

Genetic diversity and connectivity in a brooding reef coral at the limit of its distribution

Annika M. E. Noreen^{1,2}, Peter L. Harrison¹
and Madeleine J. H. Van Oppen^{2,*}

¹Coral Reef Research Centre, School of Environmental Science and Management, Southern Cross University,
PO Box 157, Lismore, New South Wales 2480, Australia

²Australian Institute of Marine Science, PMB 3, Townsville MC, Queensland 4810, Australia

Remote populations are predicted to be vulnerable owing to their isolation from potential source reefs, and usually low population size and associated increased extinction risk. We investigated genetic diversity, population subdivision and connectivity in the brooding reef coral *Seriatopora hystrix* at the limits of its Eastern Australian (EA) distribution and three sites in the southern Great Barrier Reef (GBR). Over the approximately 1270 km survey range, high levels of population subdivision were detected (global $F_{ST} = 0.224$), with the greatest range in pairwise F_{ST} values observed among the three southernmost locations: Lord Howe Island, Elizabeth Reef and Middleton Reef. Flinders Reef, located between the GBR and the more southerly offshore reefs, was highly isolated and showed the signature of a recent bottleneck. High pairwise F_{ST} values and the presence of multiple genetic clusters indicate that EA subtropical coral populations have been historically isolated from each other and the GBR. One putative first-generation migrant was detected from the GBR into the EA subtropics. Occasional long-distance dispersal is supported by changes in species composition at these high-latitude reefs and the occurrence of new species records over the past three decades. While subtropical populations exhibited significantly lower allelic richness than their GBR counterparts, genetic diversity was still moderately high. Furthermore, subtropical populations were not inbred and had a considerable number of private alleles. The results suggest that these high-latitude *S. hystrix* populations are supplemented by infrequent long-distance migrants from the GBR and may have adequate population sizes to maintain viability and resist severe losses of genetic diversity.

Keywords: *Seriatopora hystrix*; subtropical; connectivity; microsatellites; Eastern Australia

1. INTRODUCTION

Coral reefs are among the most vulnerable habitats worldwide and are increasingly threatened both by chronic and acute anthropogenic disturbances (e.g. Bellwood *et al.* 2004; Hoegh-Guldberg *et al.* 2007). Dramatic deterioration of reef health has occurred over the past few decades and is predicted to continue due to rapidly increasing sea surface temperatures (SSTs) and ocean acidification, resulting in increased bleaching frequency and severity, and reduced calcification and reef accretion (e.g. Hoegh-Guldberg *et al.* 2007; Silverman *et al.* 2009). High-latitude reefs are of particular concern as they are already considered to be a marginal reefal environment (Veron 1995; Kleypas *et al.* 1999a; Guinotte *et al.* 2003). Geographical isolation (Kleypas *et al.* 1999a; Hughes *et al.* 2003), as well as their location in areas of rapidly declining aragonite saturation (e.g. Kleypas *et al.* 1999b; Guinotte *et al.* 2003) may further contribute to their vulnerability. The capacity of corals at subtropical reefs to survive disturbances, such as mass bleaching, disease, crown-of-thorns starfish (COTS) outbreaks or chronic

stressors such as rapidly changing SSTs and ocean chemistry, will depend partly on their population genetic structure, as remote populations are predicted to have small effective population sizes, low genetic diversity and to suffer from inbreeding. Low genetic diversity reflects limited adaptive potential (Gates & Edmunds 1999; Frankham 2005), and inbreeding can significantly reduce fitness and increase the risk of extinction (Crnokrak & Roff 1999; Bijlsma *et al.* 2000).

Over an evolutionary timeframe, successful long-distance dispersal, settlement and reproduction may increase genetic diversity where the dispersed species has an existing population or, if the species is not present, constitute a founder event. Coral reefs have evolved under various intensities, modes and frequencies of disturbances, and reef coral populations have experienced severe reduction (Wakeford *et al.* 2008) to complete eradication throughout their evolutionary history (e.g. Veron 1995). Recovery of disturbed reefs depends on the density of survivors, their fecundity and the magnitude of recruitment of individuals from external sources (e.g. Harrison & Wallace 1990; Harrison & Booth 2007). Routine dispersal over ecological time-scales (i.e. ecological gene flow) is necessary for replenishment of severely disturbed reefs (e.g. Jones *et al.* 2009). If a coral species were to be completely extirpated at the isolated subtropical reefs of Lord

* Author for correspondence (m.vanoppen@aims.gov.au).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2009.1050> or via <http://rspb.royalsocietypublishing.org>.

Howe Island, Elizabeth Reef and Middleton Reef, recovery would require rafting or long-distance larval dispersal across more than 630 km of open ocean. The rapid, warm-water Eastern Australian Current (EAC) provides a vehicle for unidirectional dispersal from the Great Barrier Reef (GBR) to the subtropics, and large gyres extending several hundreds of kilometres offshore (e.g. Ridgway & Dunn 2003) could disperse larvae between subtropical inshore and offshore reefs (Wilson & Harrison 1997). Despite the potential for dispersal, previous research has demonstrated low but variable realized gene flow between coral populations from the GBR to the subtropics (Ayre & Hughes 2004) and within the subtropics (Miller & Ayre 2008). Further, no genetic variation was found in the reef coral *Seriatopora hystrix* population at Lord Howe Island population for five of the six allozyme loci tested, leading to the hypothesis that a founder event of one or very few colonists is responsible for the presence of this species at Lord Howe Island (Ayre & Hughes 2004). The high subdivision and low genetic diversity at subtropical sites has been interpreted as historical isolation and has led to predictions that localized extinctions will have 'persistent impacts over very long periods' (Ayre & Hughes 2004), and that 'the likelihood that the GBR will act as a source of recruits to [high-latitude reefs] following catastrophic events is negligible and only likely to occur over long timescales' (Miller & Ayre 2008).

The focus of this study is the brooding hermaphroditic reef coral *S. hystrix* (Pocilloporidae). *Seriatopora hystrix* has primarily sexually produced larvae (Ayre & Resing 1986; Sherman 2008) that are matured within the polyps and periodically released (Harrison & Wallace 1990). Most brooding corals, including *S. hystrix*, have larvae that are competent to settle within a few hours after release (Isomura & Nishihira 2001; Harii *et al.* 2002). This promotes settlement on, or close to, the natal reef (e.g. Tioho *et al.* 2001). Recent work on *S. hystrix* populations using highly variable microsatellite markers showed dispersal mostly occurred over small spatial scales (less than 100 m), although this was supplemented by less frequent longer-distance dispersal (Underwood *et al.* 2007). Localized dispersal generally leads to high population differentiation, and earlier research on GBR *S. hystrix* populations (using allozymes) confirms this ($F_{ST} = 0.43$, Ayre & Dufty 1994; $F_{ST} = 0.15$, Ayre & Hughes 2000). Despite the usually rapid settlement of larvae, some brooding corals produce a small proportion of larvae that have extended competency periods of over 100 days in the water column (e.g. Harii *et al.* 2002), which can translate to a dispersal potential of hundreds of kilometres (Harrison & Wallace 1990; Harrison & Booth 2007). Genetic evidence of recent longer distance dispersal events in *S. hystrix* (on the order of tens to hundreds of kilometres) exists from the Red Sea, Western Australia and Eastern Australia (EA) (Maier *et al.* 2005; Underwood *et al.* 2007; Van Oppen *et al.* 2008).

Currently, all subtropical EA reefs are Marine Parks or Nature Reserves, but, apart from Elizabeth and Middleton Reefs, are managed individually by three different states or Commonwealth departments. Whether these reefs should be managed as individual units or a network is largely dependent on the spatial scale of dispersal of

focus species (Jones *et al.* 2009). As the integral habitat-forming component of reefs, scleractinian corals are a key target taxon for genetic studies of dispersal (Jones *et al.* 2009). This is, to our knowledge, the first population genetics study to include a coral species from the Middleton and Elizabeth Reefs: these subtropical atolls have the highest number of coral species (approx. 155) and the largest reef area (approx. 8000 total hectares) south of the GBR (Hutchings *et al.* 1992; Veron 1993; Oxley *et al.* 2004; A. Noreen 2007, unpublished data). This study aims to increase our understanding of the risk of the loss of genetic diversity, inbreeding and extinction of *S. hystrix* at the southern limits of its distribution range, as well as to estimate the temporal scale of the frequency and magnitude of long-distance dispersal from the GBR.

2. MATERIAL AND METHODS

Ten DNA microsatellite loci were used to assess genetic diversity and connectivity in *S. hystrix* from four major EA subtropical reefs and three reefs in the southern GBR (figure 1). The reefs sampled in this study were subtropical Lord Howe Island, Elizabeth Reef, Middleton Reef, Flinders Reef and Broomfield, Snake and Turner Reefs from the GBR. Lord Howe Island is the southernmost true coral reef in the world (Harriott *et al.* 1995). The other true coral reefs of the EA subtropics, Elizabeth and Middleton Reefs, are located 180 and 235 km north of Lord Howe Island, respectively (figure 1).

Two habitats were sampled at each of these reefs: Lord Howe Island—lagoon and east coast; Elizabeth Reef—lagoon and channel; Middleton Reef—Blue Hole (=lagoon) and channel. These reefs have a unique mix of temperate, subtropical and tropical biota and represent the highest latitude species records for many tropical taxa (Hutchings *et al.* 1992; Veron 1993; Harriott *et al.* 1995; Oxley *et al.* 2004; P. Harrison & A. Noreen 2007, unpublished data). A large proportion of coral species sampled at these locations were sexually reproductive (Hutchings *et al.* 1992; Harrison 2008). These reefs lie in the path of the warm, south and east flowing EAC, which not only acts as a conduit for larval transport from further north, but provides the climate needed for these warm-water reefs to exist (Veron & Done 1979; Hutchings *et al.* 1992; Harriott *et al.* 1995; Harrison & Booth 2007).

Flinders Reef, near the south Queensland coast, supports a diverse coral community of at least 119 coral species growing on and around a small sandstone platform (Veron 1993; Harrison *et al.* 1998). A single site was sampled at Flinders Reef. Of all subtropical sites where *S. hystrix* are recorded, only one was not sampled. This was the Gneering Shoals, a low-diversity coral community growing on rocky substrate approximately 50 km north of Flinders Reef, which is known to have only sporadic occurrences of *S. hystrix* (Banks & Harriott 1995). An outer reef site was sampled at each of three reefs on the GBR (figure 1).

Branches of *S. hystrix* approximately 4 cm in length were collected by hand from individual colonies using SCUBA or snorkel and placed immediately in 95 per cent ethanol (see table 1 for sample sizes). DNA was extracted following Wilson *et al.* (2002). Ten loci were amplified using fluorescently labelled primers in three multiplex PCRs as described in Underwood *et al.* (2006). Samples with rare (frequency < 0.05) private alleles were PCR amplified and genotyped at least twice to confirm the allele's authenticity. In this study,

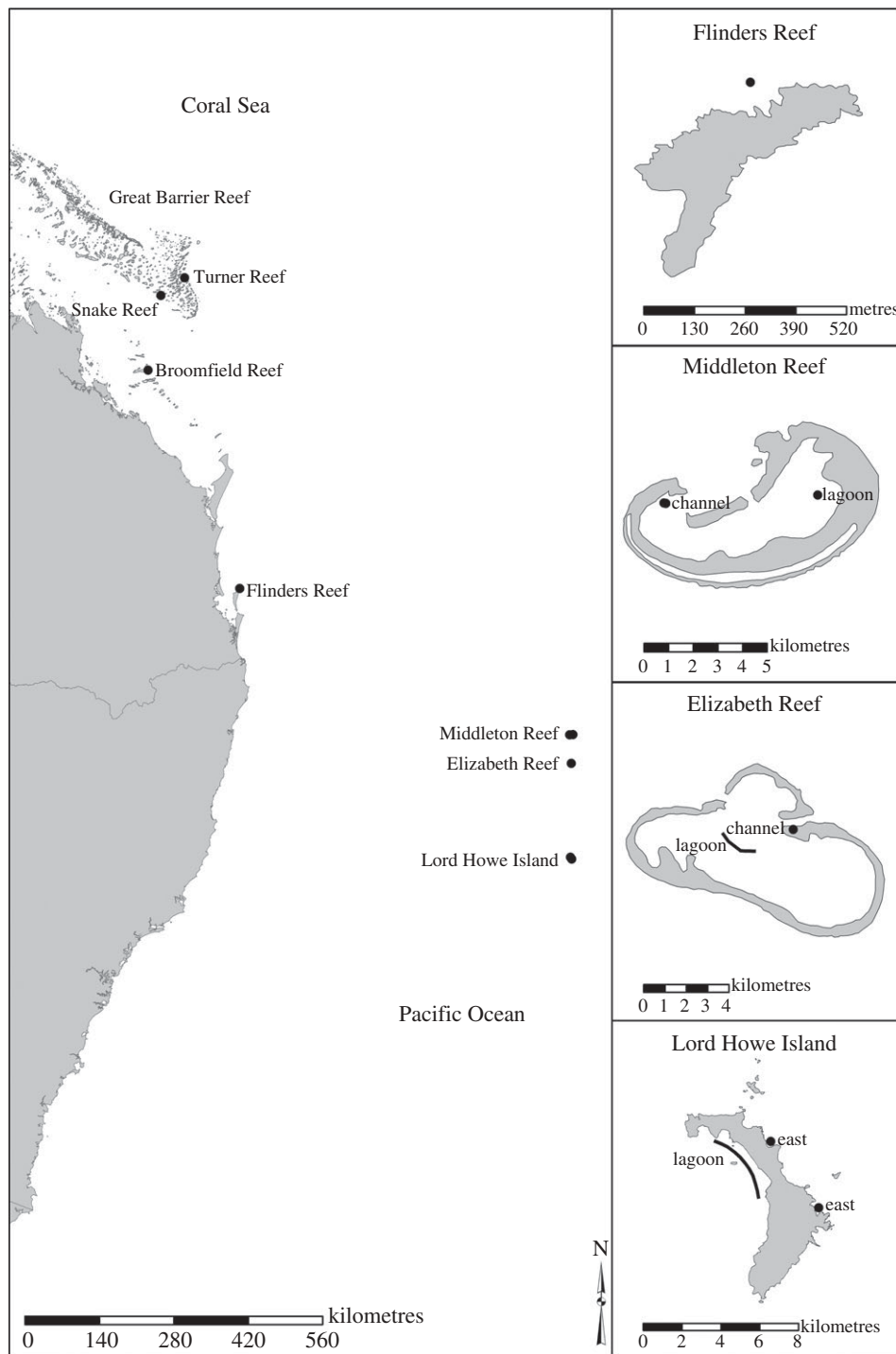


Figure 1. Map of EA showing the sampling locations of the three GBR reefs and four subtropical reefs sampled. Map details of subtropical reefs show sampling sites as points, or lines when the sampling was continuous in a given area.

rare alleles shared between two subtropical sites are defined as 'semi-private' alleles.

(a) Data analysis

GIMLET v. 1.3.3 (Valiere 2002) was used to assess the presence of clone mates; in the case that the same multilocus genotype was found more than once, all but one of the individuals were removed from the dataset prior to analysis. Tests for Hardy–Weinberg equilibrium and linkage disequilibrium (LD) were conducted in the web-based package GENEPOP v. 4.0 (Raymond & Rousset 1995) under the following Markov chain parameters: 1000 dememorization, 100 batches, 10 000 iterations per batch. FSTAT v. 2.9.3 (Goudet 2001) was used

to generate allele frequencies, allelic richness (calculated per locus) and F_{ST} values (Weir & Cockerham 1984, Infinite Allele Model). Statistical significance of pairwise F_{ST} values was based on 45 000 permutations and p -values were corrected for multiple comparisons using strict Bonferroni. BOTTLENECK v. 1.2.02 (Cornuet & Luikart 1996) was used to determine whether any recent population bottlenecks were likely to have occurred. This program uses coalescent theory to provide different models for heterozygosity compared with allelic richness. Allele numbers decrease faster than heterozygosity during bottlenecks, and a rapid decrease in allele numbers while maintaining heterozygosity creates a distinct signature. However, this pattern deteriorates after two to four generations,

Table 1. Sample size (N), proportion of unique genotypes (N_g) to total samples (N) and number and identity of fixed loci in each of the sampled sites.

site	N	N_g/N	no. fixed loci	locus fixed
Lord Howe Island lagoon	42	0.88	0	
Lord Howe Island east	22	0.91	1	Sh2_002
Elizabeth Reef lagoon	55	0.98	1	Sh3_003
Elizabeth Reef channel	35	1	1	Sh4_001
Middleton Reef lagoon	17	1	1	Sh3_003
Middleton Reef channel	37	0.97	1	Sh3_003
Flinders Reef	33	0.97	2	Sh3_003 Sh3_007
Broomfield Reef	27	1	0	
Snake Reef	37	1	0	
Turner Reef	29	1	0	

which means that BOTTLENECK will only detect recent events. A change in the allele frequency distribution ('mode shift') indicates a recent bottleneck. BOTTLENECK was run under the two-phased mutation model default settings with 10 000 replications. STRUCTURE v. 2.2 (Pritchard *et al.* 2000), a model-based clustering method, was used to find the most likely number of genetic clusters (K) and to represent these visually. Parameters used were: admixture model and independent allele frequency model with no prior information. Burn-in was 100 000 with 1 000 000 iterations for $K = 1$ to 12. For each K , the run was replicated five times, and the most likely value of K was determined following Evanno *et al.* (2005). GENECLASS v. 2.0 (Piry *et al.* 2004) was used to detect first-generation migrants under the following conditions: likelihood ratio $L_{\text{home}}/L_{\text{max}}$; criterion: Rannala & Mountain (1997), simulation algorithm: Paetkau *et al.* (2004); 10 000 simulated individuals with a threshold $p \leq 0.01$. This generated a probability of each individual being a putative migrant. These putative migrants were then removed from the original populations and reassigned to the sampled populations using the above parameters. This produced a probability for each putative migrant of originating from each of the other sampled populations.

3. RESULTS

The Lord Howe Island lagoon site had the highest number of potential clone mates of any site (table 1), where three samples of one multilocus genotype and two samples of another multilocus genotype were present among the 42 colonies collected. However, the majority of samples at all sites were derived via sexual reproduction. All but one subtropical population sampled showed a significant heterozygote deficit for zero to two loci only (figure 2), indicating that null alleles, further population substructuring (Wahlund 1928), or inbreeding (Wright 1922) are unlikely to apply to these populations. The Elizabeth Reef lagoon site showed significant heterozygote deficits in a larger number of loci (figure 2). The single significant heterozygote excess (in the Elizabeth Reef channel population) is unlikely to have biological significance. LD and significant heterozygote deficiencies were high in the GBR populations. The pairwise F_{ST} values between the two eastern Lord Howe Island sites were not significantly

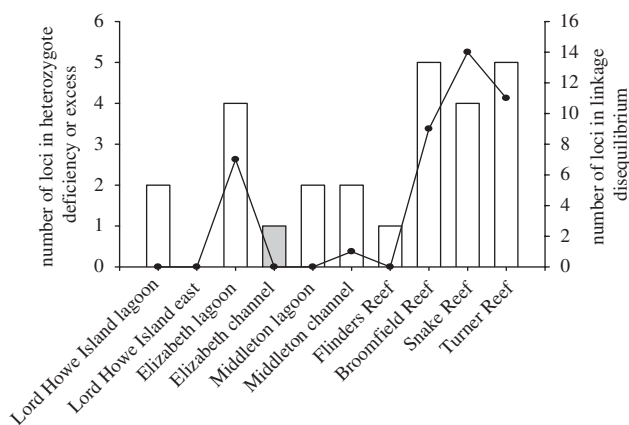


Figure 2. Number of loci with significant ($p \leq 0.01$) heterozygote deficiencies (empty bars) or excess (grey bars) and number of locus pairs showing significant ($p \leq 0.01$) LD (solid line).

different from zero ($p \geq 0.05$), and these sampling sites were therefore pooled for further analysis.

Allelic richness was significantly lower in the subtropics compared with the southern GBR (t -test, $p = 0.0009$) (figure 3). Despite spanning over 700 km in latitude, there was no significant difference in allelic richness between sites within the subtropics (ANOVA, $p = 0.42$). All subtropical sites except one (Lord Howe Island lagoon) had a locus fixed for a single allele and Flinders Reef had two (table 1). Most subtropical sites or reefs had private or semi-private alleles (table 2).

Pairwise F_{ST} values were significantly different from zero for all comparisons except one (Broomfield Reef \times Turner Reef) (table 3). Subtropical \times subtropical reefs showed a larger range of pairwise F_{ST} values ($F_{ST} = 0.034$ – 0.475) than GBR \times subtropical reefs ($F_{ST} = 0.162$ – 0.358), despite a similar number of pairwise comparisons (21 versus 22). The highest pairwise F_{ST} values observed were between Lord Howe Island east and lagoon \times Middleton Reef lagoon ($F_{ST} = 0.475$ and 0.462 , respectively). The global F_{ST} value was 0.224.

The most likely number of genetic clusters (K) as determined in STRUCTURE was seven. At all of the subtropical sites, except for the Elizabeth Reef lagoon, most individuals sampled at that site belonged to a single genetic cluster (figure 4). The Elizabeth Reef lagoon site showed a signature of admixture between two clusters: the Elizabeth Reef channel and the Middleton Reef lagoon. There were two main genetic clusters on the GBR, which were distributed among the three sampling locations, although a small number of GBR individuals also showed a genetic affinity with the Middleton and Elizabeth Reef lagoonal cluster.

The genetic assignment test detected 18 potential first-generation migrants. Eleven of those were detected in subtropical populations, and all but two of those were assigned to another subtropical population with high probability (more than 80%) (table S4 in the electronic supplementary material). One was assigned with similar probability to either another subtropical site or a GBR reef (table S4 in the electronic supplementary material). Flinders Reef did not have an immigrant or emigrant identified and is the only site sampled that showed evidence of the *S. hystrix* population having gone through

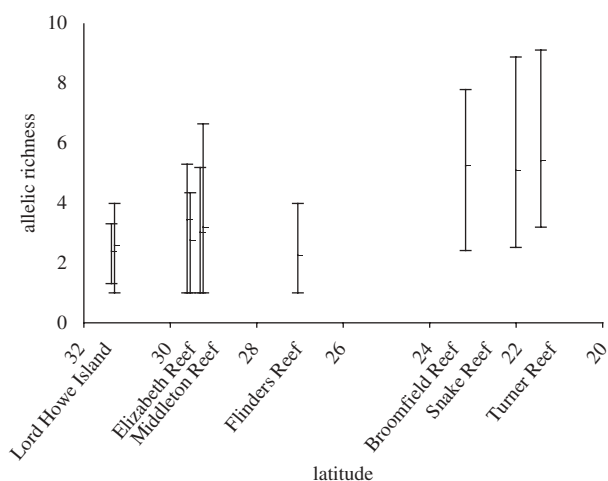


Figure 3. Allelic richness. Bars represent the range of allelic richness for 10 loci. Subtropical reefs with two sites have been separated by $0^{\circ}4'$ along the x -axis for visual clarity.

a recent bottleneck event (table S3 in the electronic supplementary material).

4. DISCUSSION

The long-term viability of populations depends at least partially on the genetic characteristics of the existing populations. Low recruitment into a population from outside sources may suffice to maintain genetic diversity (Tallmon *et al.* 2004), but is likely to be insufficient for replenishment and recovery if local populations are severely devastated (Jones *et al.* 2009). Stochasticity of recruitment as well as differences in life history, larval competency periods, settlement and survival requirements (Harrison & Wallace 1990; Harrison & Booth 2007) mean that different species will have different levels of connectivity to, and genetic diversity at, isolated locations (e.g. Miller & Ayre 2008; Underwood *et al.* 2009). Species with high population subdivision and limited dispersal are hypothesized to be more vulnerable to disturbances than populations with higher connectivity (e.g. Jones *et al.* 2009), despite some of these (such as *S. hystrix*) having a broad geographical range and large global population size. This study supports previous evidence that populations at high-latitude, isolated reefs have had historically low connectivity from each other and the GBR. Nevertheless, the data presented here show that occasional long-distance dispersal may aid in maintaining the genetic diversity of subtropical *S. hystrix* populations, and at least some subtropical reefs may support sufficiently large effective population sizes to enable them to resist severe losses of genetic diversity.

(a) Genetic diversity at subtropical reefs

Allelic richness was significantly lower at subtropical reefs than on the GBR (t -test, $p = 0.0009$), but not significantly different between subtropical sites (ANOVA, $p = 0.42$). There was no gradual decrease in allelic richness with latitude, which contrasts with onshore subtropical populations of the related coral *Pocillopora damicornis* (Miller & Ayre 2008). Allelic richness of *S. hystrix* at several GBR Reefs (using the same 10 loci) was similar to that recorded in this study (Van Oppen *et al.* 2008),

Table 2. Alleles private to one or two subtropical sites in EA.

locus	size (bp)	location(s)	frequency
Sh2_002	123	Elizabeth Reef lagoon	0.011
		Elizabeth Reef channel	0.048
		Middleton Reef lagoon	0.031
Sh3_003	86	Lord Howe Island lagoon	0.021
		Lord Howe Island east	0.024
Sh2_005	129	Middleton Reef channel	0.027
Sh3_007	101	Elizabeth Reef lagoon	0.066
		Elizabeth Reef lagoon	0.009
Sh2_008	213	Elizabeth Reef lagoon	0.010
		Middleton Reef channel	0.014
Sh3_009	166	Lord Howe Island lagoon	0.128
		Lord Howe Island east	0.105
Sh4_010	224	Elizabeth Reef lagoon	0.009

demonstrating that the subtropical genetic diversity was not substantially eroded compared to some GBR populations.

The lagoonal samples of Lord Howe Island represented the sole subtropical site in this study to retain polymorphism at all loci (table 1). Lord Howe Island *S. hystrix* had an average allelic richness per locus of 2.50, and a maximum allelic richness of 4.47 (at locus Sh2_006). This contrasts with previous research showing that *S. hystrix* was fixed for five of the six allozyme loci that were polymorphic on the GBR (Ayre & Hughes 2004). Our data therefore do not support the hypothesis that the *S. hystrix* population at Lord Howe Island is derived from one or very few colonists (cf. Ayre & Hughes 2004).

In this study, the GBR and most subtropical sites or reefs were found to have private or semi-private alleles (table 2 and table S2 in the electronic supplementary material). The high number of private alleles on the GBR may be an artefact of the small number of GBR reefs included in this study. Collectively, the subtropical sites have nine private alleles at seven loci (table 2). Private subtropical alleles were almost always rare (less than 0.05). However, an exception was an allele at Lord Howe Island, which was present in both lagoonal and eastern populations at a frequency above 0.10. Elizabeth and Middleton Reefs had seven private alleles, which were generally site-specific, although one semi-private allele was present at both Elizabeth Reef sites, and another was shared between Elizabeth and Middleton Reef. These private alleles may have entered the population through gene flow from unsampled populations, or from mutations having occurred locally. Irrespective of the origin of the private alleles, they demonstrate that these subtropical sites have sufficiently large effective population sizes to maintain low frequency alleles, rather than losing them through genetic drift. The presence of separate private alleles at sites located less than 5 km apart indicate significant historical isolation; however, the presence of semi-private alleles (shared between two subtropical sites) support low levels of gene flow between some populations.

Despite a severe COTS outbreak at Elizabeth and Middleton Reefs in the mid-1980s (J. E. N. Veron 2008, personal communication, Hutchings *et al.* 1992), there was no evidence of reduced genetic diversity

Table 3. Summary of pairwise F_{ST} values (Weir & Cockerham 1984). Permutations were run 45 000 times with strict Bonferroni corrections. After this, 98% (44) of the comparisons remained significant with 89% (40) significant at $p \leq 0.001$. LHI, Lord Howe Island; Eliz., Elizabeth Reef; Midd., Middleton Reef; Broom, Broomfield Reef.

LHI lagoon	LHI east	Eliz. lagoon	Eliz. channel	Midd. lagoon	Midd. channel	Flinders Rf.	Broom. Rf.	Snake Rf.	
0.034*									LHI east
0.233***	0.202***								Eliz. lag
0.130***	0.147***	0.146***							Eliz. chan
0.475***	0.462***	0.105***	0.391***						Midd. lag
0.233***	0.251***	0.113***	0.190***	0.285***					Midd. chan
0.241***	0.265***	0.229***	0.205***	0.384***	0.293***				Flinders Rf.
0.328***	0.294***	0.170***	0.268***	0.250***	0.232***	0.252***			Broom. Rf.
0.320***	0.285***	0.162***	0.252***	0.224***	0.204***	0.240***	0.040***		Snake Rf.
0.358***	0.339**	0.174***	0.308***	0.196**	0.212***	0.279***	0.068 ^{ns}	0.095**	Turner Rf.

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

compared with other subtropical sites or of *S. hystrix* populations having undergone a recent bottleneck. Flinders Reef was the only site sampled that showed a mode shift in BOTTLENECK, implying that its high genetic differentiation and two fixed loci may stem from a bottleneck event ca. two to four generations ago. This is supported by field evidence: the last survey of Flinders Reef was undertaken in 1993, and *S. hystrix* was only recorded at a single site and in low abundance (Harrison *et al.* 1998), whereas during the sample collection for this study (November 2007) hundreds of colonies of *S. hystrix* were sighted in a relatively small area. Thus, it is possible that *S. hystrix* at Flinders Reef has undergone a bottleneck, followed by a recent population size expansion.

(b) Genetic differentiation of subtropical reefs

Genetic signatures of both recent and historical admixture were more prominent on the GBR than in the subtropics. First, F_{ST} values between the GBR populations were significantly lower than those among subtropical populations, indicating higher historical gene flow. These populations also displayed higher heterozygote deficiencies and LD, suggestive of more recent genetic admixture. This is probably a result of their closer proximity, and the large number of other GBR reefs that could act as stepping stones between those sampled. Evidence for recent admixture was limited at subtropical locations, with one exception. The Elizabeth Reef lagoon population exhibited higher LD and heterozygote deficiencies than other subtropical reefs (figure 2), and a mix of mainly two genetic clusters in the STRUCTURE analysis (figure 4). Despite evidence of recent admixture at Elizabeth Reef lagoon site, all sites sampled at Elizabeth and Middleton Reefs were significantly differentiated, including sites at the same reef separated by only a few kilometres ($F_{ST} > 0$, $p < 0.001$, table 3). The STRUCTURE analysis indicates a deep division between the Middleton and Elizabeth lagoonal cluster and the other subtropical sites; at $K = 2$, one cluster comprised all subtropical sites except the Middleton lagoonal samples and a proportion of Elizabeth lagoonal samples, while the other cluster was the GBR, the Middleton lagoon and a proportion of Elizabeth lagoon samples. Recent work on *S. hystrix* in isolated low-latitude North Western Australian reefs has also found highly significant differentiation between sites

within a reef (Underwood *et al.* 2009). This is indicative of predominantly highly localized dispersal, which is supported by spatial autocorrelation analysis in Underwood *et al.* (2007).

Despite significant genetic subdivision between offshore subtropical sites, nine potential first-generation migrants (less than 3%) were assigned with a high probability (more than 80%) to other offshore subtropical site(s) (table S4 in the electronic supplementary material). Flinders Reef was the most genetically isolated reef in this study: it had two fixed loci (table 1), formed a distinct genetic cluster in the STRUCTURE analysis (figure 4) and did not have an immigrant or emigrant detected (table S4 in the electronic supplementary material). Hence, it is not a 'sink' population from the GBR nor a 'source' to offshore subtropical reefs further south.

The patterns of genetic subdivision and connectivity in *S. hystrix* presented here show both differences and similarities to patterns of genetic structure in other taxa. The pocilloporid coral *P. damicornis* showed high levels of subdivision among seven sites at Lord Howe Island based on allozyme data (Miller & Ayre 2004), consistent with our observation of *S. hystrix* in this study. Lord Howe Island populations of other marine organisms are generally also highly isolated (Benzie 1992; Ayre & Hughes 2004; Miller & Ayre 2008). For example, COTS populations from Lord Howe Island were the most genetically differentiated of all EA populations sampled (Benzie 1992). However, in contrast to our findings for *S. hystrix*, two other taxa sampled at Elizabeth and Middleton Reefs exhibited no significant differentiation between sites (COTS, Benzie 1992; black cod, Appleyard & Ward 2007) nor to GBR populations (Benzie 1992).

(c) Larval dispersal to subtropical reefs

Dispersal from tropical to subtropical reefs along EA over an evolutionary timeframe is supported by the occurrence of approximately 155 coral species in 42 genera shared between the GBR and the high-latitude offshore EA reefs (Veron & Done 1979; Hutchings *et al.* 1992; Veron 1993; Harriott *et al.* 1995; Oxley *et al.* 2004, P. Harrison & A. Noreen 2007, unpublished data). Although approximately a dozen coral genera have been found rafting (e.g. Jokiel 1990; deVantier 1992), larval

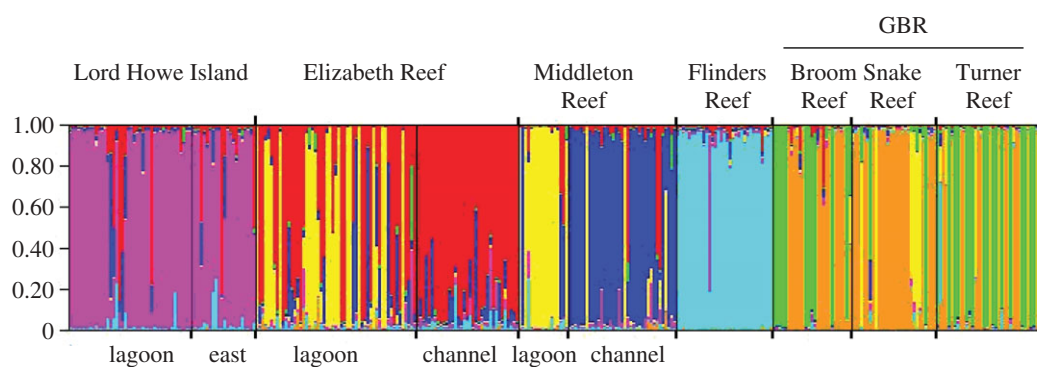


Figure 4. STRUCTURE bar plot for $K = 7$. Each vertical line represents the assignment probability of a single individual to one (or more) of the genetic clusters, without reference to where that individual was sampled.

dispersal is assumed to be the most likely origin of the majority of the primarily tropical coral species recorded at subtropical locations. In this study, both the GENECLASS and STRUCTURE results indicate that a colony of *S. hystrix* sampled at Elizabeth Reef may have originated on the GBR. While this immigrant could have originated from an unsampled subtropical population, a recent analysis of these data with a much larger dataset from GBR with the same 10 loci confirms our results. This analysis, which included the four subtropical reefs and 22 GBR reefs (an additional 19 central and southern reefs), also identified an Elizabeth Reef *S. hystrix* individual which had low probability of originating from any subtropical site and a high probability of originating from the GBR (M. J. H. Van Oppen 2009, personal communication). Independent evidence for recent long-distance dispersal events of corals from the GBR to the subtropics comes from the observation that coral species richness at Lord Howe Island and Elizabeth and Middleton Reefs has increased since the first survey of these locations in 1977 and 1981, respectively (Veron & Done 1979; Hutchings *et al.* 1992). New records include distinct species unlikely to have been overlooked during the original surveys, and whose nearest population is located on the GBR (e.g. *Australogyra zelli* Oxley *et al.* 2004). Subtropical reefs are limited by low winter water temperatures (Veron 1995), whereas increasing SSTs in this region (Lough 2008) may benefit subtropical reefs by providing a more conducive reef environment and provide opportunities for species range expansions (Buddemeier *et al.* 2004; Figueira & Booth in press). It is not inconceivable that migration from the GBR into the subtropics has increased over the past few decades as a consequence of an increase in temperature (Lough 2008) and the intensifying of the EAC (Poloczanska *et al.* 2007). The summertime recruitment of tropical GBR fish species at Lord Howe Island (Figueira & Booth in press) and more southerly locations on the New South Wales coast (Booth *et al.* 2007; Figueira & Booth in press) support our interpretation of dispersal over these large distances.

All subtropical reefs sampled in this study are Marine Parks and are managed by three jurisdictions. For *S. hystrix*, the geographical boundaries of the Marine Parks are larger than the routine dispersal range, suggesting that the present management arrangement is appropriate. This study corroborates previous findings; high levels of population subdivision between high-latitude sites and reefs. Routine gene flow over ecological

time-scales is extremely localized for *S. hystrix* and is likely to be insufficient to allow immediate recovery of subtropical reefs through reseeding from the GBR following extensive localized mortality. However, contrary to some previous studies, we suggest infrequent successful long-distance dispersal supplements coral populations on these high-latitude reefs. The levels of gene flow estimated here are likely to contribute to the maintenance of genetic diversity of these coral populations. We augment these results with the hypothesis that populations of *S. hystrix* at Lord Howe Island, Middleton Reef and Elizabeth Reef maintain adequately large populations to prevent inbreeding and resist acute loss of genetic diversity.

This project was funded by an International Society of Reef Studies/The Ocean Conservation Fellowship to A.M.E.N. A.M.E.N. is supported by a Southern Cross University International Postgraduate Research Scholarship and Stipend. We thank Southern Cross University and the Australian Institute for Marine Science for institutional support, Paul Anderson (DEWHA) for providing the opportunity to do fieldwork at Elizabeth and Middleton Reefs, Simon Hartley (SCU) for diving assistance, Greg Luker (SCU) for producing the map, Lesa Peplow (AIMS) for laboratory support and the AIMS PopGen discussion group for comments on an early draft. The comments of two anonymous reviewers substantially improved this manuscript.

REFERENCES

- Appleyard, S. & Ward, R. 2007 Genetic connectedness between black cod (*E. daemeli*) collections along the NSW coast and the Elizabeth & Middleton Reefs Reserve. Report to the Department of Environment and Water Resources, Australia.
- Ayre, D. J. & Dufty, S. 1994 Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. *Evolution* **48**, 1183–1201. (doi:10.2307/2410377)
- Ayre, D. J. & Hughes, T. P. 2000 Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution* **54**, 1590–1605.
- Ayre, D. J. & Hughes, T. P. 2004 Climate change, genotypic diversity and gene flow in reef-building corals. *Ecol. Lett.* **7**, 273–278. (doi:10.1111/j.1461-0248.2004.00585.x)
- Ayre, D. J. & Resing, J. M. 1986 Sexual and asexual reproduction of planulae in reef corals. *Mar. Biol.* **90**, 187–190. (doi:10.1007/BF00569126)
- Banks, S. A. & Harriott, V. J. 1995 Coral communities of the Gneering Shoals and Mudjimba Island, south-eastern

- Queensland. *Mar. Freshwater Res.* **46**, 1137–1144. (doi:10.1071/MF9951137)
- Bellwood, D. R., Hughes, T. P., Folke, C. & Nystrom, M. 2004 Confronting the coral reef crisis. *Nature* **429**, 827–833. (doi:10.1038/nature02691)
- Benzie, J. A. H. 1992 Review of the genetics, dispersal and recruitment of crown-of-thorns starfish (*Acanthaster planci*). *Aust. J. Mar. Freshwater Res.* **43**, 597–610. (doi:10.1071/MF9920597)
- Bijlsma, R., Bundgaard, J. & Boerema, C. A. 2000 Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. *J. Exp. Biol.* **13**, 502–514. (doi:10.1046/j.1420-9101.2000.00177.x)
- Booth, D., Figueira, W., Gregson, M., Brown, L. & Beretta, G. 2007 Occurrence of tropical fishes in temperate south-eastern Australia: role of the Eastern Australian Current. *Estuarine Coastal Shelf Sci.* **72**, 102–114. (doi:10.1016/j.ecss.2006.10.003)
- Buddemeier, R. W., Kleypas, J. A. & Aronson, R. B. 2004 *Coral reefs and global climate change: potential contributions of climate change to stresses on coral reef ecosystems*. Arlington, VA: The Pew Center on Global Climate Change.
- Cornuet, J. M. & Luikart, G. 1996 Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001–2014.
- Crnokrak, P. & Roff, D. 1999 Inbreeding depression in the wild. *Heredity* **83**, 260–270. (doi:10.1038/sj.hdy.6885530)
- DeVantier, L. M. 1992 Rafting of tropical marine organisms on buoyant coralla. *Mar. Ecol. Prog. Ser.* **86**, 301–302. (doi:10.3354/meps086301)
- Evanno, G., Regaut, S. & Goudet, J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation. *Mol. Ecol.* **14**, 2611–2620. (doi:10.1111/j.1365-294X.2005.02553.x)
- Figueira, W. F. & Booth, D. J. In press. Increasing ocean temperatures allow tropical fishes to survive over winter in temperate waters. *Global Change Biol.* (doi:10.1111/j.1365-2486.2009.01934.x)
- Frankham, R. 2005 Genetics and extinction. *Biol. Conserv.* **126**, 131–140. (doi:10.1016/j.biocon.2005.05.002)
- Gates, R. D. & Edmunds, P. J. 1999 The physiological mechanisms of acclimatization in tropical reef corals. *Am. Zool.* **39**, 30–41.
- Goudet, J. 2001 FSTAT, a program to estimate and test gene diversities and fixation indices, v. 2.9.3. Available from <http://www.unil.ch/izea/software/fstat.html>.
- Guinotte, J. M., Buddemeier, R. W. & Kleypas, J. A. 2003 Future coral reef habitat marginality: temporal and spatial effects of climate change in the Pacific basin. *Coral Reefs* **22**, 551–558. (doi:10.1007/s00338-003-0331-4)
- Harii, S., Kayanne, H., Takigawa, H., Hayashibara, T. & Yamamoto, M. 2002 Larval survivorship, competency periods and settlement of two brooding corals, *Heliopora coerulea* and *Pocillopora damicornis*. *Mar. Biol.* **141**, 39–46. (doi:10.1007/s00227-002-0812-y)
- Harriott, V. J., Harrison, P. L. & Banks, S. A. 1995 The coral communities of Lord Howe Island. *Mar. Freshwater Res.* **46**, 457–465. (doi:10.1071/MF9950457)
- Harrison, P. L. 2008 Coral spawn slicks at Lord Howe Island, Tasman Sea, Australia: the world's most southerly coral reef. *Coral Reefs* **27**, 35. (doi:10.1007/s00338-007-0302-2)
- Harrison, P. L. & Booth, D. J. 2007 Coral reefs: naturally dynamic and increasingly disturbed ecosystems. In *Marine ecology* (eds S. D. Connell & B. M. Gillanders), pp. 316–377. Melbourne, Australia: Oxford University Press.
- Harrison, P. L. & Wallace, C. C. 1990 Reproduction, dispersal and recruitment of Scleractinian corals. In *Coral reef ecosystems, ecosystems of the world*, vol. 25 (ed. Z. Dubinsky), pp. 133–207. Amsterdam, The Netherlands: Elsevier Science Publishers.
- Harrison, P. L., Harriott, V. J., Banks, S. A. & Holmes, N. J. 1998 The coral communities of Flinders Reef and Myora Reef in the Moreton Bay Marine Park, Queensland, Australia. In *Moreton Bay and catchment* (ed. I. R. Tibbetts), pp. 525–536. Brisbane, Australia: School of Marine Science, The University of Queensland.
- Hoegh-Guldberg, O. *et al.* 2007 Coral reefs under rapid climate change and ocean acidification. *Science* **318**, 1737–1742. (doi:10.1126/science.1152509)
- Hughes, T. P. *et al.* 2003 Climate change, human impacts, and the resilience of coral reefs. *Science* **301**, 929–933. (doi:10.1126/science.1085046)
- Hutchings, P. *et al.* 1992 *Reef biology: a survey of Elizabeth and Middleton Reefs, South Pacific*. Kowari 3. Canberra, Australia: Australian National Parks and Wildlife Service.
- Isomura, N. & Nishihira, M. 2001 Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. *Coral Reefs* **20**, 309–315. (doi:10.1007/s003380100180)
- Jokiel, P. L. 1990 Transport of reef corals into the Great Barrier Reef. *Nature* **347**, 665–667. (doi:10.1038/347665a0)
- Jones, G. P., Almany, G. R., Russ, G. R., Sale, P. F., Steneck, R. S., Van Oppen, M. J. H. & Willis, B. L. 2009 Larval retention and connectivity among populations of coral and reef fishes: history, advances and challenges. *Coral Reefs* **28**, 307–325. (doi:10.1007/s00338-009-0469-9)
- Kleypas, J. A., McManus, J. W. & Menez, L. A. B. 1999a Environmental limits to coral reef development: where do we draw the line? *Am. Zool.* **39**, 146–159.
- Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J.-P., Langdon, C. & Opdyke, B. N. 1999b Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* **284**, 118–120. (doi:10.1126/science.284.5411.118)
- Lough, J. M. 2008 Shifting climate zones for Australia's tropical marine ecosystems. *Geophys. Res. Lett.* **35**, L14708. (doi:10.1029/2008GL034634)
- Maier, E., Tollrian, R., Baruch, R. & Nurnberger, B. 2005 Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. *Mar. Biol.* **147**, 1109–1120. (doi:10.1007/s00227-005-0013-6)
- Miller, K. J. & Ayre, D. J. 2004 The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. *Heredity* **92**, 557–568. (doi:10.1038/sj.hdy.6800459)
- Miller, K. J. & Ayre, D. J. 2008 Protection of genetic diversity and maintenance of connectivity among reef corals within marine protected areas. *Conserv. Biol.* **22**, 1245–1254. (doi:10.1111/j.1523-1739.2008.00985.x)
- Oxley, W. G., Ayling, A. M., Cheal, A. M. & Osbourne, K. 2004 Marine surveys undertaken in the Elizabeth and Middleton Reefs Marine National Nature Reserve, December 2003. Report to the Department of Environment and Heritage, Australia.
- Paetkau, D., Slade, R., Burden, M. & Estoup, A. 2004 Genetic assignment methods for the direct, real-time estimation of migration rate using assignment methods: a simulation-based exploration of accuracy and power. *Mol. Ecol.* **13**, 55–65. (doi:10.1046/j.1365-294X.2004.02008.x)
- Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. & Estoup, A. 2004 GENECLASS2: a software for genetic assignment and first-generation migrant detection. *J. Hered.* **95**, 536–539. (doi:10.1093/jhered/esh074)
- Poloczanska, E. S. *et al.* 2007 Climate change and Australian marine life. *Oceanogr. Mar. Biol.* **45**, 407–478.

- Pritchard, J. K., Stephens, M. & Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.
- Rannala, B. & Mountain, J. L. 1997 Detecting immigration by using multilocus genotypes. *Proc. Natl Acad. Sci. USA* **94**, 9197–9221. (doi:10.1073/pnas.94.17.9197)
- Raymond, M. & Rousset, F. 1995 GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Heredity* **86**, 248–249.
- Ridgway, K. R. & Dunn, J. R. 2003 Mesoscale structure of the mean Eastern Australian Current System and its relationship with topography. *Prog. Oceanogr.* **56**, 189–222. (doi:10.1016/S0079-6611(03)00004-1)
- Sherman, C. D. H. 2008 Mating system variation in the hermaphroditic brooding coral, *Seriatopora hystrix*. *Heredity* **100**, 296–303. (doi:10.1038/sj.hdy.6801076)
- Silverman, J., Lazar, B., Long, C., Caldeira, K. & Erez, J. 2009 Coral reefs may start dissolving when atmospheric CO₂ doubles. *Geophys. Res. Lett.* **36**, (doi:10.1029/2008GL036282)
- Tallmon, D. A., Luikart, G. & Waples, R. S. 2004 The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol.* **19**, 489–496. (doi:10.1016/j.tree.2004.07.003)
- Tioho, H., Tokeshi, M. & Nojima, S. 2001 Experimental analysis of recruitment in a scleractinian coral at high latitude. *Mar. Ecol. Prog. Ser.* **213**, 79–86. (doi:10.3354/meps213079)
- Underwood, J. N., Souter, P. B., Ballment, E. R., Lutz, A. H. & Van Oppen, M. J. H. 2006 Development of 10 polymorphic microsatellite markers from herbicide-bleached tissues of the brooding pocilloporid coral *Seriatopora hystrix*. *Mol. Ecol. Notes* **6**, 176–178. (doi:10.1111/j.1471-8286.2005.01183.x)
- Underwood, J. N., Smith, L. D., Van Oppen, M. J. H. & Gilmour, J. P. 2007 Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Mol. Ecol.* **16**, 771–784. (doi:10.1111/j.1365-294X.2006.03187.x)
- Underwood, J. N., Smith, L. D., Van Oppen, M. J. H. & Gilmour, J. P. 2009 Ecologically relevant dispersal of corals on isolated reefs: implications for managing resilience. *Ecol. Appl.* **19**, 18–29. (doi:10.1890/07-1461.1)
- Valiere, N. 2002 GIMLET: a computer program for analysing genetic individual identification data. *Mol. Ecol. Notes* **2**, 377–379.
- Van Oppen, M. J. H., Lutz, A., De'ath, G., Peplow, L. & Kininmonth, S. 2008 Genetic traces of recent long-distance dispersal in a predominantly self-recruiting coral. *PLoS ONE*, **3**e3401. (doi:10.1371/journal.pone.0003401)
- Veron, J. E. N. 1993 *A biogeographic database of hermatypic corals: species of the Central Indo-Pacific genera of the world*. Australian Institute of Marine Science Monograph Series, vol. 10. Townsville, Australia: Australian Institute of Marine Science.
- Veron, J. E. N. 1995 *Corals in space and time: the biogeography and evolution on the Scleractinia*. Sydney, Australia: University of New South Wales Press.
- Veron, J. E. N. & Done, T. J. 1979 Corals and coral communities on Lord Howe Island. *Aust. J. Mar. Freshwater Res.* **30**, 203–236. (doi:10.1071/MF9790203)
- Wahlund, S. 1928 Zusammensetzung von Population und Korrelationserscheinung vom Standpunkt der Vererbungsllehre aus betrachtet. *Hereditas* **11**, 65–106.
- Wakeford, M., Done, T. J. & Johnson, C. R. 2008 Decadal trends in a coral community and evidence of changed disturbance regime. *Coral Reefs* **27**, 1–13. (doi:10.1007/s00338-007-0284-0)
- Weir, B. S. & Cockerham, C. C. 1984 Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. (doi:10.2307/2408641)
- Wilson, J. & Harrison, P. L. 1997 Sexual reproduction in high latitude coral communities of the Solitary Islands, Eastern Australia. In *Proc. 8th Int. Coral Reef Symp., Panama*, vol. 1, pp. 533–538.
- Wilson, K. *et al.* 2002 Genetic mapping of the black tiger shrimp *Penaeus monodon* with amplified fragment length polymorphism. *Aquaculture* **204**, 297–309. (doi:10.1016/S0044-8486(01)00842-0)
- Wright, S. 1922 Coefficients of inbreeding and relationship. *Am. Nat.* **56**, 330–338. (doi:10.1086/279872)