Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008
Session number 14

South east African, high-latitude coral communities, a canary for western Indian Ocean coral reefs?

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Abstract. Whether south west Indian Ocean reef coral communities are resilient to anthropogenic and natural stressors will depend on their inherent ability to adapt to change. In this study, reef coral population diversity and relatedness were investigated at varied scales using molecular methods. Genetic diversity may be used as a proxy to gauge both the population dynamics and resilience of a community and was thus measured in two corals with different reproductive modes, larval dispersal capabilities and life history strategies. We used an intron region in the nuclear DNA of *Acropora austera* and the ITS region of the ribosomal DNA of *Platygyra daedalea* to compare genetic variability between populations. In *A. austera* there appears to be regular genetic exchange between populations in the region. The ITS region has, however, been demonstrated to yield inconclusive results. Local oceanography dictates that northern reef systems may be considered the source populations of those in the south. However, local reef-coral genetic diversity and connectivity may be better judged with a combination of finer resolution molecular markers.

Key words: Indian Ocean, reef-coral, genetic diversity, connectivity.

Introduction

Many scleractinian corals produce propagules which may move between habitats suitable for recruitment in a mobile pelagic stage which has potential for long-distance dispersal. Consequently, initial hypotheses considering larval viability proposed large genetically-unstructured populations for these marine invertebrates (Avise 1998). However, measuring gene flow as a proxy for movement using allozyme studies has confirmed that although some populations of hard coral showed high levels of gene flow, they also had structured populations (Ayre and Hughes 2004, Dai *et al.* 2000, Goffredo *et al.* 2004, Hellberg 1994, Nishikawa and Sakai 2005).

Various studies of genetic variability have revealed the relatedness of life strategy to connectedness of populations (Hellberg 1994; Bastidas *et al* 2002; Goffredo *et al*. 2004; Miller and Ayre 2004, LeGoff-Vitry *et al.* 2004), population structure (Mackenzie *et al.* 2004), speciation (Fukami *et al.* 2004, Marquez *et al.* 2002) and reticulation within species (Diekmann *et al.* 2001). Genetic information may reveal unexpected patterns of population structure and gene flow in hard reef corals and thus is a powerful tool in their management.

A recent review showed that little genetic work has been conducted on marine organisms in the south west Indian Ocean (SWIO) (Ridgway and Sampayo 2005). A number of studies have subsequently been published which shed light on patterns of connectivity and structure in scleractinia and their symbionts in the SWIO (Ridgway *et al.* 2008; Souter and Grahn 2007; Mangubhai and Souter 2007; Macdonald *et al.* 2008). Oceanic waters of Maputaland in the northern KwaZulu-Natal province of South Africa harbour the southern-most communities of coral in the SWIO (see Fig. 1). These communities comprise approximately 90 species of coral (hard and soft) which form the basis for these diverse communities (Schleyer 1999). Further north, the Bazeruto Archipelago also supports a diverse assemblage of corals on accretive reef systems. A gradient of species diversity decreasing from north to south (Obura 2000), along with predominantly southerly offshore currents in the Mozambican channel (5cm.s^{-1}, Lutjeharms 2006) implies that the more diverse northern reefs may be seeding the southern reef coral systems. This, however, has not been tested with appropriate genetic marker systems. As less than 2% of the coast is under any sort of management (Wells and Ngusaru 2004), regional authorities are developing management plans for local exploited resources. We sampled the east African coastline from a number of sites between northern Maputaland, South Africa and southern Tanzania. We collected *Acropora austera*, a widespread coral with a broadcast-spawning reproductive strategy common to the scleractinia (Carroll *et al*. 2006). We also sampled a Favid,

Platygyra daedalea, which, although also a broadcast spawner, displays a life strategy with slower growth and a pattern of intra-reef dispersal different from that observed in *A. austera* (Miller and Babcock 1997).

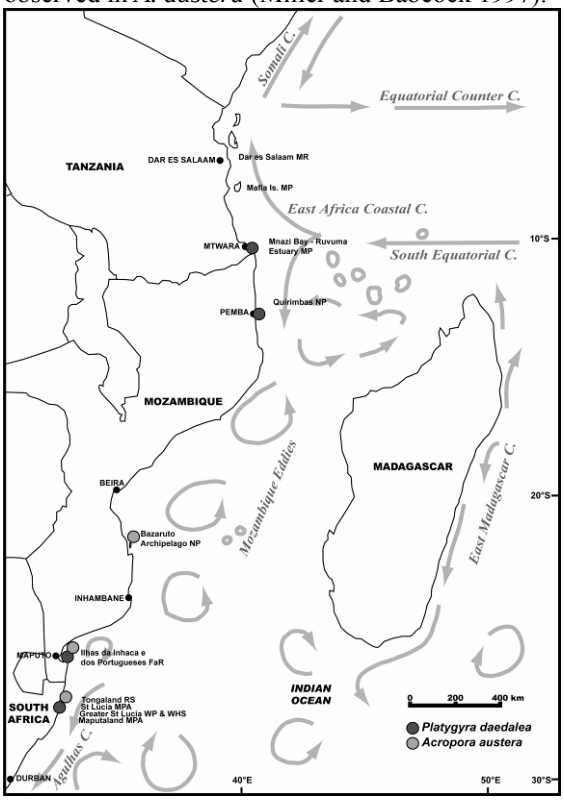

Figure 1. Currents, eddies and sampling locations of *A. austera* and *P. daedalea* in the SWIO study area.

*W*e used nuclear intron sequences similar to those developed by Concepcion *et al.* (2007) for *A. austera* specimens and ITS sequences for *P. daedalea*. ITS rDNA, however, is subject to concerted evolution, the rate of which may either obscure (Vollmer and Palumbi 2004) or resolve phylogenies (Lam and Morton 2003) and introgressive recombination (Vollmer and Palumbi 2004, Diekmann *et al.* 2001) which may further obscure inter-species variation and inter-population variation. This has, however, only been shown in certain families of the scleractinia (*Acroporidae*) (Vollmer and Palumbi 2004) whereas analysis of this region has revealed significant population differentiation in the *Faviidae* (Rodriguez-Lannetty and Hoegh-Guldberg 2002, Lam and Morton 2003), particularly within the genus *Platygyra*. We sequenced specimens from each collection locality using nuclear markers to obtain an initial estimate of nuclear genetic variability.

Materials and Methods

Corals were sampled from sites along the east African coast, using SCUBA and snorkel diving. Care was taken to avoid the collection of clone-mates by sampling colonies separated by at least 5m. Samples were immediately stored in either a dimethyl sulfoxide (DMSO) salt buffer or 70 % ethanol. All DNA was isolated using a Fermentas Life Sciences^{TM} genomic DNA purification kit as per their extraction protocol.

Acropora austera DNA was amplified using single-copy nuclear intron primers developed at the Centre for Marine Studies (CMS) at the University of Queensland (Ridgway, unpublished data). These amplify a hypervariable intron region of the carboxy-anhydrase gene. PCR reactions contained: 1µl sample template, 21.68µl dH_2O, 3µl 10X Platinum Taq PCR buffer mix, 0.9µl 50mM $MgCl_2$, 0.6µl 40mM dNTP mix, 0.84µl of each primer (10µM) and 0.12µl Platinum Taq 5u/µl (Invitrogen^{TM}) . The following thermal cycle was used for the PCR: [94°C for 2 minutes], 40 X [(94°C for 60 seconds), (51°C for 60s), (72°C for 2 m)], [72°C for 10m], [10°C∞]. Samples were sequenced on an ABI 3730 capillary sequencer at Inqaba Biotechnology^{TM}.

Platygyra daedalea DNA was amplified using the A18S and ITS4 primers developed by Takabayashi *et al.* (1998). PCRs contained: 1µl specimen DNA, 21.68µl dH_2O, 3µl 10X supertherm Taq PCR buffer mix, 1.8µl 25mM $MgCl_2$, 0.6µl 40mM dNTP mix, 0.84µl of each primer at 10µM concentration and 0.24µl supertherm Taq. The following thermal cycle was used for PCR amplification: [95°C for 10m], 40 X [(94°C for 45s), (51°C for 45s), (65°C for 1m)], [72°C for 10m], [10°C∞]. Sequencing showed that some specimens were polymorphic and that cloning the samples would be necessary in order to obtain good quality sequence data. Samples were cloned at Inqaba Biotechnology, Pretoria SA, and re-sequenced.

*A*ll sequences were aligned using Bioedit, a sequence alignment package (Hall 1999). Sequence variation was compared using Arlequin 3.11 (Excoffier *et al.* 2005) and DNAsp (Rozas *et al.* 2003). A minimum spanning network was constructed in Arlequin 3.11 (Excoffier *et al.* 2005) for *A. austera* haplotypes and drawn using MrEnt 2 (Zuccon and Zuccon 2008).

Results

*S*equencing - Samples were collected from throughout the study area (Fig. 1, Tab. 1). *A. austera* carbonic anhydrase intron DNA sequences were 155 base pairs (bp) long, had a GC composition of 43.5% and showed a total of 12 variable sites among all 51 sequences. These 51 sequences comprised 9 haplotypes, with haplotype diversity (hd) of 0.697 (std dev = 0.064), which were shared amongst the populations studied (Tab. 2). The complete ITS region (ITS1-5.8S-ITS2) was sequenced for 22 *P.*

daedalea samples. The total sequence length was 374 bp whilst the G+C content was 58.3 %. These 22 sequences comprised 15 haplotypes with hd = 0.939 (std. dev. = 0.029) and $\pi = 4.9$

Table 1. Number of specimens from each site along the east African coast analysed in this study.

Sampling location	*Acropora austera*	*Platygyra daedalea*
Two-mile reef	18	3
Nine-mile reef		2
Inhaca Island – Bareirra Vermelho	6	6
Inhaca Island – Baixo Danae	13	
Bazeruto Island Archipelago	14	
Pemba Bay		6
Mtwara		5

Table 2. *A. austera* haplotypes distributed amongst sampled sites along the east African coastline (Tmr1 = Two-mile reef site 1, Two-mile reef site 2, Bd = Baixo Danae, BV = Barreira Vermelho, Bztmr = Bazeruto Two mile reef and Bzlh = Bazeruto Lighthouse reef).

Haplotype:	Tmr1	Tmr2	Bd	Bv	Bztmr	Bzlh
Hap_1	1		1	1	1	1
Hap_2	7	4	8		6	2
Hap_3	1				1	
Hap_4	1	2	2		1	
Hap_5		1	1		1	
Hap_6		1	1	1		
Hap_7				1		
Hap_8				2	1	
Hap_9				1		
Π	0.711	1.821	1.410	5.8	2.764	0.667

For *A. austera,* analysis of molecular variance (AMOVA) carried out in Arlequin 3.11 (Excoffier *et al* 2007) yielded $F_{ST} = 0.18$ ($p < 0.01$).
Barreira Vermelho (BV) was, however, found to show significant pairwise Fst values; when it was removed from the analyses the AMOVA showed no significant structure at any level. DNAsp was used to calculate the average number of migrants per generation ΔNm (Nei 1982) = 0.71 and the inter-populational gene diversity γst = 0.26. A minimum spanning network (MSN) was constructed to illustrate relationships between haplotypes and their distribution amongst populations (Fig. 2).

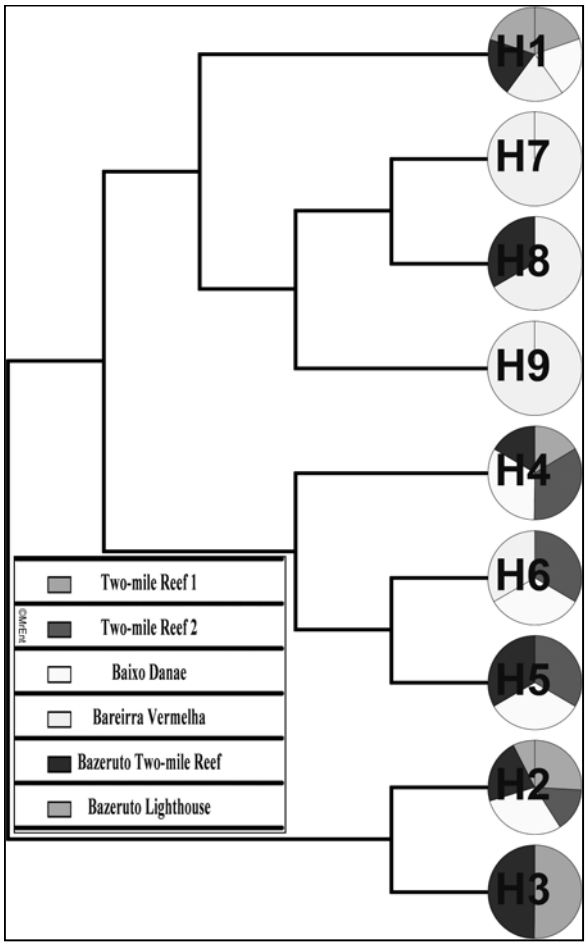

Figure 2 Minimum spanning network shows relationships between *A. austera* nuclear intron haplotypes and collection localities in the south-west Indian Ocean.

Using mismatch distributions (Fig. 3) we found that the Inhaca Island population of BV may have established equilibrium in terms of growth, in contrast to other populations (Rogers and Harpending 1992).

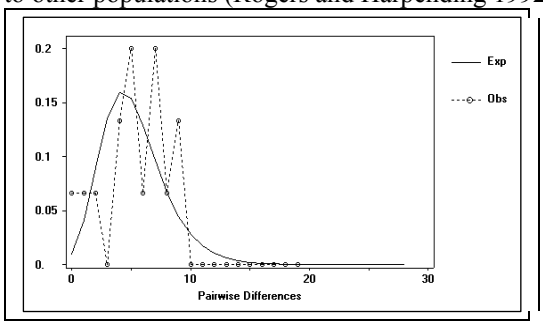

Figure 3. Pairwise mismatch distribution for population BV, generated in DNAsp (Rozas *et al* 2003).

Upon removing BV from further analyses, we found Nm = 3.87 and γst = 0.06, indicating that the remainder of the populations were less differentiated

from one another than they were from BV (Hudson *et al.* 1992). Genetic fixation indices calculated in AMOVA for *P. daedalea* were not significant. The proportional inter-population gene diversity was found to be γst = 0.29 with Nm = 0.63. We found evidence for a minimum of two recombination events within the ITS region of *P. daedalea*.

Discussion

Nuclear genetic markers from *A. austera* and *P. daedalea* showed markedly different levels of variation amongst the populations sampled, which may be attributed to the different evolutionary constraints under which they evolve. Analysis of sequence variation in the intron that was used to compare populations of *A. austera* showed that populations regularly exchange genes, with the exception of the population of Barreira Vermelho (BV), which may be isolated from the other local populations. Genetic diversity (which may be equated to nucleotide diversity) was particularly high (π = 5.8) within the BV population in comparison with other local populations (Tab. 2). This may be a consequence of the predominant current patterns offshore of Maputo Bay and the Delagoa Bight (Fig. 1) (Lutjeharms 2006), which circulate bay waters in a clockwise eddy. These local currents may inhibit the spread of propagules out of this breeding population. Pairwise F_{ST} values indicated that BV was significantly distinct from other populations sampled. Further, other *A. austera* populations in the study appear to differ in growth pattern from those at BV (Fig. 3). Once BV was removed from analyses, further tests of variability demonstrated that the remaining *A. austera* populations were panmictic. Haplotypes were shared widely between populations and there was no evidence of reciprocal monophyly (Fig. 2). This was similar to results of previous regional work on *Pocillopora verrucosa,* which showed local panmixia (Ridgway *et al.* 2001).

Local *A. austera* populations are expected to rely on migrants from the north to maintain levels of genetic diversity, in a similar fashion to that reported for *Pocillopora verrucosa* (Ridgway *et al.* 2008). This may be confirmed by a larger scale analysis which includes more populations from this region. With longer-lived coral species, such as *P. daedalea* (in comparison to *A. austera)*, it is possible that there may be enough migration to sustain a high level of genetic homogeneity throughout a larger area, as was found for *Plesiastrea versipora* in the western Pacific (Rodriguez-Lanetty and Hoegh-Guldberg 2002). However, although *P. daedalea* populations are well differentiated (γst = 0.29), the ITS region is notoriously difficult to work with (Lam and Morton 2003; Vollmer and Palumbi 2004, Mangubhai and Souter 2007) and regions with such high recombination rates may not yield conclusive results at this scale. Thus, other than to encourage further research using more conclusive molecular markers and larger population sizes, little may be drawn from analyses of the ITS region of *P. daedalea* in this study.

Southward movement the water is very gradual ($0.05ms^{-1}$ south) in the north of the study area, but the current is fast flowing from between Inhaca and Maputaland ($1.5ms^{-1}$ south, Lutjeharms 2006). These currents do not necessarily preclude movement of propagules in a northerly direction in nearshore water movement. East African reefs are thus unlikely to be connected to the same extent amongst all genera considering the different reproductive strategies amongst local scleractinians (Mangubhai and Harrison 2006). Population genetic studies of species with very different life strategies, such as *A. austera* and *P. daedalea,* will help to shed light on this issue, especially if fine-scale variability within and between populations is measured. Future research in the SWIO region would benefit from employing molecular markers which display the high resolution necessary to detect this variability.

Acknowledgements

AM thanks SAAMBR and colleagues, AM Perreira, L Celliers, T. Ridgway and the UQ CMS, WIOMSA, the SANRF, UKZN and colleagues at the CONSPEC lab and Transmap (European Commission-funded within the Sixth Framework Programme, project INCO-CT-2004-510862).

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