forces by linkage to functional genes. Hence, signatures of selection were detected by comparing locus-specific F_{ST} values to 10 000 simulated F_{ST} values according to the F_{ST} -outlier method using the Selection Workbench (Beaumont & Nichols 1996). The Microsatellite Toolkit was used to infer levels of genetic diversity, measured as observed (H_O) and expected (H_E) levels of heterozygosity (Nei 1987) and average numbers of alleles per locus and sample. Fstat 2.9.3.2 (Goudet 1995) was used to calculate allele frequencies (see Table S1 in the supplement at www.int-res.com/articles/suppl/m416p035_supp.pdf), allelic richness and inbreeding coefficients partitioned among individuals within each sample (F_{IS}), sites within total (F_{ST}), and individuals within total (F_{IT}), according to Weir & Cockerham (1984). These tests weigh allele frequencies according to sample size, thus accommodating the fact that sample sizes were not equal. Significance levels of differentiation were corrected using the false discovery rate method (FDR: $\alpha = 0.01$) (Benjamini & Hochberg 1995) for multiple comparisons. Temporal changes in genetic diversity were calculated as a percent change, since the sample size for this comparison was too small ($n = 2$) to allow for statistical analyses to be made. Differences in genetic diversity (H_E , H_O , and allelic richness) among samples were made using a 2-sided permutation statistic in Fstat 2.9.3.2 (Goudet 1995). The program calculates the average of each statistic (OSx) and then employs a permutation scheme whereby the samples are allocated at random to each group, keeping the number of samples per group constant (Sx). The p-value is calculated as the proportion of randomized datasets where $Sx > OSx$.

To validate species delineations and to visualize the uppermost level of population subdivision, the software STRUCTURE v 2.3.3 (Pritchard et al. 2000) was used to infer the most likely number of genetic clusters. The program was run without population information under the admixture model (as individuals may have mixed ancestry) and independent allele frequencies;

10 000 burn-ins and 10 000 MCM repetitions were run and K (the number of genetic clusters) was set to range from 2 to 13. The most likely value of K was inferred using the method described by Evanno et al. (2005).

RESULTS

The final data set comprised 13 population samples with a total of 489 unique MLGs (Table 1). Identical MLGs were observed in *Acropora pulchra* and *A. papillare* only. The probability of the MLGs being produced by random mating within each population was low ($< 1 \times 10^{-5}$), indicating that they are likely to be the result of asexual reproduction, most likely through fragmentation or partial mortality isolating portions of encrusting colonies. Three population samples were too small ($n < 15$ colonies) to warrant inclusion in population level statistical analyses but some non-statistical results from these populations, such as the presence of shared multi-locus genotypes, are considered in the discussion. These samples included *A. spathulata* and *A. papillare* from the 1997 collections at Pelorus and Orpheus and *A. pulchra* from the 2004 collections at Orpheus. One other member of the *Acropora aspera* group, *A. aspera*, does occur on these reefs but was not sampled in large enough numbers to warrant inclusion. Null alleles were observed in a majority of the samples, species and loci. Locus Amil5_007 contained a large proportion of null alleles across all species and populations and was therefore excluded from the analyses. Locus Apam3_166 had null allele frequencies of above 40% in all populations of *A. papillare* and *A. pulchra* and was excluded from the analyses that involved these 2 species (see Table S2 in the supplement; www.int-res.com/articles/suppl/m416p035_supp.pdf). Corrected allele frequencies could not be calculated for *A. papillare* due to the small number of population samples; hence, allele frequencies for this species remain uncorrected and thus may inflate pairwise F_{ST} values that include these 2 samples (between species comparisons and spatial comparison between Orpheus and Pelorus in 2004) (Tables 2 & 3). The overall F_{ST} value for the data set changed from 0.317 (± 0.061) before null allele correction to 0.299 (± 0.054), post null allele correction; however, the corrections did not change the significance of any of the pairwise population differentiations. It is also apparent that null alleles are a more common issue in the 1997 samples, possibly due to the longer storage of the samples prior to DNA extraction.

Locus specific F_{ST} values varied no more than expected from stochastic processes and inter-specific differentiations were an order of magnitude larger than the intra-specific values (Table 2). The 2 excep-

Table 2. *Acropora* spp. Genetic differentiation partitioned among samples within total (F_{ST}) values for each locus partitioned between intra- and inter-specific differentiations. **Bold** indicates potential F_{ST} outliers indicative of non-neutrality. Apam3_166 was removed from analyses including *Acropora pulchra* and *A. papillare*. Amil2_023 was monomorphic in *A. papillare*. na: not applicable

Locus	Amil2_002	Amil2_006	Amil5_028	Amil2_022	Amil2_023	Apam3_166	Amil2_010	Amil2_012	All
Inter-species									
<i>A. millepora</i>	0.120	-0.001	0.012	0.024	0.057	0.007	0.001	0.060	0.019
<i>A. spathulata</i>	0.054	-0.004	0.016	0.001	0.004	0.009	-0.003	-0.004	0.008
<i>A. pulchra</i>	0.028	0.026	0.030	-0.002	0.002	na	0.012	0.029	0.022
<i>A. papillare</i>	-0.01	-0.014	0.025	0.005	na	na	-0.013	0.030	0.005
Intra-species	0.402	0.031	0.149	0.288	0.533	0.168	0.307	0.685	0.337
Overall	0.360	0.030	0.134	0.251	0.489	0.148	0.261	0.647	0.299

Table 3. *Acropora* spp. Pairwise population differentiation between Pelorus Island (PI), Orpheus Island (OI) and Fantome Island (FI). 'Not applicable' (na) indicates that a pairwise comparison was not possible due to small or lacking samples. Samples are from 2004 unless otherwise indicated. **Bold** F_{ST} values are significantly different from zero ($p < 0.0096$, FDR $\alpha = 0.01$). F_{ST} : genetic differentiation partitioned among samples within total; FDR: false discovery rate

	PI/OI	OI/FI	PI/FI
<i>A. millepora</i> 1997	0.010	na	na
<i>A. millepora</i>	0.000	0.011	0.013
<i>A. pulchra</i>	na	na	0.027
<i>A. papillare</i>	0.005	na	na
<i>A. spathulata</i>	0.002	0.008	0.015

tions to this were loci Amil2_023 (across all samples) and Amil2_002 (across spatial comparisons in 2004) in *A. millepora*, which were identified as outliers (p (simulated $F_{ST} < \text{sample } F_{ST}$) > 0.95) and potentially impacted by selection (Table 2). However, the removal of these 2 loci did not change any results and both loci were therefore retained (see Table S3 in the supplement at www.int-res.com/articles/suppl/m416/p035_supp.pdf). All loci were polymorphic and the overall allelic richness, which corrects for variable sample sizes, was 7.99 (± 2.91 SD) alleles per locus (Table 1). Expected levels of heterozygosity varied across samples and species and ranged from 0.289 (± 0.107 SD) in *A. papillare* at Pelorus to 0.672 (± 0.096 SD) in the 1997 sample of *A. millepora* from Orpheus (Table 1). A significant deficit of heterozygotes in at least one sampled population was apparent in all species (Table 1).

Spatial population genetic structure

The hypothesis of panmixia could not be rejected for any pairwise comparison between Pelorus and Orpheus (Table 3). One identical multilocus genotype

in each of *Acropora papillare* and *A. pulchra* was identified from both the Pelorus and Orpheus populations, suggesting dispersal of asexual recruits or fragments in these 2 species across the 14 m deep and approximately 1000 m wide channel that separates the 2 sites. *A. pulchra* was not found in sufficient numbers at Orpheus in 2004 ($n = 6$ samples) to allow tests of pairwise differentiation between Pelorus and Orpheus, but the Pelorus population was significantly differentiated from its conspecific population at Fantome. Similarly, populations of *A. millepora* and *A. spathulata* from Fantome were significantly differentiated from their conspecific populations at the 2 northern sites (Table 3). *A. papillare* was not found in sufficient numbers for population level analyses at the site on Fantome and therefore was not included in this spatial analysis in neither 1997 nor 2004.

Temporal population genetic structure

The populations of *Acropora millepora* at Orpheus, *A. millepora* at Pelorus, and *A. pulchra* at Pelorus were genetically differentiated between 1997 and 2004 (Table 4). Changes in the levels of genetic diversity (H_E) were marginal (-1.0% for *A. millepora* at Orpheus; $+0.5\%$ for *A. millepora* at Pelorus; and $+1.1\%$ for *A. pulchra* at Pelorus). Null alleles were more common in the 1997 than in the 2004 samples of

Table 4. *Acropora* spp. Level of population differentiation between 1997 and 2004. All F_{ST} values are significantly different from zero as indicated by **bold** ($p < 0.0096$, FDR $\alpha = 0.01$). F_{ST} : genetic differentiation among samples within total; FDR: false discovery rate

	F_{ST}	p
<i>A. millepora</i> PI	0.049	0.001
<i>A. millepora</i> OI	0.017	0.009
<i>A. pulchra</i> PI	0.018	0.003

Table 5. *Acropora* spp. Differences in diversity between the studied species across all samples measured as observed (H_O) and expected heterozygosity (H_E), allelic richness (A) and inbreeding coefficient (F_{IS})

	H_O	H_E	A	F_{IS}
<i>A. millepora</i>	0.591	0.651	6.731	0.092
<i>A. spathulata</i>	0.470	0.510	5.491	0.078
<i>A. pulchra</i>	0.421	0.497	5.312	0.153
<i>A. papillare</i>	0.136	0.249	2.739	0.452
p-value	0.005	0.004	0.006	0.013

Table 6. *Acropora* spp. Matrix of pairwise differentiation between sympatric species. All F_{ST} values are significantly different from zero as indicated by **bold** ($p < 0.0096$, FDR $\alpha = 0.01$). F_{ST} : genetic differentiation among samples within total; FDR: false discovery rate

	<i>A. millepora</i>	<i>A. spathulata</i>	<i>A. pulchra</i>
<i>A. spathulata</i>	0.125		
<i>A. pulchra</i>	0.324	0.392	
<i>A. papillare</i>	0.430	0.531	0.272

the same species. One identical MLG was collected both in 1997 and in 2004 in all 3 temporal sample pairs (PID < 0.001 in all 3 instances).

Genetic diversity

Genetic diversity, estimated by allelic richness and observed and expected heterozygosities, varied significantly among species, with *Acropora millepora* being the most and *A. papillare* the least genetically diverse (Table 5). Pairwise comparisons of levels of genetic diversity revealed no significant differences between *A. millepora* and *A. spathulata*, or between *A. millepora* and *A. pulchra*.

Species boundaries

Based on F_{ST} values, the 4 species were all genetically significantly differentiated from one another (Table 6). This includes sympatric populations of *Acropora millepora* and *A. spathulata* from all 3 sites, confirming the current species status of *A. spathulata*. However, the species delineation between *A. papillare* and *A. pulchra* is less clear. At NE Pelorus, these 2 species shared one identical multilocus genotype. Furthermore, despite the observation of significant F_{ST} values, the Bayesian clustering analysis performed with the package STRUCTURE indicates the highest probability for 3 instead of 4 genetic clusters within the 4 species and fails to subdivide *A. papillare* and *A. pulchra* (Fig 4).

DISCUSSION

Spatial population genetic structure

Our study revealed similar spatial patterns of genetic structure in 4 species of coral in the *Acropora aspera* group. The apparent lack of genetic differentiation between populations at Pelorus and Orpheus Islands suggests that migration occurs across the 14 m deep and 1000 m wide channel that separates these sites. Significant genetic differentiation was detected for all pairwise comparisons between the 2 northerly populations at Pelorus and Orpheus and the one at Fantome, despite the fact that this island is located only 11 km to the south. Such small-scale differentiation is not commonly reported for broadcast spawning corals whose gametes and larvae spend at least 3 to 4 d in the water column prior to becoming competent to settle (Nishikawa et al. 2003), during which time they can disperse tens of kilometres (Willis & Oliver 1988, Gilmour et al. 2009). Furthermore, larvae of broadcast spawning corals can survive for 6 to 8 mo in the laboratory (Graham et al. 2008), suggesting that they may disperse over much greater distances. Prevailing hydrodynamic conditions in the shallow near-shore environment have a strong influence on dispersal, retention and settlement (Cowen & Sponaugle 2009), but currently available models are insufficient to predict the hydrodynamics inside the bays that we sampled (Largier 2003, Gawarkiewicz et al. 2007). Nevertheless, our results provide support to Cowen and Sponaugle's recent conclusion that realised dispersal distances are not simply a function of planktonic larval duration, but are determined by complex interactions between numerous physical and biological parameters, including hydrodynamics, selection and habitat availability (Cowen & Sponaugle 2009).

Because of lack of replication in the sampling design, it is not possible to discern whether the small-scale patterns of genetic differentiation found in this study are typical of these species or simply reflect past events at these specific sites. For example, in 2004, the site at Fantome Island was still recovering from the 1998 coral beaching event. It is plausible, therefore, that differentiation between this site and the 2 northerly sites resulted from a difference in the genetic composition of remnant populations or newly recruited colonies at each site following the 1998 bleaching and that there had been insufficient gene flow between them to erase this difference after 6 yr. Conversely, given that panmixia between Pelorus and Orpheus could not be rejected, it may be that divergence associated with similar random reductions within each population and its associated allele frequencies have been erased due to the proximity of these populations to each other.

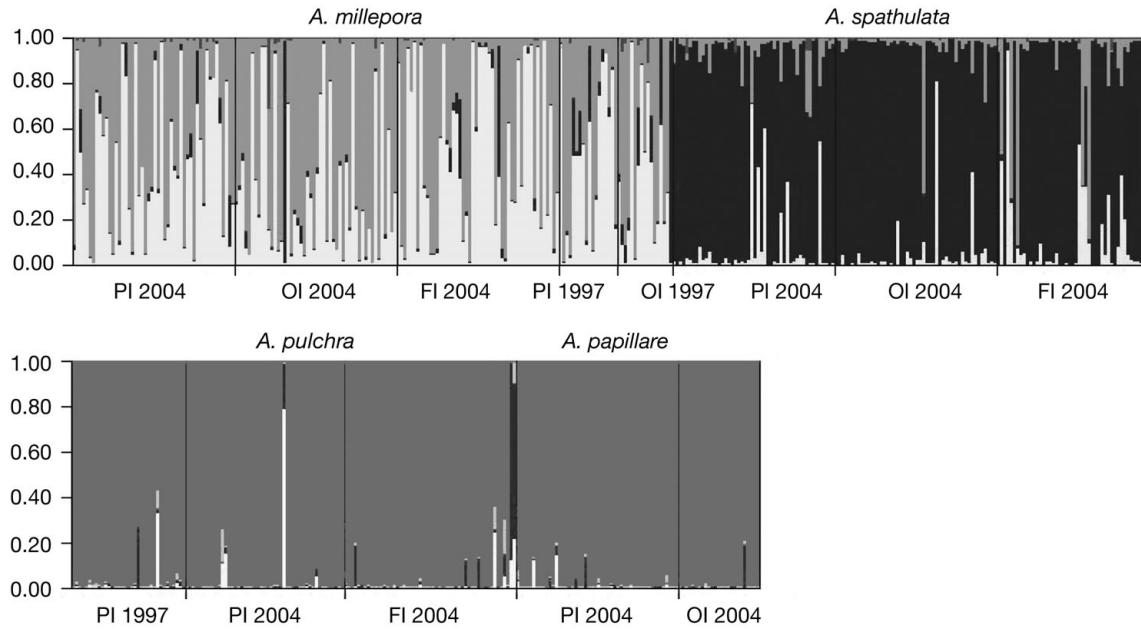


Fig. 4. Bayesian model-based cluster analysis of the *Acropora millepora*, *A. spathulata*, *A. pulchra*, and *A. papillare* populations as implemented in STRUCTURE v2.3.3 with K (most likely number of genetic clusters) being set at 4. Each individual colony is represented by a vertical bar. Sampling locations and year are given along the x-axis, and are separated by black lines (PI: Pelorus Island; OI: Orpheus Island; FI: Fantome Island). The level of genetic differentiation between *A. papillare* and *A. pulchra* is weak, but these 2 species are both clearly distinct from *A. millepora* and *A. spathulata*, which are also genetically distinct from one another

Site-specific selection is a potential explanation for genetic differentiation such as that observed between the southern Fantome population and the 2 northern populations. Although on the windward side of the island, the Fantome site is sheltered within an embayment and by an adjacent island, whereas the sites on Pelorus and Orpheus are exposed to prevailing southeasterly winds. Such habitat variation may affect the population genetic structure and distribution of species. For example, genetic differentiation in the coral *Pocillopora damicornis* between lagoonal and reef slope sites has previously been attributed to habitat-specific selection (Benzie et al. 1995). Also, *Acropora papillare*, which is restricted to shallow, exposed reef environments is only rarely encountered at the most exposed edges of NE Fantome reef, further emphasizing the different nature of this site. Localised selection may lead to divergence between populations for loci under selection and loci linked to these. Neutral loci, however, should remain panmictic (Nosil et al. 2009). The degree of divergence varied among loci in the present study with 2 loci showing F_{ST} values indicative of selection (non-neutrality) in *A. millepora* (Amil2_002, Amil2_023). Removing these 2 loci, however, did not alter the statistical significance of any pairwise comparison, supporting the idea that factors other than selection are likely to have played a part in the levels of differentiation observed here.

Temporal population genetic structure

Population genetic composition changed significantly between 1997 and 2004 at the sampled sites in both *Acropora millepora* and *A. pulchra*. High levels of bleaching-related mortality is likely to have exerted considerable selection against less thermally tolerant colonies, in the 4 species examined which is expected to have led to genetic changes within these populations at affected, non-neutral loci (Schmidt et al. 2008). However, this study is based on neutral genetic markers that showed mostly consistent levels of divergence across loci, making it more likely that the observed genetic divergence is the result of random genetic drift.

The presence of identical MLGs in both years indicates that some colonies survived the extensive 1998 bleaching, which reduced population sizes to almost zero for many *Acropora* species at these sites (Page 1999). Coral colonies that recover from bleaching tend to show decreased fecundity (Michalek-Wagner & Willis 2001, Baker et al. 2008) and re-generation of damaged tissue slows down growth (Mascarelli & Bunkley-Williams 1999). However, despite the devastating effect of the 1998 coral bleaching on these 4 species in this area, none of our temporal comparisons show an obvious reduction in genetic diversity (H_E) between 1997 and 2004. It should be noted that this change cannot be statistically tested due to the low

number of temporal comparisons and should be regarded with some caution. If a proportion of the bleached colonies retained even a small remnant of live tissue, this will contribute to the maintenance of genetic diversity as some of the original genotypes will remain within the surviving population. Recovery through regrowth of remnant tissues has been reported for staghorn colonies of *Acropora* in the southern GBR following bleaching-induced mortality (Diaz-Pulido et al. 2009). The same mechanism for recovery may be available to other *Acropora* species, including the ones studied here. In addition it is likely that larvae from unsampled reefs within dispersal distance contributed to the recovery and further contribute to the change in genetic composition. Such a scenario could explain the maintenance of overall genetic diversity and the re-sampling of identical MLGs, while allowing for an overall change in genetic composition through influx of new genetic material coupled with a change in the relative frequency and reproductive output of remnant colonies. The prevalence of temporally unique alleles is higher in the 2004 samples but this is most likely an effect of the larger sample sizes from this year, which would uncover more rare alleles compared to the 1997 collections. Indeed, a majority of these unique alleles occur at a low frequency. The skewed samples sizes should not impact on the F_{ST} values as statistical calculations were implemented in a manner that corrects for such differences.

Genetic diversity and species boundaries

Estimating genome-wide diversity requires the use of large numbers of both neutral and functional markers spread out across the genome, and may not necessarily be correlated to microsatellite diversity (Vali et al. 2008). Furthermore, the ascertainment bias in the selection of loci, which were developed for one of the study species (*Acropora millepora*), means that these markers are likely to show reduced levels of heterozygosity when applied to different species or even populations different from those for which they were developed (Vali et al. 2008). Another consequence of using markers developed for a particular species is that the prevalence of null alleles may be greater in the non-target species, further adding to a potential reduction in measured diversity. The large number of failed amplifications in locus Apam3_166 may indicate that this is indeed the case; however, other loci show similar frequencies of nulls across all 4 species. It can be postulated, however, that the seemingly low genetic diversity of *A. papillare* is a reflection of it being a rare species (Richards et al. 2008) and hence prone to loss of alleles through random genetic drift as a consequence

of small effective population size. Low genetic diversity coupled with rarity indicates that *A. papillare* may be at greater risk of extinction and less likely to adapt to a changing environment than the other 3 species studied here.

Despite often playing an important evolutionary role in both plant and animal taxa, the role of hybridisation in enhancing genetic and species diversity and ecosystem resilience remains a controversial topic (Allendorf et al. 2001). In corals, however, the link between morphological and genetic species is often unclear and in the genus *Acropora*, hybridisation and introgression have played an important evolutionary role (Willis et al. 2006). Indeed, *A. papillare* is one of at least 3 rare Indo-Pacific coral species likely to have arisen through hybridisation (Richards et al. 2008). In this study, the 4 species were collected and defined using morphological characters (Wallace 1999). *A. papillare* and *A. pulchra* typically occupy distinct habitats (reef crests versus inner reef flats, respectively), although they may co-occur on the outer reef flat and they are cross-fertile in no-sperm-choice fertilisation experiments (van Oppen et al. 2002). In this study, the 2 species shared a multilocus genotype and failed to separate as 2 distinct clusters in the Bayesian clustering analysis, despite showing significant F_{ST} values. Future research should focus on examining the genetic distinctiveness of these 2 species in detail.

In summary, we have shown that genetic structure is present at small spatial scales in species of the coral genus *Acropora* in the Palm Islands, despite the fact that these species are broadcast spawners with planktonic larvae that remain competent for several weeks. Furthermore, the 1998 coral bleaching changed the genetic composition of the 3 species that were sampled prior to 1998, but did not lead to considerable losses of genetic diversity at the loci examined.

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