REPORT

Carolyn Smith-Keune · Madeleine van Oppen

Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef

Abstract Adaptation to localised thermal regimes is facilitated by restricted gene flow, ultimately leading to genetic divergence among populations and differences in their physiological tolerances. Allozyme analysis of six polymorphic loci was used to assess genetic differentiation between nine populations of the reef-building coral *Acropora millepora* over a latitudinal temperature gradient on the inshore regions of the Great Barrier Reef (GBR). Small but significant genetic differentiation indicative of moderate levels of gene flow (pairwise F_{ST} 0.023 to 0.077) was found between southern populations of *A. millepora* in cooler regions of the GBR and the warmer, central or northern GBR populations. Patterns of genetic differentiation at these putatively neutral allozyme loci broadly matched experimental variation in thermal tolerance and were consistent with local thermal regimes (warmest monthly-averages) for the *A. millepora* populations examined. It is therefore hypothesized that natural selection has influenced the thermal tolerance of the *A. millepora* populations examined and greater genetic divergence is likely to be revealed by examination of genetic markers under the direct effects of natural selection.

C. Smith-Keune · M. van Oppen
Australian Institute of Marine Science, PMB 3,
Townsville MC, QLD 4810, Australia

C. Smith-Keune (✉)
Centre for Marine Studies, University of Queensland,
St Lucia, QLD, Australia
E-mail: Carolyn.Smith@jcu.edu.au
Tel.: +61-7-47816216
Fax: +61-7-47814585

Introduction

Identifying genetic differentiation between coral populations within archipelagic systems such as the Great Barrier Reef (GBR) is important for understanding the potential for adaptation on these discrete reefs. The GBR spans 2,100 km and almost 15° of latitude along the continental shelf off the north east coast of Australia. This complex reef system can be divided into three latitudinal regions: northern (∼10°S to 15°S), central (∼15°S to 20°S) and southern (∼20°S to 24°S). Inshore and offshore reefs occur in each region and those located offshore occur on both the mid- and outer-continental shelf. The proximity of inshore reefs to the coast makes them particularly vulnerable to anthropogenic stressors and as many as 28% of inshore reefs on the GBR have been affected by increased sediment runoff since the 1800s (Williams 2001; McCulloch et al. 2003). These unique ecosystems are also threatened by rising sea temperatures associated with global climate change.

Prolonged high temperature exposure results in coral bleaching, a generalised stress response that often leads to coral mortality (see reviews by Glynn 1993; Brown 1997; Hoegh-Guldberg 1999). Varying climate change scenarios have been used to model future trends in sea temperature for the GBR and these predict that rising temperatures will cause serious set backs to the ecology and appearance of reefs within a few decades (Hoegh-Guldberg 1999, 2004; Done et al. 2003). Extended periods of high seawater temperature have already caused mass coral bleaching on the GBR, with the most extensive episodes recorded in 1998 and 2002. Inshore reefs bleached more frequently and more severely than offshore reefs during both these events (Berkelmans and Oliver 1999; Berkelmans et al. 2004). Substantial drops in live coral cover were recorded on a number of inshore reefs because of these past bleaching events (Wilkinson 2000; Berkelmans 2001).

High levels of bleaching and mortality on inshore reefs of the GBR are a consequence of greater temperature

extremes in shallow coastal waters during mass bleaching events (Berkelmans 2001; Berkelmans et al. 2004). However, the inshore reefs of the GBR experience typically warmer conditions than offshore reefs even during summers when no bleaching is observed. A recent principal components analysis of sea surface temperatures (SSTs) revealed intersecting latitudinal and cross-shelf temperature gradients on the GBR (Done et al. 2003). This analysis showed five zones of similar maximum-monthly sea temperature during the 1990s (reproduced in Fig. 1) and reef-building corals on the GBR show signs of acclimatisation and/or adaptation to these thermal regimes. Corals on inshore reefs, as compared to offshore reefs at similar latitudes, tolerate warmer temperatures and longer periods of high temperatures before bleaching (Berkelmans 2002). Higher temperatures and/or exposure times also occur before bleaching is observed on the comparatively warm reefs of the northern GBR compared to cooler southern reefs (Hoegh-Guldberg 1999; Berkelmans 2002).

Localised adaptation of widespread coral species to different thermal regimes is likely to be driven by genetic variability, natural selection and restricted gene flow between geographically isolated populations. The present study investigates patterns of genetic differentiation between eight inshore populations of the reef-building coral *Acropora millepora* over ~1,300 km on the GBR. A mid-shelf reef in the central GBR was included for comparative purposes. The nine reefs studied are distributed among all three latitudinal regions of the GBR and they fall within three of the five GBR temperature zones reported by Done et al. (2003). These same reefs

were used for physiological studies of thermal tolerance and bleaching susceptibility of *A. millepora* (Smith 2005). Patterns of genetic differentiation are therefore interpreted in the context of geographic patterns of thermal tolerance and local thermal regimes.

Materials and methods

Sample collection

Acropora millepora colonies were sampled from one mid-shelf reef and eight inshore reefs spread over ~10° of latitude and three different temperature zones on the GBR (Fig. 1). Reefs are grouped into five general locations: Princess Charlotte Bay (PCB), Magnetic Island (MI), Davies Reef (DR), Whitsunday Islands (WS) and Keppel Isles (KI). PCB and MI are found inshore in the northern and central GBR respectively and both occur in the warmest temperature zone (zone 5). DR on the central GBR mid-shelf, and WS located inshore on the border of the central and southern GBR, lie within an intermediate temperature zone (zone 3). KI is found inshore on the southern GBR in the second coolest temperature zone (zone 2). Up to three reefs were sampled at most locations and samples sizes range from 20 to 49 colonies per reef (see Table 1 for details on reef locations and sample sizes).

Collections were made during November and December 2001. All collections were at depths of 2-3 m from a single area of reef flat of ~1,500 m^2. Single branches (~5 cm long) were removed from *A. millepora*

Fig. 1 Sampling locations and temperature zones on the Great Barrier Reef (GBR). **a** Reef locations, *PCB* Princess Charlotte Bay, *MI* Magnetic Island, *DR* Davies Reef, *WS* Whitsunday Islands, and *KI* Keppel Isles. **b** Location of five temperature zones on the GBR from Done et al. (2003). **c** Zones 1–5, based on warmest monthly-average Sea Surface Temperature (SST) from 1990 to 2000 (excluding 1998). *Box colour* matches colours in panel (**b**). *Boxes* show 25th and 75th percentiles, *lines* mark median temperature and whiskers are 90th and 10th percentiles. **b** and **c** are adapted with permission from Done et al. (2003)
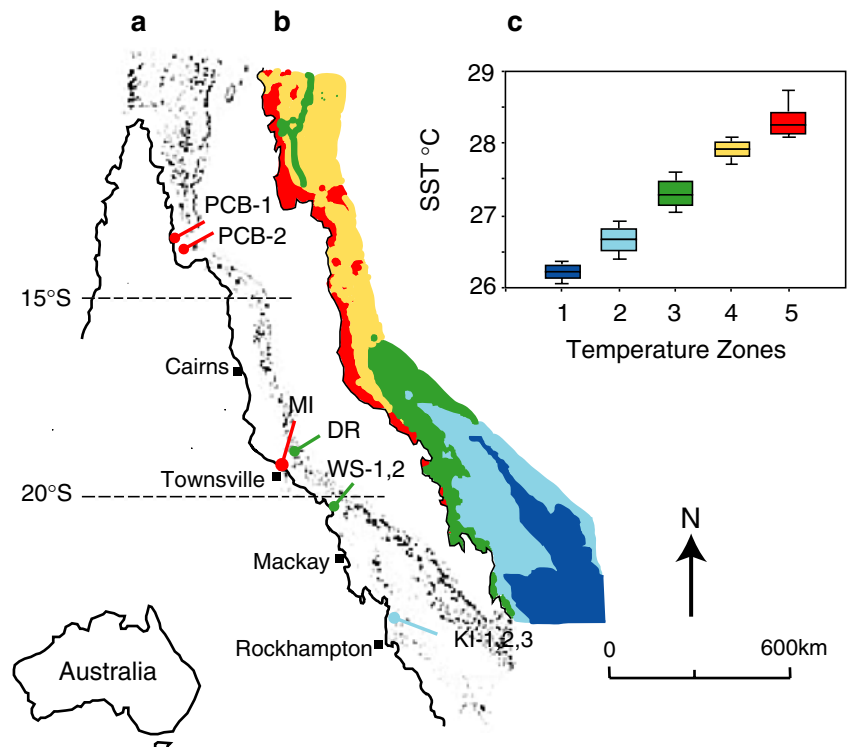
Table 1 Reef locations and summary genetic diversity statistics. Reefs are grouped by sampling location; 'PCB' Princess Charlotte Bay, 'MI' Magnetic Island, 'DR' Davies Reef , 'WS' Whitsunday Passage and 'KI' Keppel Isles

Location	Reef Name	Lat (S)	Long (E)	N	n_a	H_O	H_E
PCB-1	McDonald Reef	13°32′	143°39′	19	3.7 ± 0.7	0.564 ± 0.127	0.555 ± 0.091
PCB-2	Hannah Is.	13°52′	143°42′	20	3.8 ± 0.6	0.583 ± 0.088	0.607 ± 0.060
MI	Nelly Bay	19°9′	146°51′	45	3.8 ± 0.7	0.589 ± 0.080	0.593 ± 0.064
DR	Davies Reef	18°49′	147°38′	49	4.0 ± 0.7	0.614 ± 0.073	0.607 ± 0.034
WS-1	Day Dream Is.	20°15′	148°48′	31	4.2 ± 0.8	0.639 ± 0.095	0.615 ± 0.075
WS-2	Mid Molle Is.	20°14′	148°49′	27	4.0 ± 0.7	0.645 ± 0.057	0.630 ± 0.051
KI-1	Halfway Is.	23°11′	150°58′	20	3.3 ± 0.6	0.642 ± 0.058	0.577 ± 0.035
KI-2	Miall Is.	23°09′	150°54′	19	3.3 ± 0.4	0.663 ± 0.055	0.616 ± 0.039
KI-3	Nth Keppel Is.	23°04′	150°53′	19	4.0 ± 0.7	0.714 ± 0.054	0.627 ± 0.047

Sample size (N), number of alleles (n_a), observed heterozygosity (H_O) and expected heterozygosity (H_E), values given are means (± 1 s.e.)

colonies that were separated by a minimum of 5 m. Sampling over this spatial scale should limit the inclusion of genetically identical colonies (clone mates) produced via fragmentation of coral colonies (see Smith and Hughes 1999). Branches were snap frozen in liquid nitrogen immediately after each dive and were kept frozen at −80°C for less than 6 months prior to preparation for electrophoresis.

Allozyme electrophoresis

Host coral tissue extracts were prepared by grinding coral branches on ice in 10% sucrose (w/v), 0.1% 2-mercaptoethanol. Intact symbiotic algae were removed by centrifugation at 16,000g for 30 s. Extracts were snap frozen in liquid nitrogen and kept frozen at −80°C prior to laboratory analysis. Electrophoresis for all allozyme loci was performed simultaneously for each extract within 48 h of preparation. Electrophoresis was carried out on standard 12% (w/v) horizontal starch gels (e.g., Richardson et al. 1986). Five polymorphic loci previously identified by Ayre and Hughes (2000) were analysed, two malate dehydrogenase loci (*Mdh-1*, *Mdh-2*; EC 1.1.1.37), two leucyl-tyrosine peptidase loci (*Ltp-2* and *Ltp-3*; EC 3.4.11/13) and leucyl-proline peptidase (*Lpp*; EC 3.4.11/13). An additional polymorphic locus, malic enzyme (*Me*; EC 1.1.1.40) was also identified and scored.

Malic enzyme was run overnight at 80 V in Tris–malate pH 7.4 buffer; gel buffer 10 mM Tris, 10 mM maleic acid, 1 mM EDTA, 1 mM MgCl$_2$, 12.5 mM NaOH, electrode buffer 100 mM Tris, 100 mM maleic acid, 10 mM EDTA, 10 mM MgCl$_2$, 125 mM NaOH. Malate dehydrogenase was run overnight at 80 V in Tris–citrate buffer pH 8.0; gel buffer 27 mM Tris, 6.2 mM citric acid, electrode buffer 172 mM Tris, 39 mM citric acid. Tris–malate and Tris–citrate buffers were modified from (Selander et al. 1971). Peptidases, *Ltp* and *Lpp*, were run overnight at 110 V in Tris–EDTA–borate pH 8.4 buffer modified from Boyer et al. (1963); gel buffer 48 mM Tris, 1 mM EDTA, 37 mM boric acid, electrode buffer 150 mM Tris, 3 mM EDTA, 117 mM boric acid. Staining solutions were applied as agar overlays and followed standard recipes for all loci (e.g., Shaw and Prasard 1970; Richardson et al. 1986).

Reference samples were repeated on all gels and additional gels run to confirm the scoring of alleles. Samples that failed to show activity for *Mdh-1*, *Me*, *Ltp-3* or *Lpp* were re-run. New extracts were also prepared for several such samples to check for extract degradation. Identical patterns of isozyme activity were obtained with both the original and freshly prepared extracts. With few exceptions, these re-run and/or re-extracted samples also failed to show activity for the locus in question despite displaying strong activity for all other loci. Individuals that failed to show activity on two independent gels and which showed strong activity for at least four other loci were scored as putative null (non-active) homozygotes. Loci and visible alleles were scored alphabetically in order of decreasing mobility following the conventions of Richardson et al. (1986).

Data analyses

Departures of genotype frequencies from Hardy-Weinberg Equilibrium (HWE) were calculated as F_{IS} (Weir and Cockerham 1984), where positive and negative numbers represent deficits or excesses of heterozygotes, respectively. The significance of departures from HWE were determined by an exact probability test (Guo and Thompson 1992) employing 100,000 Markov chain iterations. Statistical independence of loci was evaluated using a Fisher exact test of linkage disequilibrium for all pairs of loci in each population using 100,000 Markov chain iterations. These tests together with F_{IS} calculations and associated probability tests were carried out using GENEPOP Version 3.1d (Raymond and Rousset 1999).

Due to strong evidence for null alleles for *Mdh-1*, *Me*, *Ltp-3* and *Lpp* (i.e. consistent failure of activity at just one of the six loci for some individuals) additional confirmation and assessment of null allele frequencies at each reef was undertaken using MICRO-CHECKER software version 2.2.1 (van Oosterhout et al. 2004a). This software assesses the likelihood of a null allele based on consistent deficits of heterozygote genotypes for each of the visible alleles at a given locus. For all four loci the presence of a null allele was supported by this analysis. Null allele frequency was calculated using Brookfield estimator 2 (Brookfield 1996) which takes

into account both the frequency of null homozygotes and the deficits of heterozygotes. Observed allele and genotype frequencies were then adjusted for each reef according to null allele frequency and assuming Hardy-Weinberg proportions as described in van Oosterhout et al. (2004b). A table of the adjusted and unadjusted allele frequencies can be found in Appendix 1.

Analysis of molecular variance (AMOVA) was used to partition variance in allele frequencies between individuals within reefs, individuals within locations and individuals between locations (Peakall and Smouse 2001). The significance of differences in allele frequencies between all pairs of reefs was determined by exact tests for population differentiation (Raymond and Rousset 1995). Exact tests were performed across all loci and for each locus separately as implemented in TFPGA version 1.3 (Miller 1997). Patterns of genetic differentiation between all pairs of reefs was further examined by calculation of Weir and Cockerham's (1984) unbiased estimate of F_{ST} which takes into account sample size at each reef. Bootstrapping over loci for 5,000 replicates was performed to determine 95% confidence intervals for F_{ST} using GDA version 1.1 (Lewis and Zaykin 2002). Significance of F_{ST} was demonstrated when lower 95% confidence intervals did not overlap with zero. Plots of pairwise F_{ST} versus geographic distance were examined for an isolation-by-distance effect and Mantel's tests were conducted using GenePop Version 3.1d (Raymond and Rousset 1999). All tests for population genetic differentiation were based on observed allele frequencies for the two loci for which null alleles were not observed (*Mdh-2* and *Ltp-2*) and adjusted allele frequencies for those affected by a null allele (*Mdh-1*, *Me*, *Ltp-3* and *Lpp*). Sequential Bonferroni correction for $\alpha = 0.05$ (Rice 1989) was used to assess the significance of all multiple tests.

Results

Genetic variation within reefs

There was a high level of genetic diversity within the reefs examined. The average heterozygosity observed within each reef was high, ranging from 0.564 ± 0.127 (mean $\pm$ SE) to 0.714 ± 0.054 after adjustment for null alleles (Table 1). Significant deficits of heterozygotes (c.f. HWE expectations) were only observed for the four loci for which there was evidence of a null allele, i.e. *Mdh-1*, *Me*, *Ltp-3* and *Lpp* (Table 2). There was only one significant excess of heterozygotes and this occurred at North Keppel Island (KI-3) for *Ltp-2* (Table 2). With the exception of Davies Reef (DR) which had significant heterozygote deficits at four of the six loci screened, all other reefs had single locus genotype frequencies that were within HWE expectations for at least four loci. Tests for linkage disequilibrium suggested that all loci were independent with no significant instances of linkage disequilibrium detected following sequential Bonferroni correction of 149 statistically valid tests.

Table 2 Single locus heterozygote deficiencies/excesses (F_{IS}) for six allozyme loci calculated from unadjusted genotype frequencies in *A. millepora* from nine reefs on the GBR

Location	MDH1	MDH2	ME	LTP2	LTP3	LPP
PCB-1	−0.059	−0.056	**0.592**	0.596	0.664	0.185
PCB−2	0.307	−0.056	**0.462**	0.363	0.391	0.314
MI	0.059	0.010	**0.255**	−0.048	**0.462**	0.385
DR	**0.484**	0.079	**0.074**	0.236	**0.536**	**0.480**
WS-1	0.102	−0.106	0.186	−0.478	0.136	**0.532**
WS-2	**0.786**	0.133	0.355	−0.064	0.131	0.335
KI-1	0.780	−0.088	0.205	0.123	**0.707**	−0.516
KI-2	0.778	0.130	0.302	−0.241	0.357	**0.077**
KI-3	**1.000**	−0.045	0.217	**−0.895**	0.327	0.660

Reefs details are given in Table 1. F_{IS} values representing significant departures from Hardy-Weinberg equilibria are in bold

The inclusion of colonies generated asexually via fragmentation was largely avoided by sampling *A. millepora* colonies on the reef flat at >5 m spatial scales as fragments of *A. millepora* on reef flat habitats rarely migrate further than 4 m and typically have low survival and reattachment rates (Smith and Hughes 1999). There were only three instances of multi-locus genotypes being repeated within reefs. The repeated genotypes only occurred twice each in all instances. Colonies with the same genotype were assumed the product of asexual reproduction in Nelly Bay (MI) as in these two instances the repeated genotypes had been sampled immediately adjacent to each other on the reef flat. The second individual of each repeated genotype was excluded from the data set. One multi-locus genotype was repeated on the reef flat of Halfway Island (KI-1) but the two colonies of the same multi-locus genotype were well separated on the reef flat in this case. These colonies were therefore considered the product of sexual reproduction as their identical genotypes may reflect the low number of alleles observed for each locus and the limited portion of the genome examined.
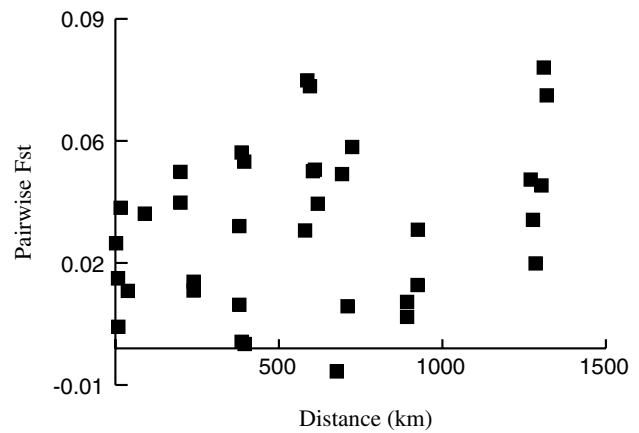
Geographic patterns of genetic differentiation

Allozyme electrophoresis of six loci indicated that a small but significant amount of genetic differentiation in *A. millepora* is due to variation between reefs and between locations (PCB, DR, MI, WS and KI) on the GBR. Analysis of molecular variance indicated that 97% of the variance in allele frequencies was due to variance within reefs ($P = 0.002$). A further 1% of the variance ($P = 0.002$) was attributable to variance between reefs within a location and the remaining 2% was due to variance between broad scale geographic locations ($P = 0.001$). There were no fixed allele differences between any of the reefs examined and only a single private allele (*Ltp-3*[e]) occurred at low frequency at Magnetic Island. There was, however, evidence for significant allele frequency differences between several of the reefs examined (Table 3). The geographic distribution of this genetic variation did not fit an isolation-by-distance model as differentiation between reefs (F_{ST}) did

not increase with increasing geographic separation (Mantel's test $P > 0.05$, Fig. 2a). The allele frequencies over all six loci were significantly different for Davies Reef on the GBR mid-shelf compared to that on all eight inshore reefs including Magnetic Island ~90 km away (Table 3). Two to three different loci contributed to these differences (Table 3). Genetic differentiation of Davies Reef from the inshore reefs is also evident from small but significant pairwise F_{ST} estimates (ranging from 0.032 to 0.073, Table 3).

There was also evidence for genetic differentiation of inshore reefs along the length of the GBR. The three inshore reefs in the Keppel Isles (southern GBR) were not significantly differentiated from one another but each had a significantly different allele frequency from the two northern-most reefs within Princess Charlotte Bay (Table 3). The difference in allele frequency was significant over all loci and for between one to three individual loci (Table 3). The Keppel Isles reefs also had significantly different allele frequency from Magnetic Island in the central GBR involving between one and three different loci (Table 3). Pairwise F_{ST} estimates support the differentiation between reefs in the Keppel Isles and the inshore reefs of the northern and central GBR with small but significant F_{ST} values (ranging from 0.023 to 0.077, Table 3). UPGMA clustering supports the relative isolation of the reefs in the Keppel Isles (data not shown). In contrast, the six allozyme loci examined gave no indication of genetic differentiation between the two inshore reefs in Princess Charlotte Bay and between these reefs and the reef in Nelly Bay, Magnetic Island (Table 3).

There was no consistent pattern of genetic similarity between fringing reefs of the Whitsunday Islands, located at the southern end of the central GBR, and inshore reefs located to the north and south. Two reefs within the Whitsunday Island group, Daydream Island (WS-1) and Mid Molle Island (WS-2) were sampled within ~2 km of one another. The *A. millepora* populations on these two reefs had significantly different allele frequencies from one another, particularly at the *Mdh-1* locus (Table 3).

Fig. 2 Geographic patterns of genetic differentiation among *Acropora millepora* populations at nine locations on the Great Barrier Reef including one mid-shelf and eight inshore reefs. F_{ST} over six allozyme loci versus geographic distance between 45 pairs of reefs

The *A. millepora* population at WS-1 appears less differentiated from reefs to the north (F_{ST} from 0.009 to 0.017) compared to those reefs to the south (F_{ST} from 0.033 to 0.053). Significant allele frequency differences between WS-1 and the southern reefs in the Keppel Isles were detected for up to three loci (Table 3). None of the six loci showed significant allele frequency differences between WS-1 and the inshore reefs to the north. In contrast, there was a tendency for *A. millepora* at WS-2 to be more differentiated from reefs to the north (non-significant pairwise F_{ST} from 0.013 to 0.032) compared to inshore reefs to the south (non-significant pairwise F_{ST} from 0.001 to 0.012). There were significant allele frequency differences for at least one locus (*Mdh-1*) between WS-2 and the three inshore reefs to the north. Fewer instances of significant allele frequency differences could be detected in comparisons between WS-2 and reefs of the Keppel Isles to the south (Table 3). An apparent inconsistency between the lack of significance of pairwise F_{ST} and the significance of exact tests for allele frequency differences may be the result of intra-locus sampling

Table 3 Pairwise measures of genetic differentiation. Below diagonal is average pairwise F_{ST} calculated over six loci

	Northern		Central		Central/southern		Southern		
	PCB-1	PCB-2	MI	DR	WS-1	WS-2	KI-1	KI-2	KI-3
PCB-1		NS	NS [4]	*** [1,2,4]	NS	*** [1]	*** [1,3,4]	*** [1,3]	*** [1]
PCB-2	0.016		NS	*** [2,3,4]	NS	NS [1]	** [1]	*** [1]	*** [1,2,4]
MI	0.012	-0.006		*** [2,3,4]	**	*** [1]	*** [1,2]	*** [1,4]	*** [1,4]
DR	**0.055**	**0.048**	**0.037**		*** [2,3]	*** [1,2,4]	*** [2,4]	*** [1,4]	*** [4]
WS-1	**0.017**	**0.009**	**0.016**	**0.04**		*** [1]	*** [1,3,4]	*** [1,4]	*** [1]
WS-2	0.032	0.013	0.018	**0.048**	0.029		NS	NS	NS [4]
KI-1	**0.069**	**0.023**	**0.04**	**0.072**	**0.051**	0.001		NS	*** [4]
KI-2	**0.077**	**0.035**	**0.049**	**0.073**	**0.054**	0.002	0.006		*** [4]
KI-3	**0.045**	**0.046**	**0.049**	**0.032**	**0.033**	0.012	0.038	0.019	

Values with 95% confidence intervals > 0 after bootstrapping over loci for 5,000 replicates are in bold. Above diagonal are summarised results of exact tests for differences in allele frequencies where 'ns' not significant
Latitudinal regions of the GBR are indicated in the top row. Reef details are given in Table 1
**$p < 0.01$, ***$p < 0.001$ over all loci. Loci with significantly different allele frequencies are indicated by $1 = Mdh-1$, $2 = Me$, $3 = Ltp-3$ and $4 = Lpp$

error associated with small sample sizes and the low values of F_{ST} obtained in this case (Waples 1998).

The frequency of null alleles made an important contribution to the patterns of genetic differentiation observed between *A. millepora* populations on the GBR. Differences in the frequency of null alleles at a number of loci may be largely responsible for the differentiation observed between Davies Reef on the GBR mid-shelf and the inshore reefs studied. The apparent absence of null alleles for *Me* at Davies Reef (Fig. 3b) and the relatively high frequency of null alleles for *Lpp* at the same reef (Fig. 3c) are likely contributors to the pattern of differentiation described. The latitudinal pattern in genetic differentiation may also be attributed to differences in the frequency of null alleles at a number of loci. A relatively high frequency of *Mdh-1* null alleles indicated for the three Keppel Isles reefs (Fig. 3a) combined with a low frequency of *Me* null alleles for the same reefs (Fig. 3b) contributes to the pattern of relative genetic isolation of these inshore reefs on the southern GBR.

Discussion

Population genetic structure is the product of opposing forces that lead to either genetic homogenization (i.e. gene flow) or divergence (i.e. genetic drift, mutation and natural selection). Neutral genetic markers, like the allozyme markers examined in the present study, are useful indicators for the extent of genetic divergence resulting from gene flow and genetic drift (Slatkin 1987). A latitudinal component of genetic differentiation was observed among the eight inshore populations of *A. millepora* examined. There was a consistent pattern of small but significant differentiation of the three inshore reefs at the southern end of the GBR (KI-1, 2 & 3) from the three inshore reefs in the northern and central GBR (PCB-1, 2 & MI). The extent of this differentiation ($F_{ST} = 0.048 \pm 0.005$; mean $\pm$ SE) was similar to that observed between Davies Reef on the mid-shelf and the inshore reefs ($F_{ST} = 0.051 \pm 0.005$). Putative null alleles contribute strongly to the patterns of genetic divergence described, however, emerging molecular data support the relative separation of warm-northern versus cool-southern populations of *A. millepora*. A recent examination of four intron sequences, using single strand conformational polymorphism (SSCP) analysis and a sub-set of the present samples, supports the genetic differentiation of PCB and MI from WS and KI (Ridgeway and Smith, unpublished data).

Gene flow is typically moderate to high, and the extent of population differentiation correspondingly low, for marine organisms with pelagic larval stages (Waples 1998; Luttikhuizen et al. 2003). In the present study, moderate levels of gene flow ($F_{ST} = 0.023$ and maxi-

Fig. 3 Spatial distributions of alleles for **a** *Mdh*-1; **b** *Me* and **c** *Lpp* enzyme encoding loci among nine *Acropora millepora* populations on the Great Barrier Reef (GBR). *Pie charts* indicate allele frequency for reefs as in Table 1 and latitudinal regions of the GBR are indicated on the far *left*. A *vertical bar* separates the inshore and mid-shelf reef sampled in the central region
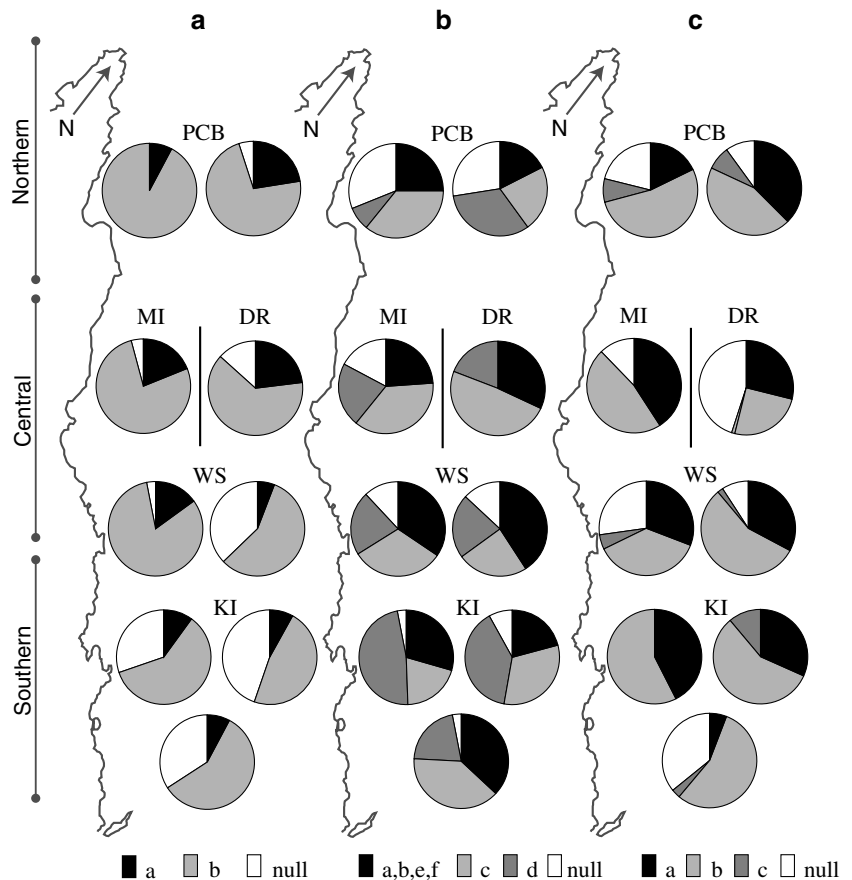
mum F_{ST} = 0.077), are inferred between the northern *A. millepora* populations (Magnetic Island and Princess Charlotte Bay) and the cool southern GBR populations (Keppel Isles). In contrast, inferred levels of gene flow between the two northern reefs within Princess Charlotte Bay, and between both of these reefs and the centrally located Magnetic Island are very high (F_{ST} not significantly different from zero), as is gene flow among the most southern reefs at the Keppel Isles. Levels of gene flow in excess of one migrant per generation are sufficient to prevent extensive population divergence (i.e. fixation of alleles) at neutral loci via genetic drift (reviewed in Slatkin 1987). The low level of population differentiation observed between the northern/central and southern regions of the GBR in the present study are consistent with weak genetic drift in the face of moderate levels of gene flow. The lack of complete panmixis does, however, suggest that past and present day barriers to gene flow have influenced genetic structure of the coral populations examined.

During past low sea level stands the GBR fauna from the northern/central and southern GBR retracted to two separated plateaus, namely the Queensland and Marion Plateaus, respectively (Davies 1994; Doherty et al. 1995; Worheide et al. 2002). Past separation and relatively slow expansion of southern GBR fauna against prevailing southward currents is a likely contributing factor to present day genetic differentiation of northern versus southern populations of several reef fish species (Doherty et al. 1995), and between northern/central and southern GBR populations of the calcareous sponge *Leucetta chagosensis* (Worheide et al. 2002). In the present day, a persistent frontal convergence zone to the south of the Whitsunday Islands (Burrage et al. 1996) may also limit gene flow between northern and central versus southern reefs on the GBR. A study of five reef building corals on the GBR mid-shelf showed greater differentiation between a central and southern reef (mean F_{ST} = 0.114) than between a northern and central reef (mean F_{ST} = 0.035) (Ayre and Hughes 2004). Genetic differentiation of southern populations of two species of clams, *Tridacna maxima* and *Tridacna derasa*, on the outer GBR shelf has also been reported (Benzie 1994). Although levels of differentiation between northern/central and southern GBR reefs are generally low, several coral reef invertebrates across the GBR shelf show evidence of genetic differentiation between these regions. If this differentiation is due to present day restrictions to gene flow then the southern inshore reefs of the GBR may be less resilient to rising sea temperatures than the more highly connected northern and central GBR reefs. This is especially important as global climate change may see the warm temperatures currently experienced in the northern GBR extending to the southern GBR by ~2050 to 2100 (Done et al. 2003).

The geographic patterns of genetic differentiation reported for *A. millepora* on the GBR are consistent with a recent physiological study. Smith (2005) examined the bleaching susceptibility and thermal tolerance of *A. millepora* from four of the same locations (PCB, MI, WS and KI) used in the present genetic study. A thermal stress experiment conducted after several weeks of laboratory acclimation under identical temperature conditions identified the relative thermal tolerance of *A. millepora* from these locations as Princess Charlotte Bay = Magnetic Island > the Whitsunday Islands > the Keppel Isles (Smith 2005). This ranking of thermal tolerance is in keeping with local thermal regimes (as shown in Fig. 1) and the pattern of genetic differentiation reported here for the host coral populations. In particular, the high and equal thermal tolerance ranking of *A. millepora* from Princess Charlotte Bay and Magnetic Island is consistent with the lack of genetic differentiation between these populations and their co-occurrence in the warmest temperature zone. The genetic differentiation of the Keppel Isles from both of these populations is also consistent with its low thermal tolerance (Smith 2005) and its location within the second coolest temperature zone on the GBR.

The present allozyme study has examined patterns of genetic differentiation for just one component of the coral–algal holobiont, namely the coral host. Specific taxonomic differences in symbiotic algal strain are also observed for *A. millepora* in the locations examined (Smith 2005). In particular, the Magnetic Island population of *A. millepora* hosts *Symbiodinium* Clade D algal symbionts (as determined by nuclear ribosomal DNA ITS1 type). Possession of this particular clade of symbionts may contribute to relatively high thermal tolerance in *A. millepora* (Berkelmans and van Oppen 2006) and other scleractinian corals (Glynn et al. 2001; Baker et al. 2004; Fabricius et al. 2004; Rowan 2004). However, *Symbiodinium* D is not the predominant algal strain in thermally tolerant populations of *A. millepora* in Princess Charlotte Bay. The warm-adapted population of *A. millepora* in Princess Charlotte Bay in fact shares *Symbiodinium* C2 symbionts with the less thermally tolerant Whitsunday Islands population (Smith 2005). Dissimilarity between the geographic distribution of algal symbiont strains and thermal tolerance of *A. millepora* populations suggests factors other than algal genotype may determine thermal tolerance in most of the populations examined.

Strong natural selection will overcome the homogenizing effects of gene flow (Wright 1931; Slatkin 1987) and population structure of quantitative traits (i.e. Q_{st}) often exceeds the extent of genetic differentiation observed with allozyme and other neutral markers (Merila and Crnokrak 2001). Natural selection in the face of moderate to high gene flow is known to have shaped quantitative traits such as salinity tolerance in toads (Gomez-Mestre and Tejedo 2004) and morphological variation in mollusks (Wilding et al. 2001; Luttikhuizen et al. 2003). Thermal tolerance is a quantitative trait for which an underlying genetic component has been identified in other organisms (e.g. Ottaviano et al. 1991; Perry et al. 2001; Hoffmann et al. 2002). In the case of *Drosophila melanogaster* populations along the east

coast of Australia, divergent selection from environmental temperature extremes has been implicated as the driving force behind a latitudinal cline in thermal tolerance (Hoffmann et al. 2002) and a corresponding cline in the frequency of alleles encoding classic heat shock response proteins (Bettencourt et al. 2002; Frydenber et al. 2003). Given the concordant patterns of thermal tolerance, slight genetic differentiation at neutral allozyme markers and divergent summer temperature regimes identified in the present study it is therefore possible that natural selection plays a role in shaping the thermal tolerance of *A. millepora* on the GBR.

The present study of *A. millepora* raises the interesting possibility that thermal tolerance in this reef building coral species could have an underlying genetic component on which natural selection may operate. Determination of the extent to which natural selection shapes thermal tolerance in this species will require additional studies on a greater number of populations throughout the range of thermal regimes on the GBR. Such studies should examine phenotypic variance in thermal tolerance between populations, correlations between this quantitative trait and both upper and lower temperature extremes, as well as patterns of genetic differentiation at candidate thermo-resistance genes or associated quantitative trait loci. Controlled experiments estimating the heritability of thermal tolerance in a range of populations would also be of substantial value given recent debate about the impact of global warming and rising sea temperatures (including extreme temperature events) on coral reefs. In light of the present data it is arguably time to move towards a more quantitative genetics approach aimed at providing a deeper understanding of the heritability and molecular-genetic basis of variance in thermal tolerance within coral species like *A. millepora*.

Appendix

Table 4.

Table 4 Observed and adjusted allele frequencies for six polymorphic allozyme loci for Acropora millepora collected on nine reefs of the GBR

	PCB-1		PCB-2		MI		DR		WS-1		WS-2		KI-1		KI-2		KI-3	
MDH1																		
a	0.08	**0.08**	0.23	**0.23**	0.20	**0.19**	0.27	**0.23**	0.15	**0.15**	0.10	**0.06**	0.13	**0.10**	0.15	**0.08**	0.11	**0.08**
b	0.92	**0.92**	0.78	**0.73**	0.80	**0.77**	0.74	**0.63**	0.85	**0.82**	0.90	**0.57**	0.87	**0.60**	0.85	**0.47**	0.89	**0.58**
Null		**0.00**		**0.05**		**0.04**		**0.13**		**0.03**		**0.37**		**0.30**		**0.44**		**0.34**
MDH2																		
a	0.14	**0.14**	0.13	**0.13**	0.16	**0.16**	0.28	**0.28**	0.27	**0.27**	0.19	**0.19**	0.10	**0.10**	0.19	**0.19**	0.21	**0.21**
b	0.28	**0.28**	0.43	**0.43**	0.32	**0.32**	0.32	**0.32**	0.37	**0.37**	0.35	**0.35**	0.48	**0.48**	0.50	**0.50**	0.45	**0.45**
c	0.58	**0.58**	0.45	**0.45**	0.52	**0.52**	0.41	**0.41**	0.36	**0.36**	0.46	**0.46**	0.43	**0.43**	0.31	**0.31**	0.34	**0.34**
ME																		
a	0.11	**0.08**	0.11	**0.08**	0.14	**0.12**	0.09	**0.09**	0.12	**0.10**	0.22	**0.19**	0.18	**0.18**	0.05	**0.05**	0.21	**0.21**
b	0.00	**0.00**	0.03	**0.03**	0.01	**0.01**	0.02	**0.02**	0.07	**0.07**	0.06	**0.06**	0.05	**0.05**	0.00	**0.00**	0.00	**0.00**
c	0.53	**0.36**	0.32	**0.23**	0.44	**0.37**	0.49	**0.49**	0.37	**0.32**	0.28	**0.24**	0.20	**0.20**	0.34	**0.32**	0.42	**0.39**
d	0.14	**0.08**	0.47	**0.33**	0.27	**0.22**	0.19	**0.19**	0.25	**0.22**	0.26	**0.22**	0.50	**0.48**	0.45	**0.39**	0.21	**0.21**
e	0.19	**0.14**	0.08	**0.08**	0.14	**0.11**	0.19	**0.19**	0.13	**0.12**	0.13	**0.11**	0.08	**0.08**	0.16	**0.16**	0.13	**0.13**
F	0.03	**0.03**	0.00	**0.00**	0.00	**0.00**	0.01	**0.01**	0.07	**0.07**	0.06	**0.06**	0.00	**0.00**	0.00	**0.00**	0.03	**0.03**
Null		**0.31**		**0.28**		**0.17**		**0.00**		**0.12**		**0.13**		**0.03**		**0.08**		**0.03**
LTP2																		
a	0.53	**0.53**	0.35	**0.35**	0.36	**0.36**	0.39	**0.39**	0.47	**0.47**	0.46	**0.46**	0.53	**0.53**	0.53	**0.53**	0.53	**0.53**
b	0.47	**0.47**	0.65	**0.65**	0.64	**0.64**	0.61	**0.61**	0.53	**0.53**	0.54	**0.54**	0.48	**0.48**	0.47	**0.47**	0.47	**0.47**
c	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**
LTP3																		
a	0.47	**0.31**	0.40	**0.28**	0.45	**0.32**	0.49	**0.21**	0.24	**0.21**	0.39	**0.26**	0.33	**0.18**	0.33	**0.24**	0.23	**0.14**
b	0.36	**0.25**	0.40	**0.28**	0.27	**0.19**	0.29	**0.15**	0.48	**0.42**	0.44	**0.28**	0.58	**0.33**	0.31	**0.21**	0.33	**0.19**
c	0.11	**0.08**	0.11	**0.10**	0.20	**0.16**	0.18	**0.09**	0.19	**0.16**	0.15	**0.11**	0.08	**0.05**	0.36	**0.26**	0.23	**0.17**
d	0.06	**0.06**	0.11	**0.10**	0.06	**0.04**	0.01	**0.01**	0.06	**0.05**	0.02	**0.02**	0.00	**0.00**	0.00	**0.00**	0.13	**0.11**
e	0.00	**0.00**	0.00	**0.00**	0.02	**0.02**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**
f	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.03	**0.01**	0.04	**0.03**	0.00	**0.00**	0.00	**0.00**	0.00	**0.00**	0.07	**0.06**
Null		**0.31**		**0.25**		**0.27**		**0.52**		**0.13**		**0.33**		**0.45**		**0.29**		**0.33**
LPP																		
a	0.22	**0.18**	0.43	**0.38**	0.47	**0.41**	0.54	**0.29**	0.42	**0.31**	0.37	**0.33**	0.43	**0.43**	0.32	**0.32**	0.06	**0.06**
b	0.69	**0.53**	0.50	**0.45**	0.53	**0.47**	0.44	**0.25**	0.52	**0.37**	0.61	**0.56**	0.58	**0.58**	0.58	**0.58**	0.91	**0.56**
c	0.08	**0.08**	0.08	**0.08**	0.00	**0.00**	0.03	**0.01**	0.07	**0.05**	0.02	**0.02**	0.00	**0.00**	0.11	**0.11**	0.03	**0.03**
Null		**0.21**		**0.10**		**0.12**		**0.45**		**0.27**		**0.09**		**0.00**		**0.00**		**0.36**

Adjusted allele frequencies are in bold. Adjustments have been made only where there was evidence for null homozygotes and a consistent deficit of heterozygotes across all alleles

References

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54:1590–1605

Ayre DJ, Hughes TP (2004) Climate change, genotypic diversity and gene flow in reef-building corals. Ecol Lett 7:273–278

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. Nature 430:741

Benzie JAH (1994) Patterns of gene flow in the Great Barrier Reef and Coral Sea. In: Beaumont AR (eds) Genetics and evolution of aquatic organisms. Chapman & Hall, London, pp 67–79

Berkelmans R (2001) Bleaching, upper thermal limits and temperature adaptation in reef corals. PhD Thesis, James Cook University of North Queensland, p 179

Berkelmans R (2002) Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. Mar Ecol Prog Ser 229:73–82

Berkelmans R, Oliver JK (1999) Large-scale bleaching of corals on the Great Barrier Reef. Coral Reefs 18:55–60

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. Proc R Soc Lond B Biol Sci (in press)

Berkelmans R, De'ath G, Kininmonth S, Skirving WJ (2004) A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation, patterns and predictions. Coral Reefs 22:74–83

Bettencourt BR, InYoung K, Hoffmann AA, Feder ME (2002) Response to natural and laboratory selection at the *Drosophila hsp70* genes. Evolution 56:1796–1801

Boyer SH, Fainer DC, Watson-Williams EJ (1963) Lactate dehydrogenase variant from human blood: evidence for molecular sub-units. Science 141:642–643

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol 5:453–455

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16:S129–S138

Burrage DM, Steinberg CR, Skirving WJ, Kleypas JA (1996) Mesoscale circulation features of the Great Barrier Reef Region inferred from NOAA satellite imagery. Remote Sens Environ 56:21–41

Davies P (1994) Evolution of the Great Barrier Reef. Aust Geol 92:21–24

Doherty PJ, Planes S, Mather P (1995) Gene flow and larval duration in seven species of fish from the Great Barrier Reef. Ecology 76:2373–2391

Done T, Whetton P, Jones R, Berkelmans R, Lough J, Skirving W, Wooldridge S (2003) Global climate change and coral bleaching on the Great Barrier Reef. CSIRO, Australian Institute of Marine Science and CRC Reef research centre, Townsville

Fabricius KE, Mieog JC, Colin PL, Idip D, van Oppen MJH (2004) Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. Mol Ecol 13:2445–2458

Frydenber J, Hoffmann AA, Loeschcke V (2003) DNA sequence variation and latitudinal associations in *hsp23*, *hsp26* and *hsp27* from natural populations of *Drosophila melanogaster*. Mol Ecol 12:2025–2032

Glynn PW (1993) Coral reef bleaching: ecological perspectives. Coral Reefs 12:1–17

Glynn PW, Mate JL, Baker AC, Calderon MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Nino-Southern Oscillation Event: spatial/temporal patterns and comparisons with the 1982–1983 event. Bull Mar Sci 69:79–109

Gomez-Mestre I, Tejedo M (2004) Contrasting patterns of quantitative and neutral genetic variation in locally adapted populations of the natterjack Toad *Bufo calamita*. Evolution 58:2343–2352

Guo SW, Thompson EA (1992) Performing the exact test for Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839–866

Hoegh-Guldberg O (2004) Coral reefs in a century of rapid environmental change. Symbiosis 37:1–32

Hoffmann AA, Anderson A, Hallas R (2002) Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecol Lett 5:614–618

Lewis PO, Zaykin D (2002) Genetic data analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors available from http://www.hydrodictyon.eeb.uconn.edu/people/plewis/software.php

Luttikhuizen PC, Drent J, Van Delden W, Piersma T (2003) Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. J Evol Biol 16:260–272

McCulloch M, Fallon S, Wyndham T, Hendy E, Lough J, Barnes D (2003) Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. Nature 421:727–730

Merila J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. J Evol Biol 14:892–903

Miller MP (1997) Tools for population genetic analyses (TFPGA) Version 1.3, available as free software from http://www.marksgeneticsoftware.net/

Ottaviano E, Gorla MS, Pe E, Frova C (1991) Molecular markers (RFLPs and HSPs) for genetic dissection of thermotolerance in maize. Theor Appl Genet 81:713–719

Peakall R, Smouse PE (2001) GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Austalia http://www.anu.edu.au/BoZo/GenAlEx/

Perry GM, Danzmann RG, Ferguson MM, Gibson JP (2001) Quantitative trait loci for upper thermal tolerance in outbred strains of rainbow trout (*Oncorhynchus mykiss*). Heredity 86:333–341

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Raymond M, Rousset F (1999) GENEPOP Version 3.1d, latest versions of this free software available from http://www.wbiomed.curtin.edu.au/genepop/

Rice WR (1989) Analysing tables of statistical tests. Evolution 43:223–225

Richardson BJ, Baverstock PR, Adams M (1986) Allozyme electrophoresis: a handbook for animal systematics and populations studies. Academic, Sydney

Rowan R (2004) Thermal adaptation in reef coral symbionts. Nature 430:742

Selander RK, Smith MH, Yang SY, Johnson WE, Gentry JB (1971) Biochemical polymorphism and systematics in the genus *Peromyscus* 1. Variation in the old field mouse (*Peromyscus polinotus*) Studies in Genetics VI. University of Texas Publication 7103, pp 49–90

Shaw CR, Prasard R (1970) Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem Genet 4:297–320

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792

Smith CR (2005) The role of genetic and environmental variation on thermal tolerance of a reef-building coral, *Acropora millepora*. PhD thesis, University of Queensland, p 166

Smith LD, Hughes TP (1999) An experimental assessment of survival, re-attachment and fecundity of coral fragments. J Exp Mar Biol Ecol 235:147–164

van Oosterhout C, Hutchinson WF, Willis DPM, Shipley PF (2004a) Micro-checker version 2.2.1 An application for checking microsatellite data for null alleles available from http://www.microchecker.hull.ac.uk

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004b) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Am Genetic Assoc 89:438–450

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Wilding CS, Butlin RK, Grahame J (2001) Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. J Evol Biol 14:611–619

Wilkinson C (2000) The 1997–98 mass coral bleaching and mortality event: 2 years on. In: Wilkinson C (eds) Status of coral reefs of the world: 2000. Australian Institute of Marine Science, Townsville, pp 21–34

Williams DM (2001) Impacts of terrestrial run-off on the GBRWHA. Report to CRC Reef, Townsville, Queensland

Worheide G, Hooper JNA, Degnan BM (2002) Phylogeography of western Pacific *Leucetta 'chagosensis'* (Porifera: Calcarea) from ribosomal DNA sequences: implications for population history and conservation of the Great Barrier Reef World Heritage Area (Australia). Mol Ecol 11:1753–1768

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97–159