Connectivity of two scleractinian corals in the south west Indian Ocean.

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ABSTRACT

Generations of hard corals have built the complex reef ecosystems that harbour a huge diversity of sea-life in the world's shallow tropical oceans. These undergo both sexual and clonal reproduction, and may contain signatures in their genomes which help to decipher the riddles of past population dynamics and evolutionary history. Two species of coral, *Acropora austera* and *Platygyra daedalea*, were collected from sites along the east African coastline from Kenya in the north to Maputaland, South Africa in the south, and from the Chagos Archipelago. Sequences of two different DNA regions were tested, in a preliminary study, for their potential ability to elucidate connectivity and differentiation among these coral populations. These were the nuclear ribosomal ITS region of *P. daedalea* populations, and a previously-unused marker, the carbonic anhydrase 3/550 nuclear intron of *A. austera*. These molecular markers indicated high levels of connectivity amongst populations in a preliminary study based on limited sample sizes and a subset of populations. It was decided to further explore the variability of the carbonic anhydrase 3/550 intron, which showed evidence of subdivision and structuring within Mozambique populations relative to South African populations, in a study in which both the sample size per site and the number and range of sampled sites were increased. ITS sequences, although highly variable, revealed no population differentiation in *P. daedalea;* STR markers were used in subsequent studies of population differentiation in this species. Populations of both *A. austera* and *P. daedalea* showed signs of high connectivity along the region of the coastline sampled in this study. However, there appeared to be a disjunction in ecological connectivity between reefs in Maputaland, South Africa and those in southern Mozambique, between Durban and Maputo where the Agulhas Current originates. This was reinforced in *A. austera* populations which displayed a region of genetic discontinuity between Inhaca Island and Maputaland reefs of the central reef complex, in the region of Rabbit Rock. Northern reef complexes also harboured unique haplotypes in contrast to southern reefs which shared all haplotypes with those in the north, an indication that northern reefs have seeded the southern (Maputaland) reefs. *P. daedalea* populations appeared evolutionarily panmictic over scales relevant to this study. Evidence for fine-scale structure indicated that populations were separated from one another over ecologically relevant time-scales. These populations were defined by both their habitats and their sampling location. There was a possibility that the *Platygyra* species complex included cryptic species that were not distinguishable from *P. daedalea*. However, the disjunction in the connectivity between northern and southern population groups was also evident in the population structure of *P. daedalea*. There was a net immigration of propagules of both *P. daedalea* and *A. austera* into populations north of the disjunction between groups, where the prevailing current regime is dictated by the Mozambique Channel eddies. In contrast populations to

the south of the disjunction (the southern population group) which are subject to the swiftly flowing Agulhas Current, showed a net emigration of propagules from Maputaland reefs. These emigrants were likely to be lost to inhospitable habitat south of the marginal Maputaland region. Although there was evidence for migration of both *Platygyra* and *Acropora* propagules between the Bazaruto Archipelago reefs and certain Maputaland reefs, genetic exchange between Mozambique and Maputaland reefs appeared to be limited and may have occurred primarily at evolutionary rather than demographic levels. Managers may need to treat the regional Maputaland reefs as separate stocks and manage them accordingly, as the relative isolation of these corals in the central and southern reef complexes in Maputaland, South Africa, means that they are at risk to losing species to evolutionary extinction. It is also important that reef health in northern Mozambique and Tanzania is maintained as, despite evidence of a break in demographic connectivity, between reefs in these regions and those in Maputaland, there was evidence to suggest that reefs were connected at evolutionary scales, thus maintaining levels of genetic diversity on southern African reefs.

for my wife Paula,
my son Joseph,
and my parents, Susan and Ian,
with lots of love.

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PREFACE

The experimental work described in this thesis was carried out in the Oceanographic Research Institute and the School of Biological & Conservation Sciences, University of KwaZulu-Natal, Durban, from June 2005 to July 2010, under the supervision of Professor M.H Schleyer and Professor J. Lamb.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1
Chapter 2: Macdonald AHH, Lamb J, Schleyer MH. (2009) South east African, high-latitude coral communities, a canary for western Indian Ocean coral reefs? Proc. 11th International Coral Reef Symp. Ft. Lauderdale, Florida, 7-11 July 2008 Session number 14
AHH Macdonald conducted legwork, experimental work and was lead author. Prof J. Lamb assisted in authoring the paper and provided funding for experimental work and Prof. MH Schleyer assisted in authorship, contributed funding to the experimental work and contributed legwork in sampling material.

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Publication 3
Chapter 4: Macdonald AHH, Lamb J, Schleyer MH (In prep.) Population structure of *Platygyra deadalea* from the south-east African coastline. In preparation.
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Publication 4
Chapter 5: Macdonald AHH, Lamb J, Schleyer MH (In prep.) Marine protected area-based management of hard-coral communities in the south west Indian Ocean. In preparation.
AHH Macdonald conducted legwork, experimental work and was lead author. Prof. J. Lamb assisted in authoring the paper and provided funding for experimental work and Prof. MH Schleyer assisted in authorship, contributed funding to the experimental work and contributed legwork in sampling material.

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1. Introduction

1.1 Hard corals

Hard, reef-building corals (Cnidaria, Anthozoa, Scleractinia) are simple metazoans with an internal calcium carbonate skeleton that is deposited layer upon layer to create large colonies, and ultimately reefs in shallow tropical oceanic waters. An individual coral colony consists of a multitude of polyps feeding heterotrophically and providing the organism with nutrients. Corals are, however, successful in competition with other benthic invertebrates because algae living within the tissue of the organism produce carbohydrates autotrophically. This symbiotic relationship with the algal genus *Symbiodinium* has developed into a successful strategy for dominating the shallow oligotrophic benthic habitat and has facilitated the development of the most biodiverse habitats in the world's oceans. Coral reefs are constrained by a narrow set of environmental variables (Bellwood and Hughes 2001) which are being affected by global warming (Hoegh-Guldberg 1999, Hoegh-Guldberg *et al*. 2007). For this reason corals are declining in abundance (Bruno and Selig 2007) and hard, reef-building corals are the subject of much scientific scrutiny and concern.

1.2 Western Indian Ocean reef corals

The western Indian Ocean (WIO) is home to a rich diversity of hard (scleractinian) and soft (alcyonacean) corals, the former comprising the basis for coral reef-systems in the shallow, warm waters of the region. These coral reefs and their associated resources are important to human communities throughout the WIO both for exploitation (food security and other resources) and for income through non-extractive use (van der Elst *et al*. 2005, Schleyer and Tomalin 2000). Around 50% of local fisheries production in areas with coral reefs may be attributed, either directly or indirectly, to coral reefs (Linden *et al*. 2002). Non-extractive income attributable to coral reefs, such as that derived from tourism, also contributes significantly to local community income and is negatively affected by damage to reefs (Cesar *et al*. 2002). Fishing malpractice in various forms such as dynamiting, the indiscriminate use of poisons, seine-netting, trawling and over-fishing has led to the decline of catches and the destruction of coral reef habitat throughout the region (Wells and Ngusaru 2004; Linden *et al*. 2002; McClanahan *et al*. 2000). Coral reef health is deteriorating both in this region, the Indian Ocean and globally. Eutrophication of previously-oligotrophic waters, arising from an influx of terrestrial sediment due to deforestation, poor agricultural practice

and inadequate land resource management threatens reefs already damaged by man (Obura *et al.* 2002). Global climate change is a further threat to coral reef health. Recently, during an El Nino Southern Oscillation (ENSO) Event, reef systems around the world manifested stress as water temperatures rose above the monthly average means (16% of the worlds reefs were destroyed in the 1997-98 ENSO) (Wilkinson 2002). Signs of coral-bleaching became evident on reef systems world-wide and, although there has been subsequent recovery in many, up to 100% of the coral died on others (Wilkinson 2002). Certain reef systems in the WIO were amongst the most damaged, with 100% hard coral cover mortality in places (Linden *et al*. 2002). Although some have shown evidence of recovery, this has been irregular within and amongst regions (Tamelander 2002; Obura *et al.* 2002). Combinations of anthropogenic stressors such as eutrophication and blast fishing, and indirect stressors such as global climate change, are causing reef productivity to decline and recovery to slow (McClanahan *et al*. 2000; Tamelander 2002). Observations over the last 50 years indicate that reef-coral health is deteriorating worldwide (Bruno and Selig 2007), and current trends suggest that these systems will no longer be able to sustain the exploitation to which they are currently subjected (Hoegh-Guldberg *et al*. 2007).

Reefs on the east African coastline range from true accretive reef systems in the north to reef-coral communities in the south. South Africa's coral communities were relatively unscathed in recent global declines of reef health (Schleyer *et al*. 2008); it was evident during past bleaching events that levels of bleaching on Maputaland reefs were significantly lower than those which occurred elsewhere in the Indian Ocean (Wilkinson 2000, 2008). The reason for this is unclear, but the possibility that the health of South Africa's reefs may be influenced in the long-term by reefs further north has not been wholly discounted, and evidence is accumulating that these coral reefs are connected to distant reefs and communities (van Oppen *et al*. 2008).

The northern reef-coral resources of Kenya, Tanzania and Mozambique are subject to little management and high levels of extractive effort from local subsistence-based communities (van der Elst *et al*. 2005). If these havens of biodiversity are not managed sustainably there is a risk that their resources may become depleted beyond the point of recovery in the short-term (on time-scales affecting humans), and may not even recover completely in the long-term (Law and Morton 1996). Thus, the appropriate management of coastal and oceanic resources is vital to their perpetuity. The creation of marine protected areas (MPAs) has been identified as a suitable management method. Both South Africa and Tanzania have committed to increasing current MPA coverage of their coastlines to 20% (Wells and Ngusaru 2005). Tanzania has established a timeline and hopes to accomplish this goal by 2025. Currently less than 2% of the study area is covered by MPAs; there are plans to increase this with the goal of protecting regions that encompass high levels of biodiversity (Wells and Ngusaru 2005). However, knowledge of the spatial genetic structure of

constituent populations is necessary if marine organisms are to be protected adequately (Palumbi 2003, Jones *et al.* 2007).

1.3 Population genetics of marine invertebrates

Populations of marine organisms are more genetically structured at spatial scales of hundreds of kilometres than would be expected of organisms that are dispersed in oceanic currents during larval development (Palumbi 2003), and are therefore likely to manifest irregular patterns of recruitment and dispersal (Grosberg and Cunningham 2001). Most of these animals disperse during the larval stage in their life cycle, when they are difficult to track. Thus very little is known about their dispersal and recruitment strategies, which are crucial to an understanding of connectivity between populations. If individual larval taxis is considered to have little influence on dispersal, then ocean currents and near-shore water movement may be considered to be of the utmost importance (Palumbi 2003). Certain larvae, however, are active swimmers and are able to detect suitable areas for recruitment (Fadlallah 1983). Using physical parameters alone in considering larval dispersal may thus be invalid (Miller and Ayre 2008). As tracking larvae in the ocean is difficult, another mode of monitoring dispersal is necessary, especially in light of recent studies that relate population genetic structure to larval dispersal potential (see Ayre and Hughes 2000, Baums *et al.* 2005 and Miller and Ayre 2008).

The best way of monitoring dispersal would be one that considers successful recruits and clarifies the relationships between individuals within meta-populations at different sampled reefs. In the case of sessile marine invertebrates, the location of mature individuals is generally an indication of the original site to which they recruited as larvae; although fragmentation may occur in some instances, relationships between individuals in different populations should be assessed in this light. There are, however, limitations to this approach of sampling adults (Ayre and Miller 2004, Miller and Ayre 2008). Discontinuities between life-history traits and observed genetic heterogeneity in the wild (Ayre and Miller 2004) illustrate the need for exhaustive research before drawing conclusions about modes of reproduction. Nevertheless, it is important to take the life strategies of marine organisms into account when devising programmes for their protection. One reason for this is that marine organisms are vulnerable to disturbances at different stages in their ontogeny. Both hard and soft corals have a larval stage during which dispersal may occur (Fadlallah 1983). It is important to consider the pelagic dispersal of larvae when designing MPAs for these organisms, as large-scale distribution of propagules (between communities) may occur in this stage.

Traditionally, tagging systems have been used to gather data on ontogenic displacement of cohorts and to aid studies of recruitment of marine vertebrates (Thorrold *et al.* 2002). As this is

difficult in the case of marine invertebrates, and perhaps not possible for scleractinian corals, other methods must be used for the assessment of population dynamics. In this study, variability in genetic composition was used as a naturally-occurring marker for assessment of the spatial distribution of corals. Such data allows inferences, based on evolutionary theory, about colonization events, population demographics and linkages between discontinuous populations (Hellberg *et al.* 2002, Palumbi 2003). Marine organisms have been the subject of a number of systematic- and population-oriented studies using genetics as the basis for comparison (Tab. 1.1).

Research on the spatial relationships between populations of hard corals is proliferating rapidly (Baums *et al.* 2005; Chen *et al.* 2002; Gutierrez-Rodriguez and Lasker 2004; MacKenzie *et al.* 2004; Whitaker 2006; Magalon *et al*. 2005; Nishikawa and Sakai 2005; Nishikawa *et al.* 2003; Goffredo *et al.* 2004; Ng and Morton 2003; Takabayashi *et al.* 2003). Finding good molecular markers in the Cnidaria has, however, proven difficult as they manifest low rates of molecular evolution in genes popularly used to assess relationships in other phyla (Macdonald 2005; Marquez *et al.* 2003a; 2003b; Knowlton 2000, Eytan *et al.* 2009). Research in this field was initially based on allozyme markers (Stoddart 1984, Ayre and Hughes 2000), moved to DNA markers that have demonstrated usefulness in other orders (Shearer *et al*. 2002) and is currently centered on nuclear DNA markers, the most popular being simple tandem repeats (STRs or microsatellites) (see Tab. 1.1). The most recent research in this field indicates that microsatellites are useful in determining variability within and between populations of Scleractinia (Miller and Howard 2004; Baums *et al.* 2005a; 2005b; Chen *et al.* 2002; MacKenzie *et al.* 2004, Magalon *et al*. 2005, van Oppen *et al.* 2008, Underwood *et al*. 2009). It has also been shown that microsatellites may be inherited in a Mendelian fashion in certain of the Scleractinia (Baums *et al.* 2005a). The levels of variation are high enough to show differences between individuals in a population and, as they are inherited in a predictable fashion, inter-population dynamics may be considered and compared to Hardy-Weinberg proportions. For example, Baums *et al.* (2005b) found that Caribbean acroporids comprised two distinct macro-populations and recommended that these groups be separately managed. Magalon *et al.* (2005) found that populations of *Pocillopora meandrina* showed levels of genetic differentiation that are congruent with current knowledge of larval dispersal. Possible re-colonisation events in these corals have led to some unusual population structures and should be considered in the formulation of future management initiatives.

1.4 Population genetics of scleractinians

Population genetic investigations of corals have largely made use of allozymes (Tab 1.1), whereas such work in other taxa has moved on to more explicit, definitive markers which are better understood in terms of inheritance and linkage. Allozyme studies continue to be popular in coral

research because many of the markers used in other taxa have proven ineffective when applied to hard corals. Until recently, there has been a lack of studies of coral population genetics due to lack of effective markers, difficulty in accessing and sampling scleractinian populations and expenses associated with this type of molecular study (Tab 1.1).

It is apparent from Table 1.1 that there is a need for more genetic studies of reef coral populations in the WIO region, using a combination of nuclear markers for more accurate assessment of population structure. Nuclear markers which exhibit high levels of variation, such as nuclear ribosomal internal transcribed spacer (ITS) sequences, microsatellites and single copy nuclear intron sequences have recently been used to investigate the diversity and connectivity of populations of a number of scleractinian genera (Tab. 1.1). It is therefore possible to select species with different life strategies to contrast general levels of connectivity amongst coral reefs.

This study focuses on two coral species, *Platygyra daedalea* and *Acropora austera*, that are distributed throughout the WIO (Veron 2000), and for which there are well-developed genus-level molecular-markers (Maier *et al.* 2001; Takabayashi *et al.* 2003, Severance *et al.* 2004; Magalon *et al.* 2004; Mackenzie *et al.* 2004; Baums *et al.* 2005a, Wang *et al*. 2008). Miller and Howard (2004) developed STR markers for both *Platygyra daedalea* and *Goniastera favulus*; of the 27 STR markers they identified, only ten (five in each) were effective possibly because, as Marquez *et al*. (2003a) suggest, STRs may be rare in scleractinians. Subsequent to the experimental work in this study, further microsatellites isolated from the *Acroporidae* were found to be largely tri-nucleotide repeats rather than di-nucleotide repeats (more common in other taxa) (Wang *et al*. 2008).

Hypervariable intron sequences from single-copy nuclear DNA have been used in studies of fine-scale genetic differentiation amongst acroporids (Vollmer and Palumbi 2007, van Oppen *et al*. 2002). These markers have the advantage of being neutral, co-dominant and coral-specific, without the disadvantages associated with multi-copy regions. The data generated may be examined using standard F-statistics as for allozyme data.

Table 1.1 Population genetic studies of scleractinian corals pertinent to this study of *Acropora austera* and *Platygyra daedalea* in the western Indian Ocean.

Species	Scale (km)	Current velocity ($1.5ms^{-1}$)	PLD (hrs)	Fixation index (F_{ST})	Region	Marker system	Reference
Agaricia agaricites	1000	NR	NR	0.139	Carribean	AFLP	Brazeau *et al.* 2005
Acropora hyacynthis, A. cytherea, A. tenuis	100	NR	NR	0.025 (AcAh), 0.427	GBR, Australia	Allozymes	Marquez et al. 2002
Acropora millepora , A. valida, A. cytherea, A. hyacinthus, A. cuneata, A. palifera, Pocillopora damicornis, Seriatopora hystrix, Stylophora pistillata	1000	NR	NR	0 0 0 0 0 0 0 0.15 0.09	GBR, Australia	Allozymes	Ayre and Hughes 2000
Acropora nasuta, Stylophora pistillata	1000	1.5	NR	0.015-0.066, 0.142-0.215	Ryukyu's, Japan	Allozymes	Nishikawa *et al*. 2003
Acropora valida, Pocillopora damicornis, Seriatopora hystrix, Styllophora pistillata, Acropora cuneata	1000	NR	NR	0.2	GBR, Lord Howe Island, Australia	Allozymes	Ayre and Hughes 2004
Balanophyllia elegans	1000	NR	NR	0.283	Pacific, USA	Allozymes	Hellberg 1994

Goniastrea aspera	1000	NR	NR	NS.	West Pacific	Allozymes	Nishikawa and Sakai 2005
Mycedium elephantotus	1000	NR	NR	0.155	West Pacific	Allozymes	Dai *et al*. 2000
Species	Scale (km)	Current velocity ($1.5ms^{-1}$)	PLD (hrs)	Fixation index (F_{ST})	Region	Marker system	Reference
Pocillopora damicornis	10	NR	NR	0.102	HLR, Australia	Allozymes	Miller and Ayre 2004
Pocillopora damicornis	10	NR	NR	0.044	GBR, Australia	Allozymes	Ayre and Miller 2004
Pocillopora damicornis, Goniastrea australensis	1000	NR	NR	0.24 0.00	GBR, HLRs, Australia	Allozymes	Miller and Ayre 2008b
Pocillopora verrucosa	100	1.5	30	NS.	South Africa	Allozymes	Ridgway *et a*l 2001
Platygyra sinensis	100	NR	NR	0.017	West Pacific	ITS rDNA	Ng and Morton 2003
Acropora cervicornis		NR	NR	0.130 - 0.067	Caribbean	mDNA nDNA	Vollmer and Palumbi 2007
Acropora austera	1000	0.05-1.5	144	0.079	Southern Mozambique, South Africa	scnDNA	Macdonald *et al* 2009
Acropora nasuta	1000	NR	NR	0.02	GBR, Australia	scnDNA	Mackenzie *et al*. 2004
Acropora palmata	1000	NR	NR	0.036	Caribbean	STRs	Baums *et al*. 2005b
Acropora tenuis, Seriatopora hystrix	1000	NR	NR	0.034 0.234	East Indian Ocean, Australia	STRs	Underwood *et al*. 2009

Goniastrea favulus, *Platygyra daedalea*	<10	NR	NR	0.199 0.19	GBR, Australia	STRs	Miller & Ayre 2008a
Platygyra daedalea	1000	>1	66	NS.	Kenya, Tanzania	STRs	Souter and Grahn 2008
Species	Scale (km)	Current velocity ($1.5ms^{-1}$)	PLD (hrs)	Fixation index (F_{ST})	Region	Marker system	Reference
Platygyra daedalea	2500	0.05-1.5	66	0.049	Kenya, Tanzania, Mozambique, South Africa	STRs	Chapter 4
Pocillopora damicornis	1000	NR	NR	0.023	East Africa	STRs	Souter *et al*. 2009
Pocillopora meandrina	2000	NR	NR	0.02 – 0.16	South Pacific	STRs	Magalon *et al*. 2005
Pocillopora verrucosa	1000	0.05-1.5	30	0.054	Southern Mozambique, South Africa	STRs	Ridgway *et al* 2008
Seriatopora hystrix	1000	NR	NR	0.201	GBR, Australia	STRs	Van Oppen *et al.* 2008
Seriatopora hystrix	100	NR	NR	0.095	East Indian Ocean, Australia	STRs	Underwood *et al*. 2007
Seriatopora hystrix	1000	NR	NR	0.089	Red Sea	STRs	Maier *et al.* 2005
Montastraea annularis, *M. faveolata*		NR	NR	0.38 NS.	Caribbean	STRs and RFLP	Severance and Karl 2006

PLD = pelagic larval dispersal (Harii *et al*. 2002, Miller and Mundy 2003, Harrison and Wallace 1990); NR = not reported.

1.5 Population genetics of scleractinians in the western Indian Ocean region

Population connectivity of both vertebrates and invertebrates from the WIO has been investigated in several studies (Grant *et al.* 1992, Ridgway *et al.* 1998, Forbes *et al.* 1999, Fratini and Vanini 2002). The genetics of scleractinian populations in the WIO have been examined using allozyme (Ridgway *et al.* 2001) and microsatellite (Souter and Grahn 2008, Ridgway *et al*. 2008, Souter *et al*. 2009) marker systems. Nevertheless, the region remains understudied at best, and there is little understanding of regional patterns of connectivity. Ridgway *et al*. (2001; 2008) have studied P*ocillopora verrucosa* exclusively, using allozymes in the Maputaland area and STRs in a study including a population from Bazaruto Island. Souter and Grahn (2008) showed that there were genetic differences between island and lagoonal populations of *Platygyra daedalea* in Kenya and northern Tanzania, and in a subsequent study Souter *et al*. (2009) have shown that *Pocillopora damicornis* populations are subject to fine-scale differentiation in Kenya and Tanzania. Only Ridgway *et al*. (2008) have studied a scleractinian population (of *Pocillopora verrucosa*) in Mozambique.

Between the diverse accretive reefs of Kenya and Tanzania and the marginal reefs of Maputaland, South Africa lie large tracts of coral reef in Mozambique. These reefs may prove to be important intermediate stepping-stones (Hellberg *et al*. 2002) which provide a pathway for northern reefs to supply southern reefs with genetically-diverse propagules and population subsidies. However, with only one study of scleractinian population genetics (*Pocillopora verrucosa*) covering this region (Ridgway *et al*. 2008), little is known about the diversity and importance of these reefs from this perspective. This study of coral population genetics will attempt to ascertain the health of some Mozambican reefs and their role in maintaining Maputaland coral reef communities.

1.6 Aims of study

Understanding reef-coral population dynamics of the WIO at inter- and intra-population levels will help to illustrate general patterns of exchange of coral larvae and the locations of any barriers to dispersal. The aim of this study is to assess the dynamics of reef coral populations from the Chagos Archipelago, Kenya, Tanzania, Mozambique and South Africa using molecular markers and appropriate evolutionary methods of analysis. This research is intended to complement previous research on scleractinia from the region (Ridgway *et al.* 2001; 2008,

Souter and Grahn 2008, Souter *et al*. 2009). It was designed to establish whether the current marine protected area systems are adequate for sustaining coral genetic diversity and connectivity, whether management within established reserves is adequate or whether more marine protected areas should be designated. Importantly, this study examines an area spanning approximately 6750 km, extending from Maputaland, on the north east coast of South Africa to the Chagos Archipelago and covering 3250 km of the coastline of east Africa (Fig. 1.1). This comprises a substantial area for the examination of patterns of connectivity between populations. Considering modern trends in the development of MPA networks, this study may help elucidate whether current MPA distribution on the east African coast is adequate for the long- and short-term maintenance of genetic diversity amongst scleractinian populations.

1.7 Thesis structure

Chapter 2

The initial work was based on some of the sites sampled at the beginning of the project and may be regarded as an exploratory study. It explores the utility of nuclear sequence markers, namely the nuclear ribosomal ITS1-5.8SrRNA-ITS2 region of *Platygyra daedalea* and the carbonic anhydrase 3/550 intron of *Acropora austera* for studies of genetic variation at intra-population level. This initial exploration of data was followed by a more extensive study of genetic variability in both taxa (see Chapters 3 and 4).

Chapter 3

This comprises a study of genetic diversity and connectivity between populations of *A. austera* based on the carbonic anhydrase 3/550 nuclear intron sequence marker. The sample size was increased over that reported on in Chapter 2 for existing sites, and other east African sites were included in the sample. The sequence alignment was analysed to measure genetic diversity, connectivity and population structure amongst sites. Breaks in population connectivity and regional centres of diversity were identified.

Chapter 4

Microsatellite primers developed for *P. daedalea* were used to amplify five loci from 350 specimens collected thoughout the study area. Deviations from Hardy-Weinberg Equilibrium, population structure and rates of immigration were measured.

Chapter 5

Trends in migration and connectivity revealed in the experimental studies on *Acropora austera* and *Platygyra daedalea* are analysed in conjunction with data from other studies on scleractinian genetics and current patterns in the WIO. These are interpreted in terms of the need for and effectiveness of currently existing MPAs.

Chapter 6

This comprises a summary and overall conclusions and recommendations for the management of scleractinian coral in this region.

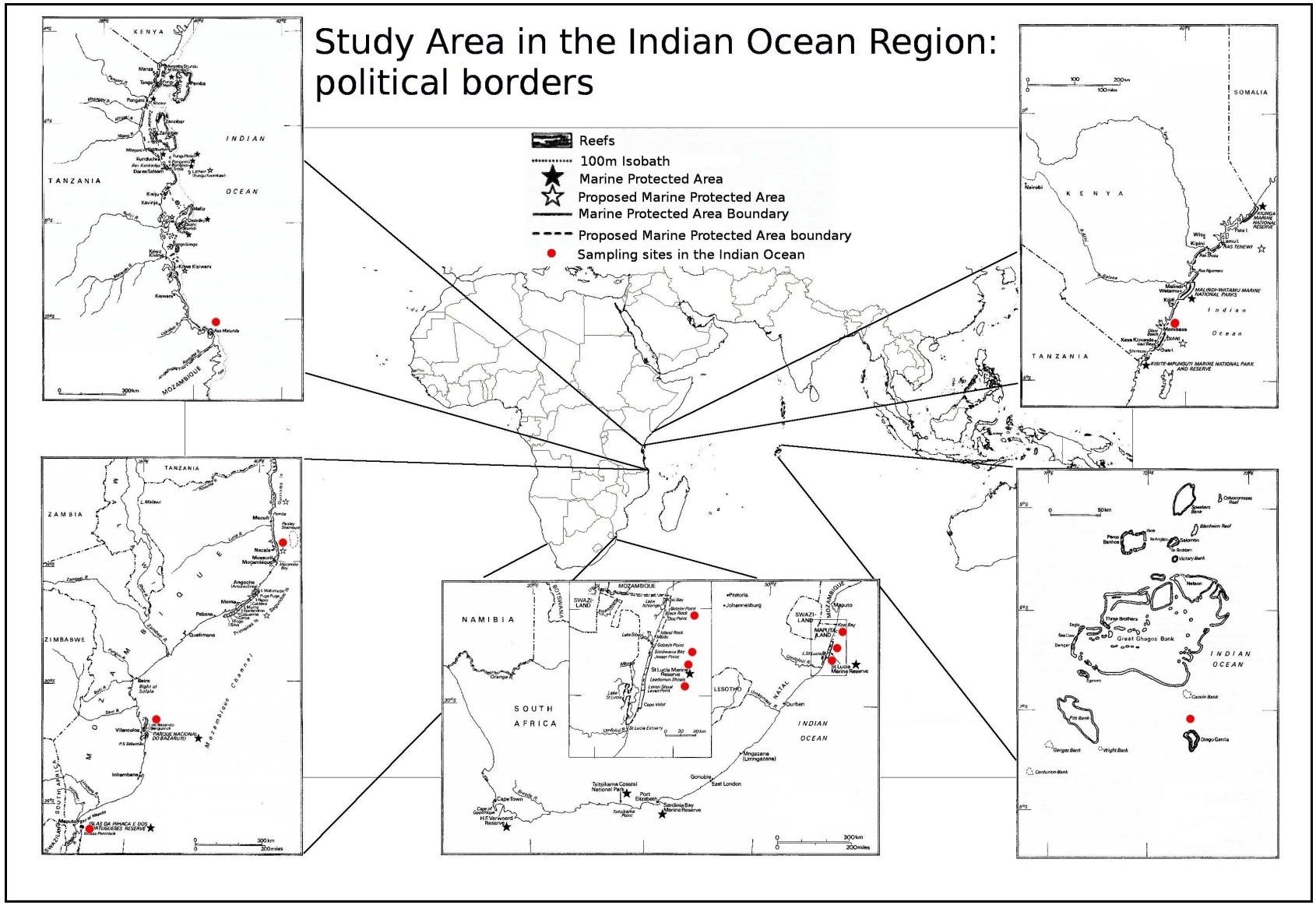

Figure 1.1 Study area in the Indian Ocean showing political boundaries, marine protected areas and sites from which *Acropora austera* and *Platygyra daedalea* samples were collected (Maps: UNEP/IUCN 1988).

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

2.1 Summary

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 genetic diversity and relatedness were investigated at varied geographic scales using molecular methods. Genetic diversity may be used as a proxy to gauge both the population dynamics and resilience of a community and, in this exploratory study, was measured in two corals with different reproductive modes, larval dispersal capabilities and life history strategies. DNA sequences of the nuclear carbonic anhydrase 3/550 intron region of *Acropora austera* and the nuclear ribosomal ITS region of *Platygyra daedalea* were used to estimate the genetic variability within and between populations of these species. A total of 51 *A. austera* sequences comprised 9 haplotypes, with a haplotype diversity of 0.697. *Acropora austera* populations at Inhaca Island harboured the highest levels of unique haplotypes and appeared to have attained genetic equilibrium. There was also evidence of regular genetic exchange between *A. austera* populations in the region with a large number of individuals sharing haplotypes between populations despite significant measures of population structure (F_{ST} = 0.18). Analysis of the nuclear ribosomal ITS sequences of 22 *P. daedalea* specimens showed high-variability; comprising 14 haplotypes with a haplotype diversity of 0.939. There was no evidence of population genetic structure (F_{ST} no different from zero), indicating that these populations were panmictic. Local oceanography dictates that northern reef systems may be considered the source populations of those in the south. With levels of genetic exchange between many populations of both *A. austera* (ΔNm = 0.71 - 3.87) and *P. daedalea* (ΔNm = 1.31) greater than one individual per generation, it appears that populations of *A. austera* and *P. daedalea* may be reliant on subsidies from other reefs for maintenance of diversity. It was decided not to pursue the ITS sequencing investigation of *P. daedalea* owing to a lack of resolution, uncertainty in interpretation of the results due to potential recombination within these repeated sequences, and the availability of STR primers for *Platygyra*.

2.2 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 (Avise

1998). 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 showed significant levels of population structure (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 between life strategy and 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 is thus 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 western Indian Ocean (WIO) (Ridgway *et al.* 2008; Souter and Grahn 2007; Mangubhai *et al.* 2007; Macdonald *et al.* 2008). Only one study, Ridgway *et al*. (2008), has focused on the SWIO and these authors sampled only a single Mozambican population. Mozambique is host to a great diversity of hard corals and thus represents a gap in contemporary knowledge of scleractinian connectivity.

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. 2.1). These diverse communities comprise approximately 90 species of hard and soft coral (Schleyer 2000). Further north, the Bazaruto Archipelago also supports a diverse assemblage of corals on accretive reef systems. A gradient of species diversity decreasing from north to south (Obura 2000), combined with predominantly southerly offshore currents in the Mozambican channel ($0.05 m.s^{-1}$, 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. Currently less than 2% of the coast is under any sort of management (Wells and Ngusaru 2004), although regional authorities are developing management plans for local exploited resources.

In this study, the east African coastline was sampled at a number of sites between northern Maputaland, South Africa and southern Tanzania. Two corals were sampled: *Acropora austera* is a widespread coral with a broadcast-spawning reproductive strategy common to the scleractinia (Carroll *et al*. 2006). *Platygyra daedalea,* a Faviid, is also a broadcast spawner but 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).

The hypervariable carbonic anhydrase 3/550 intron (Ridgway unpublished data), similar to that developed by Concepcion *et al.* (2007), was tested for use in assessments of genetic diversity and structure of *A. austera* specimens. The ITS1-5.8S-ITS2 region of *P. daedalea* was sequenced to assess its utility in assessments of genetic diversity and structure amongst east African populations of this species. ITS rDNA has been reported to be 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 thus tested ITS sequences for use with *Platygyra* sp., as they have been used in previous studies for this purpose (Lam and Morton 2003), whereas it has been shown that the ITS region is an unreliable population-level marker in the *Acroporidae* (Vollmer and Palumbi 2004). The carbonic anhydrase intron has been demonstrated to be a single-copy region and so appropriate for use in genetic analyses of the *Acroporidae* (Ridgway unpublished data).

The aim of the work reported on in this chapter was to obtain an initial estimate of the genetic diversity and structure of populations of *Acropora austera* and *Platygyra daedalea* in the SWIO. This preliminary study was also designed to assess the utility of the novel carbonic anhydrase 3/550 nuclear intron marker and the nuclear ribosomal ITS region in assessing genetic diversity and structure in *A. austera* and *P. daedalea* respectively. We expect that *A. austera* will comprise a single large population in the study area as it is fast-growing and "weedy" in nature, whereas we expect that *P. daedalea* will comprise several populations as it is comparatively slow-growing and does not spread by fragmentation.

2.3 Materials and methods

All coral specimens were identified in the field according to Veron (2000). Corals were sampled from sites along the east African coast, using SCUBA and snorkel diving (Tab. 2.1). Care was taken to avoid the collection of clone-mates by sampling colonies separated by at least 5m (Ridgway *et al.* 2001). Samples were immediately stored in either a dimethyl sulfoxide (DMSO) salt buffer (0.25 M EDTA; 20% (v/v) DMSO, saturated with NaCl) or 70 % ethanol. All DNA was isolated using a Fermentas Life Sciences^{TM} genomic DNA purification kit as per

their extraction protocol.

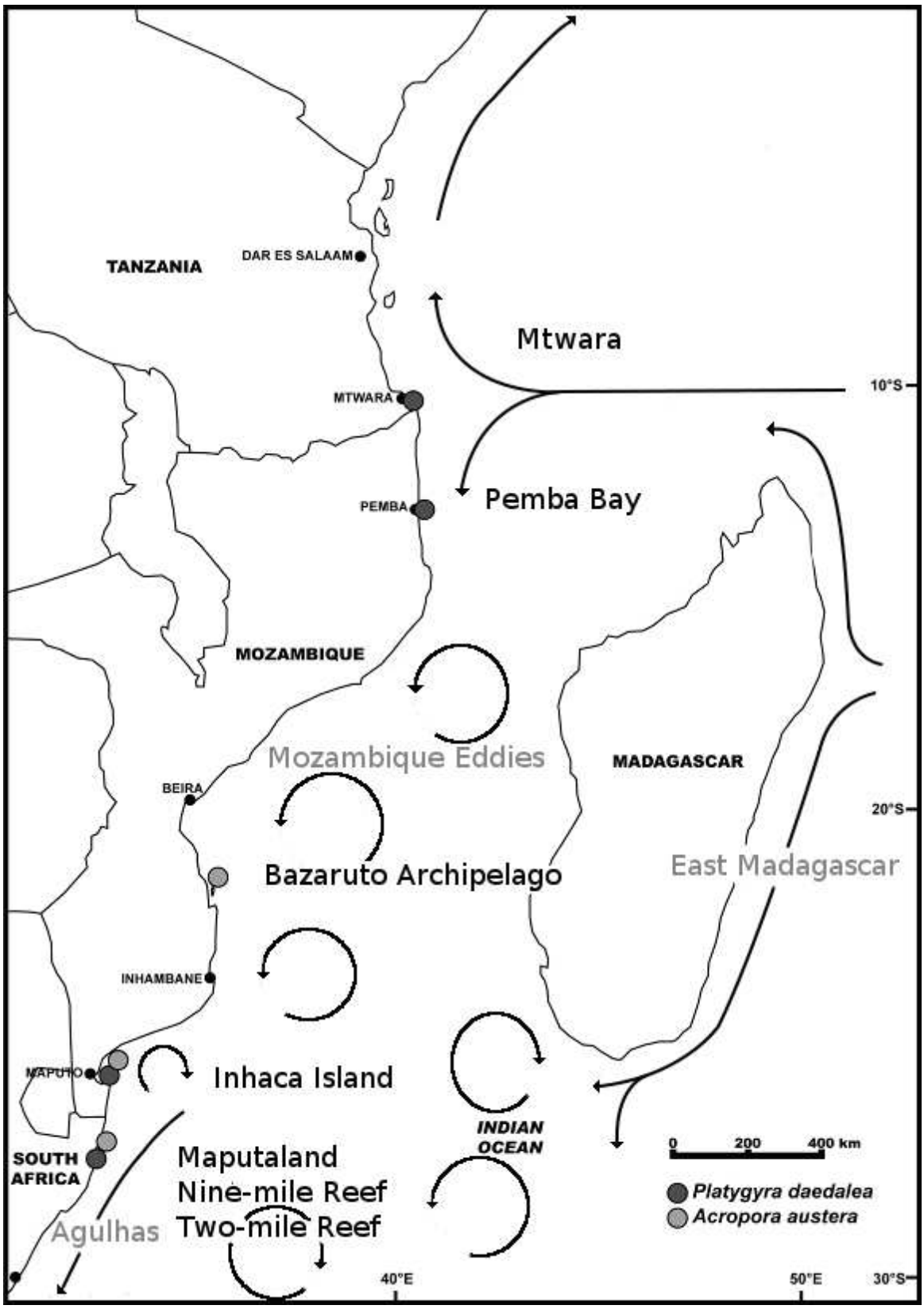

Figure 2.1 Currents, eddies and sampling locations of *Acropora austera* and *Platygyra daedalea* in the south western Indian Ocean study area.

Table 2.1 Number of specimens from each site along the east African coast analysed in this study of genetic diversity and structure in *Acropora austera* and *Platygyra daedalea*.

Sampling location	*Acropora austera*	*Platygyra daedalea*
Two-mile reef, SA 27°31'29" S 32°41'18" E	18	3
Nine-mile reef, SA 27°24'43" S 32°43'40" E		2
Inhaca– Bareirra Vermelho, Moz. 25°55'46" S 32°55'30" E	6	6
Inhaca– Baixo Danae, Moz. 26°01'54" S 32°52'37" E	13	
Bazaruto, Moz. 21°48'27" S 35°30'15" E	14	
Pemba Bay, Moz. 12°54'39" S 40°30'02" E		6
Mtwara, Tanzania 10°16'30" S 40°23'35" E		5

Carbonic anhydrase 3/550 intron sequencing

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 3/550 intron region of the carbonic-anhydrase gene. PCR reactions contained: 1µl sample template (as extracted), 22.7µl 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^{1} (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 Biotechnical Industries (Pty) Ltd., P.O. Box 14356, Hatfield 0028, Pretoria, South Africa.

ITS region sequencing

The nuclear ribosomal ITS1-5.8S-ITS2 region of *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 10µMconcentration 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 would be necessary in order to obtain good quality sequence data. Samples were cloned at Inqaba Biotechnical Industries (Pty) Ltd., P.O. Box 14356, Hatfield 0028, Pretoria, South Africa. A single clone of each sequence was obtained from cultures using the pGEM-T easy vector cloning ssytem (Promega Corporation).

All sequences were edited and aligned using the Bioedit Sequence Alignment Editor 7.0.9.0 (Hall 1999) and by eye for polymorphism considered to be the result of sequence reaction error. Sequence variation was compared using Arlequin 3.11 (Excoffier *et al.* 2005) to calculate nucleotide diversity (Pi (π)) and pairwise F_{ST} and DNAsp 4 (Rozas *et al.* 2003) to calculate haplotype diversity (Hd), guanine and cytosine (G + C) composition and the number of variable sites, with gaps (a site of insertion or deletion) considered a fifth state. Insertions or deletions were considered as a fifth state as all variation in these sequences were considered important for ascertaining fine-scale genetic distance within and between populations.

Construction of neighbour-joining, maximum likelihood and Bayesian haplotype trees

An unrooted haplotype tree was created for the *A. austera* sequence dataset, as there are no carbonic anhydrase 3/550 intron sequences available on the NCBI Genbank which could be used as outgroups. However it was possible to create a rooted haplotype tree for the *P. daedalea* ITS sequence dataset; sequences that aligned most closely to south east African *P. daedalea* in a BLAST search of the GENBANK archive were used as outgroups in phylogeny reconstruction. These ITS sequences were from colonies of *Platygyra* sp. collected in Japanese and Hong Kong waters (AF481891, AF481895, AB214159, AB214160). A species distantly related to *Platygyra* within the *Faviidae* (Huang *et al.* 2009), *Diploastrea heliopora* (AB441396), was used to root the trees.

Mr Modeltest (Nylander 2004) was used to find the best-fit model of molecular evolution for use in analyses with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), whilst Modeltest (Posada and Crandall 1998) was used to find the model of evolution best suited to search for trees using PAUP 4b10 (Swofford 1998). The model of evolution chosen for the *A. austera* haplotype dataset was the Kimura (1980) simple model. For the *P .daedalea* haplotype dataset the Jukes and Cantor (1969) and the Kimura (1980) simple models were chosen, for the Bayesian and parsimony analyses respectively.

A Bayesian tree of haplotyes was constructed for each species using 4 Markov chains run for 1 million generations each, sampling every 10 generations. The first 10 000 trees were discarded as burn-in and the rest of the genealogies were used to construct a 50% majority rule consensus tree. Neighbour-joining and maximum likelihood trees were constructed in Paup 4b10. In each case nodal support was estimated with 100 bootstrap iterations.

For both the *A. austera* and *P. daedalea* datasets, analyses of molecular variance (AMOVAs) were carried out in Arlequin 3.11 (Excoffier *et al*. 2005). South African samples were used as a southern group and the remainder as northern groups to gauge whether corals in South Africa are isolated from coral populations which occur further north in the WIO. DNAsp was further used to estimate migration between populations (ΔNm) using the algorithm of Hudson *et al*. (1992), and to estimate interpopulational gene diversity (γ_{ST}) (Nei 1982). Pairwise distance matrices were calculated in PAUP (Swofford 1998). Mismatch distributions of sequences from both species were analysed in order to discern whether populations were at equilibrium (Rogers and Harpending 1992). The number of recombination events that may have occurred in the *P. daedalea* ITS1-5,8S-ITS2 region was calculated in DNAsp (Rozas *et al*. 2003; Hudson and Kaplan 1985).

2.4 Results

A. austera carbonic anhydrase intron sequences were trimmed to a length of 155 base pairs (bp). They had a G+C composition of 43.5%. There were 14 variable sites, with an overall nucleotide diversty (π) = 2.2 (+/- 1.9). These 51 sequences comprised 9 haplotypes, with a haplotype diversity (hd) of 0.697 (+/- 0.064) (Fig. 2.2). The mean distance between haplotypes was 0.37% (±0.23). Barreira Vermelha hosted two unique haplotypes, whilst the other 7 haplotypes were shared amongst the local reefs. Haplotype 2 was the most common and occurred at all sites except Barreira Vermelha (at Inhaca Island).

P. daedalea ITS1-5.8S-ITS2 sequences were trimmed to 180 bp and had a G+C content of 68.3%. These 22 sequences showed 9 variable sites with π = 4.9 (± 1.4), and comprised 14 haplotypes. The haplotype diversity (hd) was 0.939 (± 0.029) (Fig. 2.3), whilst the mean distance between haplotypes was 0.35% (± 0.19). Nine of the 14 haplotypes of south east African *Platygyra daedalea* were unique, with haplotype 8, the most common haplotype, shared between Nine-mile Reef, Inhaca Island and Mnazi Bay.

Phylogenetic and phenetic analyses of an alignment of *A. austera* carbonic anhydrase 3/550 intron haplotypes produced congruent trees (Fig. 2.2). There was only one well-supported group, comprising haplotypes 7, 8 and 9, with support-values; Bayesian (1.00), neighbour-joining (96%), maximum-likelihood (99%). These haplotypes were found off the Mozambican coast at Barreira Vermelha (7, 8 and 9) and Bazaruto Two-mile Reef (8).

Phylogenetic and phenetic analyses of an alignment of *P. daedalea* ITS1-5.8S-ITS2 haplotypes produced essentially congruent trees (Fig 2.3) which contained no well-supported groups and took the form of a “comb”. The outgroup taxa, *Platygyra* species from south east

Asia (haplotypes 15, 16, 17) formed an unsupported monophyletic group. *Platygyra daedalea* from the SWIO (haplotypes 2 - 14) did not form a monophyletic group.

Analysis of molecular variance (AMOVA) of *A. austera* carbonic anhydrase 3/550 nuclear intron sequences yielded $F_{ST} = 0.18$ ($p < 0.05$) (Tab. 2.2). Samples from the stand at Barreira Vermelho (BV) were found to be significantly differentiated (pairwise F_{ST}, $p < 0.05$); when this population was removed from the analyses the AMOVA showed no significant structure at any level. The average number of migrants per generation (ΔNm) was 0.71 and the inter-population gene diversity (γ_{ST}) was 0.26 when the Inhaca Island populations were included in the analysis. Upon removing Barreira Vermelha (BV) from further analyses, we found $\Delta Nm = 3.87$ and $\gamma st = 0.06$, indicating that the remainder of the populations were less differentiated from one another than they were from BV. Using mismatch distributions it was established that the *Acropora austera* Inhaca Island population of BV may have established equilibrium in terms of growth (data not shown), in contrast to other populations, which did not appear to be at equilibrium (Rogers and Harpending 1992).

Table 2.2 Comparison of the AMOVAs of *Acropora austera* 3/550 carbonic anhydrase intron and *Platygyra daedalea* ITS sequences from the south east African coastline. The 'south' population group comprised samples from Maputaland, South Africa, whilst the 'North' group comprised samples from Mozambican waters.

	Acropora austera			*Platygyra daedalea*		
Source of variation	F_{ST}	Component variance in %	$p < 0.05$	F_{ST}	Component variance in %	$p < 0.05$
Populations in two geographic groups (North and South)	0.18		*	0.09		
Among groupings		-7.47			0.1	
Among populations within groupings		25.71	*		8.9	
Within populations		81.75	*		91	

In the case of *P. daedalea*, FST values produced by AMOVA were not significant (Tab. 2.2). The proportional inter-population gene diversity was found to be $\gamma st = 0.29$. *P. daedalea* did not display significant levels of variation in AMOVA between groups divided into northern and southern population groupings (Tab. 2.2) or at any other level of population structure. The inter-population migration was greater than one individual per generation (ΔNm) = 1.31. There was evidence for a minimum of two recombination events within the ITS region of *P. daedalea*.

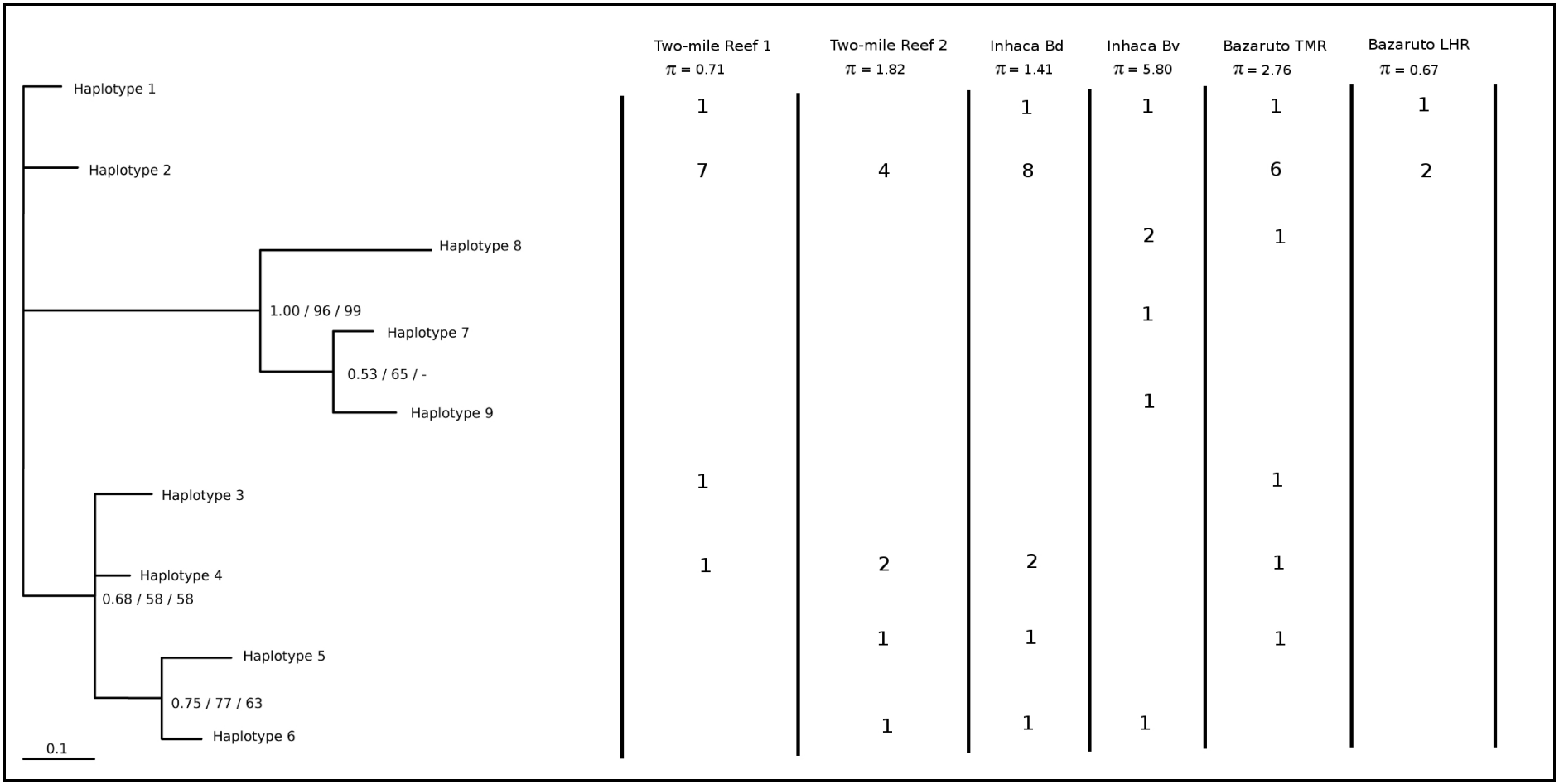

Figure 2.2 Unrooted tree illustrating relationships between carbonic anhydrase 3/550 intron haplotypes of *Acropora austera* from the south west Indian Ocean. This tree represents the results of congruent Bayesian likelihood, neighbour-joining and maximum likelihood analyses. Nodal support values are, in order, Bayesian posterior probabilities/ neighbour-joining bootstrap values (100 iterations) / maximum-likelihood bootstrap values (100 iterations). Columns indicate locality and number of haplotype copies obtained from each.

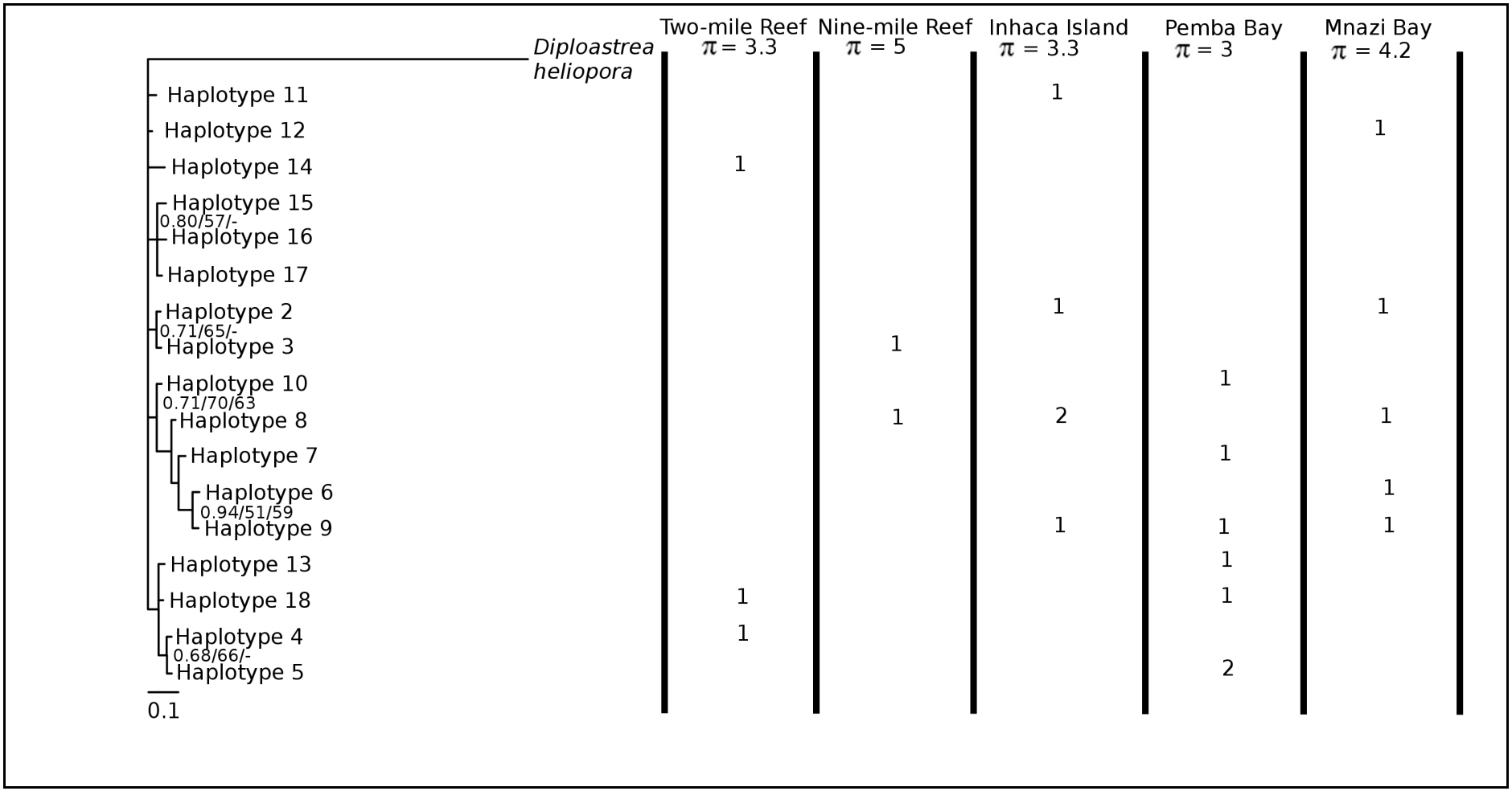

Figure 2.3 Rooted tree illustrating relationships between ITS1-5.8S-ITS2 haplotypes of *Platygyra daedalea* from the south west Indian Ocean and east Asian haplotypes from Genbank (accession numbers; AF481891, AF481895, AB214159, AB214160) and a *Diploastrea heliopora* ITS sequence (AB441396). This tree represents the results of congruent Bayesian likelihood, neighbour-joining and maximum likelihood analyses. Nodal support values are, in order, Bayesian posterior probabilities/ neighbour-joining bootstrap values (100 iterations) / maximum-likelihood bootstrap values (100 iterations). Haplotypes 15, 16 and 17 represent the outgroup *Platygyra* sp. from south east Asia. Columns indicate locality and number of haplotype copies obtained from each.

2.5 Discussion

Nuclear genetic markers from *A. austera* (carbonic anhydrase 3/550 intron) and *P. daedalea* (ITS region) showed markedly different levels of genetic variation (*A. austera*, π = 2.2 (+/- 1.9); *P. daedalea*, π = 4.9 (+/- 1.4)), which may be attributed to the different evolutionary constraints under which they evolve. Thus although different life strategies in *A. austera* and *P. daedalea* may have contributed to the observed differences in the distribution of sequence variation, the overall level of variation is not directly comparable as the two different markers are almost certainly subject to different evolutionary constraints. As might be expected, the more variable *P. daedalea* ITS dataset showed a higher haplotype diversity (0.939) than did the *A. austera* carbonic anhydrase intron dataset (0.697). There was also evidence of recombination within the *P. daedalea* ITS dataset, which might constrain the interpretations which can be made from this repeated DNA region.

Analysis of sequence variation in the *A. austera* carbonic anhydrase 3/550 intron showed little structure, with the exception of a strongly supported clade of 3 haplotypes (7, 8, 9) which are unique to Mozambican populations. The stand at Barreira Vermelho (BV) from Inhaca Island contains two private haplotypes (7, 9) indicating that it may be isolated from other local stands and populations. This is supported by the AMOVA results (Tab. 2.2), which indicate that the BV population is significantly differentiated from all other populations, and that there is no significant structure among any of the other populations of *A. austera*. Further, mismatch distribution analysis indicates that the *Acropora austera* Inhaca Island population of BV may have established equilibrium in terms of growth (data not shown), in contrast to other populations, which did not appear to be at equilibrium. With the exception of the Inhaca (BV) population, it appears that most *A. austera* populations regularly exchange genes, at an average of 3.87 migrants per population per generation, and are panmictic.

Genetic diversity (which may be equated to nucleotide diversity) in *A. austera* was particularly high (π = 5.8) within the BV population in comparison with other local populations (mean π = 1.5 +/- 0.9; Fig. 2.2). This may be a consequence of the predominant current patterns offshore of Maputo Bay and the Delagoa Bight (Fig. 2.1) (Lutjeharms 2006), which circulate bay waters in a clockwise eddy. These local currents may play a role in stabilising sea-surface temperatures in this area by causing upwelling (Lutjeharms 2006), which in turn may make these populations less prone to bleaching-induced mortality.

Platygyra daedalea was somewhat similar to *Acropora austera* in that the among-population and population-group F_{ST} values were not significantly different from zero (Tab. 2.2), indicating panmixia. Although a failure to recover genetic structure amongst populations does

not necessarily indicate panmixia, and small sample sizes may not help to identify polymorphism present in a population, there was no evidence that the low diversity in populations was attributable to ancestral polymorphism. Panmixia was supported by the absence of supported clades in congruent Bayesian, neighbour-joining and maximum-likelihood analyses (Fig. 2.3). Further, the genetic variation in *P. daedalea* (Fig. 2.3) did not display any well-defined relationship to specific geographic sampling locations. Of interest is the failure of phenetic phylogenetic analyses to resolve south east Asian *Platygyra* sp. from those found in the SWIO, which brings the usefulness of the marker into question.

Thus trees derived from analyses of both *A. austera* and *P. daedalea* sequence data indicated a considerable degree of panmixia among populations, except for the BV Inhaca Island population of *A. austera* and (Fig. 2.2; Fig. 2.3). Thus it appears that these populations are exchanging genes on a regular basis or that they may have recently been established in a single wave of recruitment from populations elsewhere. The well-distinguished *A. austera* clade (Fig. 2.2) was entirely from Mozambican localities. Thus, except for a clade of *A. austera* haplotypes (Fig. 2.2), both phylogenetic trees reinforce the idea of panmictic population structure suggested by Ridgway *et al.* (2001) using allozyme markers to study *Pocillopora verrucosa*, a coral with a life-history strategy more similar to *A. austera* than *P. daedalea*, on South African coral reefs. However, the rate and mode of the ribosomal DNA evolution in corals may compromise any inferences about population sub-division, or lack thereof, from the *P. daedalea* dataset.

South African *Acropora austera* populations might be expected to rely on current driven migration from the north to maintain levels of genetic diversity, in a similar fashion to that reported for *Pocillopora verrucosa* (Ridgway *et al.* 2008). This is consistent with the finding that all southern (Maputaland) haplotypes are also found in the northern (Mozabican) waters, but not *vice versa* as Mozambican reefs harbour private haplotypes. The existence of private (northern) haplotypes could indicate the existence of certain barriers to southward current-driven gene flow. Alternatively it may reflect the inability of certain haplotypes to survive in the marginal southern environment. This may be confirmed by a larger-scale analysis which includes more populations from this region (see Chapter 3).

With *Platygyra daedalea*, a longer-lived coral than *A. austera* and *P. verrucosa*), 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, this was not the case, as analysis of haplotype data showed a low level of haplotype sharing among localities, with 9 out of 14 haplotypes unique to particular locations. The high level of haploype diversity (Hd = 0.939) obtained with the *P. daedalea* sample set and the failure to resolve south east Asian and SWIO

samples is evidence of the difficulties associated with analyses based on this region (Vollmer and Palumbi 2004). Further, PCR products were cloned in order to obtain sequences free of polymorphisms. The ITS region is notoriously difficult to work with (Lam and Morton 2003; Vollmer and Palumbi 2004, Mangubhai *et al.* 2007) and regions with such high recombination rates may not yield conclusive results at this scale. Thus analyses of the ITS region of *P. daedalea* in this study should be interpreted with caution. For this reason, and because of the availability of simple tandem repeat (STR) primers for *Platygyra*, it was decided to pursue the investigation of genetic diversity in this species with microsatellite markers (see Chapter 4).

This study has helped to elucidate the potential usefulness of a novel genetic marker, the carbonic anhydrase 3/550 nuclear intron, for studies of genetic diversity and structure of populations of *Acropora austera* in the SWIO. It has laid the groundwork for a study based on more extensive sampling, which will include samples from a greater geographical range (the WIO) and greater sample sizes per population (see Chapter 3). Analyses of ITS sequences from populations of *Platygyra daedalea* in the SWIO have revealed extremely high diversity, low levels of haplotype sharing and a failure to resolve samples from the SWIO and south east Asian waters. These results indicate that the usefulness of the ITS region for further study of the genetic variation and structure of *P. daedalea* might be limited. It was therefore decided to continue the investigation of genetic variability in a more extensive (numerically and geographically) sample using STR markers (see Chapter 4). This study has also provided information on the genetic diversity of highly-understudied Mozambican reefs, and identified the *Acropora austera* population at Inhaca Island (BV) as being genetically-isolated form the other, more panmictic populations studied.

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3. *Acropora austera* connectivity in the south western Indian Ocean assessed using nuclear intron sequence data.

3.1 Summary

Acropora austera populations were sampled from six localities on the south east African coastline (Bazaruto in the north to the southern limit at Leadsman Shoal in Maputaland, South Africa). The hypervariable carbonic anhydrase 3/550 intron marker was sequenced in order to ascertain levels of genetic variability and connectivity within and between reefal populations. A phylogram of haplotypes revealed the presence of exclusive haplotypes in the more northerly populations at Inhaca Island and Bazaruto Island in Mozambique. All haplotypes found in the southern reefs (Maputaland, South Africa) were shared with populations from Inhaca Island and Bazaruto Island. Inhaca Island had two private alleles, the only fixed differences between populations in this study. Measures of genetic variation such as haplotype diversity and nucleotide diversity were also significantly higher in the north of the study area. Levels of haplotype sharing suggest significant connectivity between populations in South Africa and southern Mozambique, which we suggest may be important in sustaining genetic diversity in the more southerly down-current South African *A. austera* populations. Further, Maputaland centres of *A. austera* diversity such as Two-mile Reef and Rabbit Rock need to be managed as source populations for other local reefs. Measures of population sub-division confirmed that there was a significant amount of fixation of allele frequencies amongst populations. Although fine, this level of differentiation in a marker from the nuclear genome of a hard coral indicates that on evolutionary scales there is a level of isolation between *A. austera* populations in southern Mozambique and those in Maputaland, South Africa. The Rabbit Rock population was found to be significantly isolated compared with the more connected populations at Bazaruto Island, Inhaca Island, Two-mile Reef, Red Sands Reef and Leadsman Shoal.

3.2 Introduction

The primary reef-building corals of the Indo-Pacific belong to the family *Acroporidae* (Veron 2000). They are distributed circum-globally, from the western Atlantic to the Pacific and Indian Oceans (Veron 2000). Regionally, acroporids are amongst the most susceptible corals to climate-change-mediated mortality (Wilkinson 2008). On South African reefs, the acroporids are widespread and diverse (Riegl 1996). True accretive coral reefs occur in shallow, warm oligotrophic waters whereas the cooler, deeper waters of Maputaland are considered marginal in

terms of the physiological tolerance of hard corals (Scleractinia) (Kleypas *et al*. 1999).

The diverse coral communities found on South African reef systems are the result of a combination of environmental and topographic factors. The fast-flowing and warm Agulhas Current moves water from between Maputo and Durban in a southerly direction along the east African coastline (Lutjeharms 2006). This current maintains warm water temperatures close to the coastline due to the narrow continental shelf margin (De Ruijter *et al*. 1999) in this area and may serve as a conduit for the dispersal of the pelagic larvae of invertebrates.

Some corals found on these high-latitude reefal communities are characterised as established residents and others as transient opportunistic colonists (Harriot *et al.* 1994). Opportunistic corals are considered to have recruited by a chance migratory event and to be unlikely to establish a permanent breeding population of con-specific corals; they are thus transient (Harrison and Wallace 1990; Harriot *et al.* 1994). *Acropora austera* is a resident species of hard coral in the marginal reefal environments of Maputaland, South Africa (Riegl 1993; Schleyer 2000). The large conspecific populations of this coral species have, however, been observed to suffer from rapid degradation and may be at risk, considering worldwide trends in reef coral mortality (Bruno and Selig 2007).

Although not quite ubiquitous, *A. austera* is distributed widely in the Indo-Pacific and represents an important life strategy in the array displayed by the Scleractinia. *A. austera* is a fast-growing colonist and forms large beds amongst local reef coral communities which have displayed vulnerability to mass mortality events (pers. obs.). In this study, the inter- and intra-population dynamics within this common South African reef coral were investigated using genetic markers as a proxy for levels of relatedness.

Connectivity amongst reef corals, at both evolutionary and demographic levels, is fundamental for the survival of these benthic organisms (Steneck *et al*. 2009). Elsewhere, the question of connectivity amongst reef corals has been addressed at various geographic scales (Hellberg 1994; Ayre and Hughes 2000; Ayre and Miller 2004; Miller and Ayre 2004) and using an assortment of molecular markers (Vollmer and Palumbi 2007; Severance and Karl 2006). Furthermore, the question of connectivity has been applied to a number of different scenarios such as marine protected area (MPA) connectivity (Miller and Ayre 2008), climate change (Munday *et al.* 2009), isolated reefs (Ayre and Hughes 2000; Underwood *et al.* 2009) and the recovery of reefs (Underwood *et al.* 2007).

It is important that reef corals are connected to one another on evolutionary scales, thus increasing diversity by genetic exchange, as this allows such populations to adapt to changing environmental parameters (Munday *et al.* 2009). At demographic levels, it is important that reefs subject to high mortality are seeded by healthier reefs in the neighbourhood (Steneck *et al.* 2009).

Those responsible for creating MPAs must consider both of these scales (evolutionary and demographic) to ensure adequate protection of such resources (Steneck *et al.* 2009). Studies have now been conducted on various scleractinian species in east Africa (*Platygyra daedalea, Pocillopora damicornis*) and South Africa (*Pocillopora verrucosa*) (Souter and Grahn 2008; Souter *et al.* 2009; Ridgway *et al.* 2001; 2008). Mozambique is, however, poorly understood in terms of scleractinian population genetics with only one population (Bazaruto Island) having been studied (Ridgway *et al.* 2008) in this country with 3500km of coastline. The east and south east African coastal regions remain, nevertheless, understudied despite work on connectivity in other taxa (Benzie 1999; Benzie *et al.* 2002; Fratini and Vanini 2002).

We looked at multiple levels of connectivity between populations of *Acropora austera*, at scales varying from tens to thousands of kilometres. All of the specimens collected in this study were from marine protective areas (MPAs). Specimens collected from the Bazaruto Archipelago in the north were from true accretive coral reefs, whereas those collected from the south are considered to be from marginal communities approaching the limit of reef coral distribution in the south-west Indian Ocean.

Interpretation of acroporid genetic data is notoriously challenging as these corals harbour unusual evolutionary patterns within their genomes (van Oppen *et al*. 1999; 2000; 2001; 2002; 2004; Vollmer and Palumbi 2002; 2004). These include hybridisation of species, recombination and introgression within genomic regions as well as high levels of polymorphism within multi-copy regions and conflicting patterns of evolution in the mitochondria. Thus, many marker systems used in other taxa are not applicable to the Scleractinia. Novel molecular markers have been designed for use in genetic analyses of scleractinian corals, particularly by those working on corals from the Caribbean Sea (Wang *et al*. 2008; Vollmer and Palumbi 2007; Severance and Karl 2006; Baums *et al*. 2005a; 2005b), the Pacific Ocean (Underwood *et al*. 2007; Miller and Howard 2004) and the Red Sea (Maier *et al*. 2005). Comparatively few molecular markers have been designed or tested for use in genetic analyses of corals in the western Indian Ocean (WIO).

Recently-designed primers allow PCR-amplification of introns situated within the carbonic anhydrase gene (Ridgway *et al*. unpublished data). These co-dominant single-copy nuclear genes may harbour interpretable inheritance patterns and offer many of the advantages of sequence-based analyses with few of the disadvantages of either mitochondrial genes or protein-based assays. Such sequence analyses are cheaper to implement than microsatellites.

Hard coral communities in South Africa have been protected from exploitation since their initial exploration in the 1970s (Heydorn 1972). These reefs were not subject to any anthropogenic perturbation prior to this and, although they have subsequently become the focus of large-scale recreational fishing and diving, there has been no large-scale direct extractive pressure on the

benthic communities (Schleyer and Tomalin 2000). This is in contrast to southern Mozambican reefs which have, until recently, been subjected to large scale extraction of resources. Southern Mozambican reef systems have also endured more extensive mortality associated with coral bleaching events (Costa *et al*. 2005). The Mozambican reefs are situated farther north than the South African reefs, closer to the tropics and have a higher level of associated scleractinian species-diversity (Riegl 1996). Considering that local oceanic current systems generally move water in a north-to-south direction (Fig. 1), we expected corals in the south to have been seeded by northern reef-coral populations.

Ridgway *et al*. (2001) proposed that South African *Pocillopora verrucosa* populations are panmictic, but over larger scales Ridgway *et al*. (2008) propose a measure of disjunction between populations from South Africa and southern Mozambique. The results of this study will help to clarify whether there is any genetic discontinuity between *Acropora austera* populations in southern Mozambique and South Africa. *Acropora austera* is regarded as opportunistic amongst South African reef corals. It is a broadcast-spawning coral (Carroll *et al*. 2005), and although there is no information on its pelagic larval duration (PLD) prior to recruitment, a PLD of 144 hrs may be inferred based on studies of other acroporids (Harrison and Wallace 1990). Considering regional current speeds and directions (Fig. 3.1), all populations are within the range of a single dispersal event. On this basis, *A. austera* may be expected to comprise a single large population spanning the south east African coastline, from the Bazaruto Archipelago to Maputaland (Fig. 3.1). Established MPAs from this region were sampled in this study in order to evaluate inter-MPA connectivity of populations of this scleractinian coral.

3.3 Materials and methods

Acropora austera was identified according to Veron (2000) and regional taxonomical work by Riegl (1993). Specimens were collected by SCUBA- or snorkel-diving at least five metres from one another in a random fashion to mitigate the collection of clone-mates propagated by local fragmentation. Collection locations included the Bazaruto Archipelago (21°48’24”S / 35°30’15”E) and Inhaca Island (26°01’54”S / 32°52’37”E) in southern Mozambique as well as the following Maputaland locations; Rabbit Rock (27°04’44”S / 32°51’08”E), Two-mile Reef (27°31’29”S / 32°41’18”E), Red Sands Reef (27°43’57”S / 32°38’31”E) and Leadsman Shoal (27°54’45”S / 32°35’56”E) (Fig. 3.1).

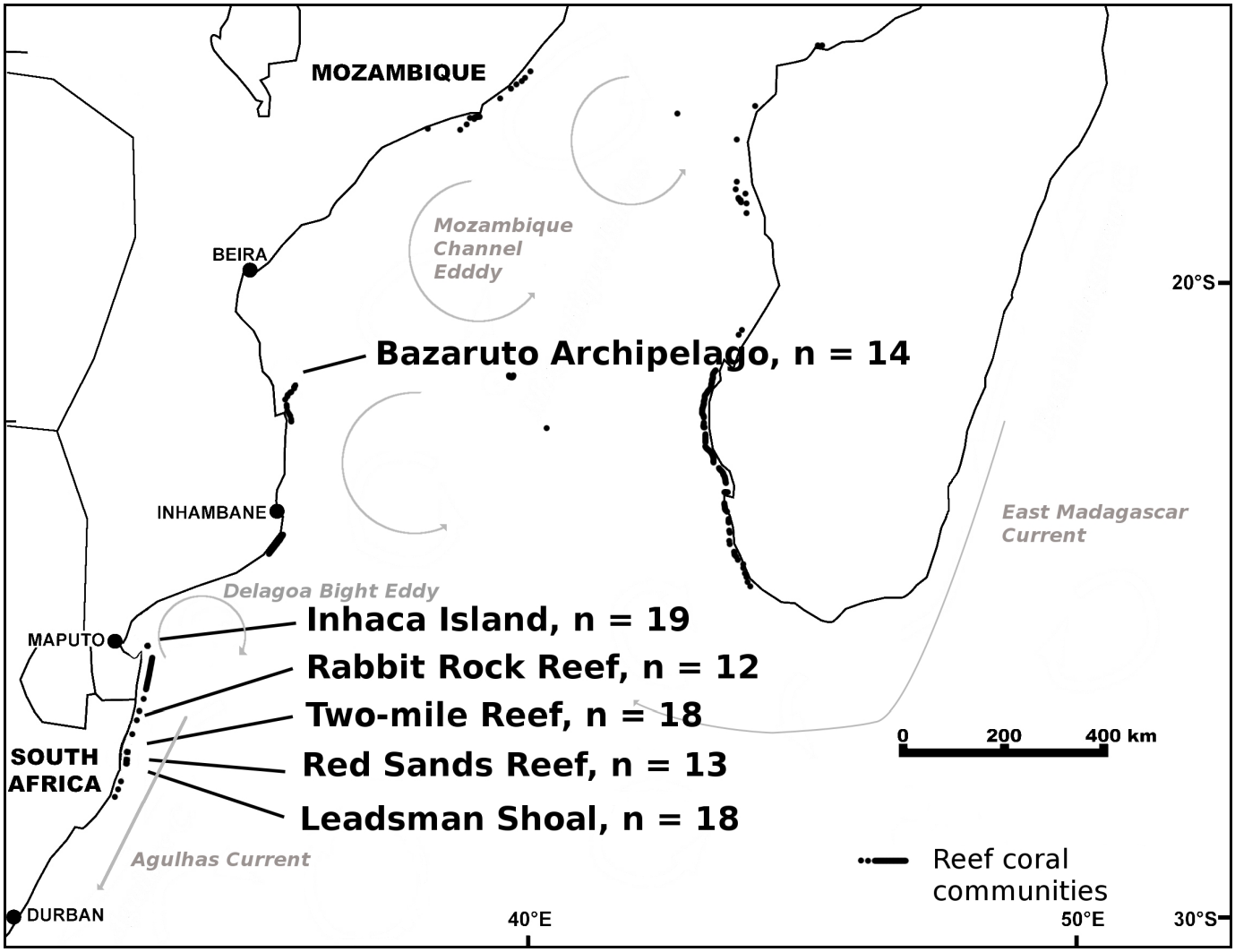

Figure 3.1 Sampling sites and sample sizes of *Acropora austera* collected from the south western Indian Ocean. Contemporary currents and oceanic gyres are indicated in grey on the map.

Fragments, five centimetres long, were removed from the apical tips of healthy colonies and stored in either a dimethyl sulfoxide (DMSO) salt buffer (0.25 M EDTA; 20% (v/v) DMSO, saturated with NaCl) or 70% alcohol (EtOH) in the field and subsequently at room temperature. DNA was extracted using a Fermentas DNA purification kit (www.fermentas.com) according to the manufacturer's instructions.

PCR Amplification

Acropora austera DNA was amplified using primers developed at the Centre for Marine Studies (CMS) at the University of Queensland (Ridgway *et al.* unpublished data). The primers, which amplify a hypervariable intron region of the carbonic-anhydrase gene, were 3-550 F: 5'-TGG CTT GTG TGT ATT GGG ATT C-3' and 3-550 R: 5'-GGC TTC AAA GCT GCA TTT TCT-3' (Ridgway *et al.* unpublished data). 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^{1} (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 using an ABI 3730 capillary sequencer at Inqaba Biotechnical Industries (Pty) Ltd., P.O. Box 14356, Hatfield 0028, Pretoria, South Africa.

Sequence alignment, editing and analysis

Between 200 and 300 bp of the 3/550 locus of the carbonic anhydrase gene were amplified and sequenced for 94 *Acropora austera* specimens. The sequence electropherograms were edited and aligned using BioEdit (Hall 1999). All aligned sequences were checked by eye and compared to their respective chromatograms. The final alignment was trimmed to a length of 157 bp, removing primers and additional nucleotides. Haplotypes were reconstructed from the sequenced genotypes using Phase (Stephens *et al.* 2001). Descriptive statistics were calculated using DNAsp 4 (Rozas *et al.* 2003); these included haplotype and nucleotide diversity (Nei 1987), and Tajima's D statistic, which was calculated to assess whether the 3/550 intron region was evolving as a neutral marker (Tajima 1989). Gaps were used for comparison in final analyses. DAMBE 5.2.5 was used to assess levels of saturation among sample sequences (Xia *et al.* 2003).

Phylogenetic analyses

MrModelTest version 2 (Nylander 2004) and Modeltest 3.7 (Posada and Crandall 1998) were used to search for the model of evolution which best fit the dataset. The TVMef model of Tavare` (1986) was selected for used in construction of neighbour-joining (NJ) and maximum likelihood (ML) trees in PAUP v.4b10 (Swofford 1998), whereas the K80 model (Kimura 1980) was used in Bayesian tree searches, carried out using MrBayes 3.1.2 (Ronquist and Heulsenbeck 2003). Additional parameters used were; equal base frequencies and transition / transversion ratios specified by the Akaike Information Criterion. The NJ and ML trees were bootstrapped for 100 iterations. The Bayesian tree of haplotypes was constructed using 4 Markov chains run for 1 000 000 generations each, with sampling every 10 generations. The first 10 000 trees were discarded as burn-in and the rest of the genealogies were used to construct a 50% majority rule consensus tree.

Population genetic analyses

Genepop was used to calculate deviations from Hardy-Weinberg equilibrium in allele frequencies (Rousset and Raymond 1995). Arlequin was used to conduct analyses of molecular variance (AMOVA) (Excoffier *et al.* 2005), grouping the samples both by region and according to the results of regional studies (Tab. 3.1) (Ridgway *et al*. 2008). These researchers found that Kosi Bay, part of the northern reef complex of Maputaland (Schleyer 2000), may have been a point of transition between populations of *Pocillopora verrucosa* in South Africa and Mozambique. One of the sites in this study, Rabbit Rock, is part of this northern reef complex; it was decided to test whether there was a break in genetic structure of *A. austera* associated with this region. Pairwise F_{ST} values were obtained using the distance method in Arlequin (Excoffier *et al.* 2005) by

permuting the haplotypes between populations, giving a null distribution against which a p-value was calculated from the proportion of F_{ST} values larger than or equal to the observed F_{ST}. A Mantel test was performed in GenAlex (Peakall and Smouse 2006) to test for isolation-by-distance.

Immigration rates for *Platygyra daedalea* were calculated in Migrate 3.0 (Beerli 1998; 2004; 2006; Beerli and Felsenstein 1999; 2001). Populations were grouped into Mozambican and Maputaland meta-populations and migration rates between these groups were tested. The models of migration tested were northerly gene flow (Mozambique to Maputaland) and southerly gene flow (Maputaland to Mozambique). A Bayesian approach using the "infinite allele model" was used to search for appropriate genealogies with 50 000 recorded iterations in increments of 100 steps; 5000 000 different parameter value combinations were sampled (burn-in, 10 000), with an exponential adaptive heating scheme and a swapping interval of 1 (Beerli 2008). Uniform priors for both theta and migration values were used. This was replicated for independent runs with a different random seed for each run; confidence intervals were different from zero in all estimates.

3.4 Results

The 94 *Acropora austera* samples comprised 9 carbonic anhydrase 3/550 intron sequence haplotypes (Genbank Accession Numbers: GB121969 – GB121977). Reconstruction from the sequenced genotypes using Phase (Stephens *et al.* 2001), yielded 15 haplotypes and 188 sequences. The trimmed, aligned sequence length was 157 bp and the G + C content 43.3%. There were 18 variable sites, with an average of 2.09 nucleotide differences between sequences. The nucleotide diversity per site (π) was 0.01 ($\pm$ 0.001). The per-population ranges of genetic diversity indices (Tab. 3.1) were; informative sites, 4 – 14; number of haplotypes, 2 – 8; haplotype diversity, 0.27 – 0.79; number of variable sites, 1.08 – 3.96 and nucleotide diversity (π) 0.007 – 0.022; the aforementioned measures all showed the Inhaca Island population to have the highest mean diversity. The Inhaca Island population contained two private alleles (haplotypes 12 and 14), the only fixed differences between populations in this study (Fig. 3.2).

The overall haplotype diversity was 0.59 ($\pm$ 0.2); the most prolific haplotype (2) was found in all populations and constituted 60% of the sequences (Fig. 3.2). Haplotypes 15 (12% of sequences) was also found throughout the study area. Two haplotypes were unique to Inhaca Island (12, 14), two to Leadsman Shoal (1, 6) and two to Rabbit Rock (5, 8). Six haplotypes (1, 6, 7, 8, 10, 5) were unique to Maputaland (South Africa) reefs, with the remainder found in one or both of the Mozambican reefs (Inhaca Island, Bazaruto Archipelago); only 3 haplotypes were exclusive to the Mozambican Reefs (12, 13, 14). Values calculated for Tajima's D statistic were not significant and confirm that the 3/550 locus of the carbonic-anhydrase gene conforms to neutral expectations (data not shown).

Phylogenetic analyses

The 50% majority-rule consensus Bayesian phylogram illustrating evolutionary relationships among the 15 *Acropora austera* study haplotypes (Fig. 3.2) was largely unresolved and contained only one well-supported clade, comprising haplotypes 12, 14 (both exclusive to Inhaca) and 13 (found on Inhaca Island and the Bazaruto Archipelago). This clade was well-supported in Bayesian analyses (posterior probability = 1.00) as well as in congruent neighbour-joining (bootstrap 97%) and maximum likelihood (bootstrap 92%) analyses.

Population genetic analyses

Heterozygosity was significantly different from Hardy-Weinberg expectations in five of the six populations considered in this study (Tab. 3.1). Four populations showed heterozygote deficits (Bazaruto, Inhaca, Two-mile and Red Sands), whilst the population at Rabbit Rock showed a heterozygote excess. Calculations of thevariability that may be attributed to various population groupings considered geographic locality, the presumed origin of the Agulhas Current, between Maputo and Durban on the east coast of southern Africa (Fig. 3.1) (Lutjeharms 2006), and previous research in this region (Ridgway *et al.* 2008). Thus, we tested for differences amongst groups comprising a northern population of Inhaca Island and the Bazaruto Archipelago, a southern group comprising Two-mile Reef, Red Sands Reef and Leadsman Shoal and Rabbit Rock was included in separate tests as part of either the northern or southern group (Tab. 3.1). Ridgway *et al*. (2008) found a potential break in connectivity between southern Mozambique and South Africa in the region of the northern reef complex. Variability among north vs. south population groups (F_{CT}) was not statistically significant ($p > 0.05$), regardless of the group placement of the Rabbit Rock population. F_{ST} values were significant when Rabbit Rock was grouped with the southern ($F_{ST} = 0.113$; $p < 0.05$) and northern ($F_{ST} = 0.099$; $p < 0.05$) populations. In each case within-population variation contributed most to the variance (90.08%, Rabbit Rock with Moz.; 88.72%, Rabbit Rock with SA). Significant molecular variance and pairwise F_{ST} between populations (Tab. 3.2) indicate that Rabbit Rock allele frequencies are most different from other populations (Leadsman Shoal, Red Sands Reef, Two-mile Reef, Inhaca Island and Bazaruto Island) and that Inhaca Island also has significantly different allele frequencies from South African reef systems, but not Bazaruto Island (in Mozambique). A Mantel test was not significant ($p > 0.05$) with $R^2 = 0.0006$ indicating no measurable isolation-by-distance.

Migration between populations of *Acropora austera* is summarised in Figure 3.3. The mean effective number of migrants per generation (Nm) from Mozambique to Maputaland was 63.2 (14.4-118.1) whereas the Nm from Maputaland to Mozambique was 15.8 (2.1-34.8) (Fig. 3.3). The log probability harmonic mean of north to south gene flow was higher than that for south to north gene flow (Fig. 3.3).

Table 3.1 Genetic diversity indices for *Acropora austera* populations sampled from various locations in the southwest Indian Ocean. Observed heterozygosity values in bold indicate those significantly different ($p < 0.05$) from expectations under Hardy-Weinberg Equilibrium.

Genetic diversity indices	AMOVA Group assignment	N	Number of informative sites (S)	Number of haplotypes	Haplotype diversity (Hd)	Average number of differences (K)	Nucleotide diversity (π)	Expected heterozygosity (He)	Observed heterozygosity (Ho)	Number of alleles
Leadsmans Shoal	South	18	8	7	0.43	1.29	0.008	0.063 ±0.023	0.061 ±0.022	1.4 ±0.1
Red Sands Reef	South	13	4	2	0.27	1.08	0.007	0.052 ±0.024	**0.031 ±0.014**	1.2 ±0.1
Two-mile Reef	South	18	4	6	0.66	1.23	0.008	0.060 ±0.031	**0**	1.2 ±0.1
Rabbit Rock Reef	South/ North	12	7	7	0.73	2.39	0.015	0.115 ±0.045	**0.163 ±0.066**	1.4 ±0.1
Inhaca Island	North	19	14	8	0.79	3.96	0.022	0.193 ±0.035	**0**	1.7 ±0.1
Bazaruto Archipelago	North	14	13	6	0.65	2.33	0.013	0.113 ±0.026	**0**	1.7 ±0.1

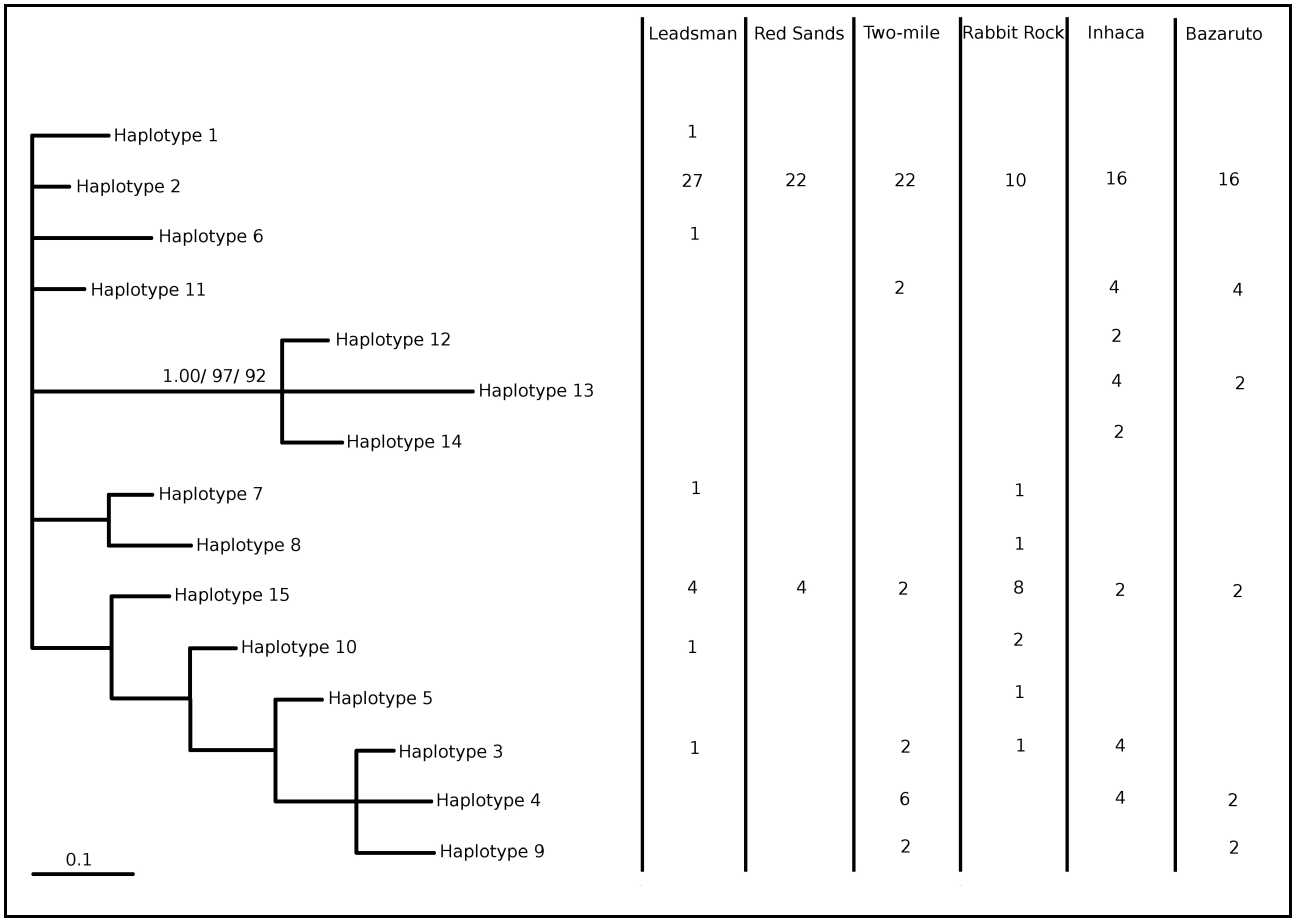

Figure 3.2 Unrooted phylogram illustrating evolutionary relationships among *15 reconstructed Acropora austera* 3/550 nuclear intron haplotypes, showing the distribution of haplotypes amongst sampled localities in the western Indian Ocean. The scale of genetic distance between haplotypes is given below the phylogram.

Table 3.2 Pairwise F_{ST} values between populations of *Acropora austera* collected from the south east African coastline, based on carbonic anhydrase 3/550 intron sequence data. Significant values ($0.05 \geq p$) are indicated in bold text. Ls = Leadsman Shoal; Rs = Red Sands Reef; Tm = Two-mile Reef; RR = Rabbit Rock; In = Inhaca Island and BA = Bazaruto Archipelago.

	Leadsman	Red Sands	Two-mile	Rabbit Rock	Inhaca	Bazaruto
Leadsman						
Red sands	-0.03083					
Two-mile	0.03373	0.03099				
Rabbit Rock	**0.16878**	**0.16705**	**0.1508**			
Inhaca	**0.12358**	**0.11034**	**0.08685**	**0.13467**		
Bazaruto	0.03173	0.02261	0.00477	**0.13952**	0.01492	

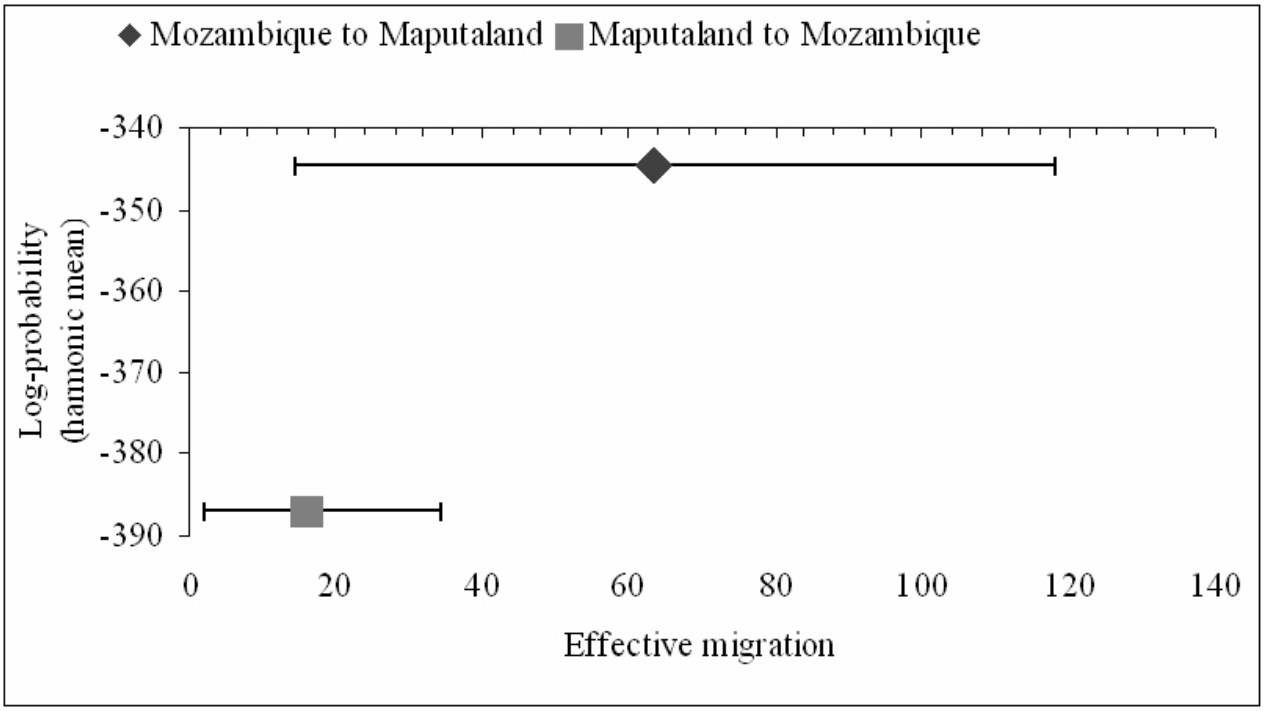

Figure 3.3 Effective migration of *Acropora austera* from Mozambique to Maputaland, South Africa, as calculated using the 3/550 nuclear intron region in a coalescent framework. Log probability of the model of gene flow is given for northerly and southerly gene flow patterns.

3.5 Discussion

Acropora austera displays panmixia amongst most sampled populations in south east African coastal waters, as indicated by analyses of molecular variance. There are two exceptions however; Rabbit Rock in the northern reef complex of Maputaland is significantly differentiated from all other reefs, and Inhaca Island (Mozambique) populations are significantly differentiated from those in Maputaland. This is indicative of a break in connectivity in the region of the South Africa / Mozambique border – the region where the strong southward Agulhas current originates. Although northern reefs may be isolated at fine scales from populations in the south, there is also a level of connectivity between extant populations in this region. Rabbit Rock may be unique either because it is situated in the area within which the Agulhas Current impinges on the African coast (Lutjeharms 2006), or because it is smaller and deeper than other reefs sampled in this region. Inhaca Island populations may be somewhat isolated due to a semi-stationary gyre in Maputo Bay, the Delagoa Bight Eddy (Fig. 3.1). MPAs in this region should be managed with the understanding that their health may influence adjacent reef coral populations reliant on their propagules for maintainance of genetic diversity and population density.

Carbonic-anhydrase 3/550 intron sequences

Nuclear intron sequences have been used for the study of scleractinians, although primarily for investigating phylogenetics (Mackenzie *et al*. 2004; van Oppen *et al*. 2001), rather than

population genetics (Vollmer and Palumbi 2007). The carbonic anhydrase 3/550 intron marker, however, is hyper-variable and informative at the population level in *A. austera* from southern African coastal waters. Its potential to identify hybrids amongst samples is limited when used as a single marker and, considering the high frequency of hybridization in *Acropora*, must be tested with more molecular markers (Richards *et al*. 2008).

Phylogenetics

The tree displaying phylogenetic relationships between haplotypes and sampled populations (Fig. 3.2) is largely unresolved and contains only a single well-resolved clade. This is composed of haplotypes (12, 13, 14) found only in specimens collected from the north of the study area (the Inhaca and Bazaruto Islands from Mozambique). Haplotypes 12 and 14 were private alleles, exclusive to Inhaca Island, whilst haplotype 13 occurred on both Inhaca Island and the Bazaruto Archipelago. Private haplotypes from Leadsman Shoal (1, 6) and Rabbit Rock (5, 8) may be indicative of a degree of genetic isolation in these populations relative to populations further north. Phylogenetic relationships among the remaining haplotypes were unresolved. Haplotypes 2 and 15 were found in all sampled localities (Fig. 3.2).

Population genetics

A. austera populations appear to attenuate in genetic diversity from north to south of the south east African coastline, with populations in the north showing higher levels of haplotype and nucleotide diversity (Tab. 3.1, Fig. 3.2). However, regression analysis revealed no relationship between nucleotide diversity and distribution ($R^2 = 0.2$, $p = 0.4$) and a Mantel test for isolation-by-distance revealed no significant correlation between genetic distance and separation of reefs (Smouse *et al*. 1986). As oceanic currents in the region move in a southerly direction, it is expected that stochastic long-distance dispersal events will have resulted in the recruitment of reef corals from the north of the range to populations in the south. This gradient in genetic diversity with latitude has been reported on the Great Barrier Reef (Ayre and Hughes 2000) and in other marine invertebrates (Kelly and Eernisse 2007). Lower indices of genetic diversity in southern populations (Red Sands and Leadsman Shoal) are consistent with their establishment by chance recruitment. Recruitment along a current-mediated gradient of recruitment success would result in more recruits persisting to adulthood closer to the point of dispersal (in the north), whilst the longer the larvae are carried in the current, the fewer will survive to recruit in the southern regions where the habitats are more marginal (Wares and Pringle 2008).

When the data were treated as a single large population, the shape of the mismatch distribution curve (data not shown) conformed to that expected of a population recently reduced in size (Rogers and Harpending 1992). It is possible that *A. austera* populations are dwindling in size

throughout the study area, and that southern African *A. austera* are a remnant of a once-larger and more diverse population which occurred throughout the southern-most extent of the area in which the Scleractinia are physiologically tolerant.

Structure and connectivity

There are fine-scale but significant ($p < 0.05$) levels of structure amongst the northern and southern groups of populations, regardless of whether the geographically-intermediate population at Rabbit Rock is assigned to the South African ($F_{ST} = 0.113$) or Mozambican ($F_{ST} = 0.092$) populations. Although the overall F_{ST} between these groups appears small, this level of significant structure in marine populations with a pelagic larval stage and the potential to disperse over long distances indicates that the populations harbour fixed differences and are perhaps isolated from one another on evolutionary time-scales (Palumbi 2003). These results are comparable with those of other studies of corals in the region (Ridgway *et al.* 2008) and reinforce the idea that breaks in connectivity between *A. austera* populations in the north (southern Mozambique and northern Maputaland) and those in the south (South Africa, from the central reef complex south) are reflected by a degree of genetic subdivision. It should be noted, however, that Bazaruto was not significantly differentiated from populations further south other than Rabbit Rock (Tab. 3.2). Further north, on the east African coastline, Souter and Grahn (2008) found no significant partitioning of molecular variance in *Platygyra daedalea* populations, based on microsatellite data. The result of this study is thus important regionally and, considering that pairwise F_{ST} values indicate significant differences between southern Mozambican and South African coral communities, the population at Rabbit Rock may be regarded as characteristic of a region of discontinuity between the north and the south.

Northern *A. austera* populations (Bazaruto Archipelago and Inhaca Island) displayed relative homogeneity reflected by high levels of migrants per generation and no significant fixation in allele frequencies between them (Tab. 3.2) despite separation by a large distance (> 500 km). Populations in the south of the study area (Two-mile Reef, Red Sands Reef and Leadsman Shoal) appear to be similarly connected (Tab. 3.2), possibly linked to one another by the fast-flowing southward Agulhas Current, which originates somewhere between Maputo and Durban (Lutjeharms 2006). In order for fixed differences to become established in populations of this nature, there must be barriers to dispersal amongst populations.

A. austera populations at Inhaca Island, the southernmost Mozambican population and Rabbit Rock, the northernmost South African population, exhibit some degree of isolation in pairwise F_{ST} values (Tab. 3.2). Indeed, the Inhaca Island *A. austera* population is the most diverse and genetically distant of the regional populations, although it does not show any level of isolation from the reefs of the Bazaruto Archipelago. The Inhaca Island samples also displayed the highest haplotype and nucleotide diversity of the populations sampled, which may be a consequence of the

partial geographic isolation of the population, possibly by virtue of its location within Maputo Bay.

Inhaca Island is situated at the southern cape of the Maputo Bay that is formed by the Delagoa bight, within which a lee eddy is situated (Quartly and Srokosz 2004) (Fig. 3.1). The Delagoa Bight eddy may serve to isolate *A. austera* at Inhaca Island from elsewhere by retaining larvae within its cyclonic currents and limiting their spread to other regions. The Delagoa Bight eddy may also regulate temperatures around Inhaca by means of regular upwelling events which cool water in this vicinity, buffering the Inhaca population and allowing it to survive environmental perturbation.

The *A. austera* population at Rabbit Rock displays an excess in heterozygosity (Tab. 3.1) and is differentiated from other reefs as indicated by significant pairwise F_{ST} values (Table 3.2). Together these indicate a degree of genetic isolation of the Rabbit Rock corals. The Rabbit Rock reef habitat provides an anomalous context for the survival of *Acropora austera*. This reef is deeper than those from which this species of coral was collected in the central and southern reef complexes (Fig. 3.1). Rabbit Rock is also isolated from nearby reefs by large breaks in suitable habitat on either side that might otherwise have facilitated its connectivity to larger systems (Schleyer pers. comm.). The relative disjunction of Rabbit Rock from other local reefs may stem from a combination of its small size, depth, isolation and stochastic spawning events in which recruitment through chemo-taxis or other reef-oriented recruiting strategies may be confounded by fast moving currents.

Although fixation of alleles in a population has traditionally been interpreted as an underlying result of isolation, there are alternative scenarios which may have lead to the establishment of such unique populations. Sweepstakes reproduction success has been proposed as a mechanism for generating exaggerated fixation of allele frequencies amongst populations (Hedgecock 1994). It is possible that the Rabbit Rock and Inhaca Island populations were created by the chance recruitment of a random assortment of successful propagules. Both Rabbit Rock and Inhaca Island are isolated due to a combination of the oceanographic regime, their geographic situation and habitat availability. They may have evolved a unique assortment of genotypes, due to their level of isolation.

Regardless of their cause, the observed patterns of genetic subdivision between populations are important sources of information for management of these resources. South African reefs were relatively unscathed in recent bleaching events (Schleyer *et al.* 2008), whereas western Indian Ocean reefs experienced some of the most severe bleaching recorded worldwide (Souter *et al.* 2005). Northern reefs, including those in Mozambique which harbour most of the sampled *A. austera* genetic diversity, appeared more vulnerable to bleaching-associated mortality (Goreau *et al.* 2000).

Gene-flow estimates

Effective migration (Nm), as reflected by the number of migrants entering populations in the *Acropora* data-set (Fig. 3.3), was considerably greater from north (Mozambique) to south (Maputaland) than vice versa, confirming that Mozambican populations are likely to be a net source of propagules for populations to the south. These coalescent-based estimations indicate that the direction of gene-flow is probably mediated by oceanic current flow.

The continued survival of *A. austera* in South Africa may depend on the maintenance of southern Mozambican coral reefs. Of the south Maputaland reefs sampled, most of the genetic diversity of *A. austera* is found on Two-mile Reef; this is one of the most heavily-dived reefs in the South African complex of reef communities (Schleyer and Tomalin 2000), with relatively high levels of genetic diversity (in relation to Red Sands and Leadsman Shoal) in at least one species (*A. austera*). In light of the possible isolation between some Mozambican and South African reef corals (Ridgway *et al.* 2008; this study) as well as the apparently self-seeding nature of reef corals (Sammarco and Andrews 1989; Underwood *et al*. 2009), consideration should be given to limiting recreational activities on this reef to maintain local levels of genetic diversity, pending investigations of nearby upstream reefs. Mozambican reefs (Bazaruto Archipelago, Inhaca Island) and northern Maputaland reefs (Rabbit Rock and Kosi Bay Reef) are the most likely upstream source of diversity for reef corals in South Africa and it would be advantageous if they were managed accordingly to sustain South African coral diversity.

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4. Population structure of *Platygyra daedalea* on the south-east African coastline.

4.1 Summary

Platygyra daedalea was collected from Indian Ocean coral reefs, mainly from the east African coast between Mombasa Marine Park (Kenya) in the north and Maputaland (South Africa) in the South. Simple-sequence repeats from five independent loci in the nuclear genome were used to measure differentiation between populations of *P. daedalea.* Of 350 specimens successfully amplified for one or more simple-sequence repeats, only 231 amplified at three or more loci. These 231 remaining specimens comprised a data-set including null-alleles. Overall heterozygosity was high, He = 0.8, and the mean number of alleles across loci, per population was 4.31. The large numbers of null alleles encountered across loci may be attributed to possible parapatric divergence reflected in the nuclear genome in this genus. Ten populations of the nineteen sampled in this study showed signs of sweepstakes reproductive success, with heterozygote excesses and deviation from expectations of Hardy-Weinberg equilibrium. Populations in this study conformed closely to expectations of a panmictic metapopulation. Fine-scale structure amongst sub-populations was detected (F_{ST} = 0.049), although admixture was evident. Cryptic speciation may also play a role in patterns of gene-flow inferred from these data. Northern reef systems may be degraded, assuming that northern tropical populations are inherently more diverse that marginal high-latitude systems at the boundary of tropical Scleractinian distribution.

4.2 Introduction

The level of genetic connectivity between coral reefs determines the extent to which they may rely on one another for the maintenance of genetic diversity (Hellberg *et al.* 2002). Genetic diversity, in turn, serves as a buffer to environmental and extraneous perturbations that may otherwise threaten reef coral health. The connectivity of reef coral populations downstream of one another relative to oceanic current flow is of particular interest to southern African coral reef ecologists.

Reef systems influenced by unidirectional current systems may be connected to one another in a stepwise fashion. Reef corals on the east African coast, from southern Tanzania to South Africa, are subject to predominantly south-moving currents and gyres (in the Mozambican channel, Fig. 1) (Lutjeharms 2006). Within this context, reef-building corals range from species-rich, accretive systems in the north to hard coral communities with lower species complements in the

south (Riegl 1996, Veron 2000).

Reef corals have a pelagic larval stage and thus the potential to disperse large distances and maintain open populations (Avise 1998). However, there is mounting evidence that most reef-building corals recruit locally (Miller and Mundy 2003), making it likely that corals have spread from a region of origin to the boundaries of their distribution in a stepwise fashion. The "Stepping-stone model" (Nei 1972) would appear to best describe regional coral dispersal in coastal waters such as these, where populations adjacent to one another are connected by occasional migrants along a current-mediated passage. As suitable habitat becomes increasingly less common approaching the temperate zone, so do species of reef-building coral (Bellwood and Hughes 2001).

Although there is evidence for stochastic long-distance larval dispersal in the *Scleractinia*, which might mask signals of local structure, the general consensus is that these events rarely take place (Underwood *et al.* 2009). The most abundant reef-building corals on low-latitude reefs appear to have adopted a broadcast-spawning strategy whereby they release gametes into the water column in a single mass-synchronised event (Babcock *et al* 1994). Recruitment studies indicate that, although local South African hard coral communities (high latitude reefs) do not spawn *en masse* as described above, they do employ a similar strategy (Mangubhai and Harrison 2006) and undergo subsequent periods of high-intensity recruitment (Glassom *et al.* 2006). Simulations of the dispersal of the pelagic propagules of reef corals indicate that most are retained on natal reefs for periods that ensure they are likely to recruit locally (Black *et al.* 1990).

Although the spawning events in the south west Indian Ocean are not as clearly punctuated as those in the Pacific or Caribbean, they appear nonetheless to be stochastic. Difficulties associated with monitoring the dispersal of propagules make it necessary to use a proxy, such as molecular markers, to gauge the relatedness of corals.

Initial studies of reef coral connectivity made use of allozyme frequencies and tested fixation and differentiation within and between reef coral populations at varied scales of separation using Wright's F-statistics (Ayre and Hughes 2000; 2004; Bastidas 2002; Goffredo *et al.* 2004; Hellberg 1994). Recent studies of reef coral connectivity have used simple-tandem repeats (STRs or microsatellites) in different genera and at different scales as they show sufficient variation in allele frequency to distinguish fine-scale differences between reef coral populations (Baums *et al.* 2005; Gutierrez - Roriguez and Lasker 2004; Magalon *et al.* 2004, 2005; Souter and Grahn 2008; Starger 2007). Baums *et al.* (2005) found that meta-populations of Caribbean reef corals were regionally-isolated from one another and thus warranted management as separate resources. Ridgway *et al.* (2008) used microsatellites to measure genetic diversity (and Wright's F-statistics) within and between reef complexes of *Pocillopora verrucosa* from Bazaruto Island (Mozambique), Kosi Bay, Sodwana Bay and southern Maputaland (South Africa) and found fine-scale differentiation between

Mozambican and South African populations.

Platygyra daedalea was chosen as a suitable study organism owing to the availability of effective markers, which were developed for use on Great Barrier Reef (GBR) corals (Miller and Howard 2004) and used to study east African species by Souter and Grahn (2008). Recruitment studies have recently shown that *Platygyra daedalea* settles relatively quickly (60 – 66 hrs after planulae become viable), which makes local recruitment likely (Miller and Mundy 2003). Local populations are therefore likely to be self-seeding; however with occasional migrants, levels of differentiation between populations may be expected to be low. It is plausible that reef populations situated in either naturally-protected areas (deeper reef or otherwise-inaccessible habitats) or marine protected areas would seed adjacent populations subject to anthropogenic stressors, contributing to local diversity. This hypothesis is tested in this study for populations in the WIO using the STRs developed by Miller and Howard (2004).

The aim of this study is to quantify the amount and partitioning of genetic diversity in extant Indian Ocean populations of *P. daedalea* from the east African coast and Chagos Archipelago, found in both legislated marine protected areas and reefs under no official management (Wells and Ngusaru 2004). The diversity and level of connectivity between these populations will serve as an indicator of the resilience of local *P. daedalea* populations, both within and adjacent to MPAs. It is hypothesised that *P. daedalea* comprises a single population of reef corals along the east African coastline, and that southern African populations represent the fringe of this large population. As such, southern African populations are reliant on tropical populations for maintenance of genetic diversity and demographic subsidy.

4.3 Materials and methods

Corals were sampled from sites along the east African coast, using SCUBA and snorkel diving (Fig 4.1). 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 salt buffer (0.25M EDTA; 20% (v/v) DMSO, saturated with NaCl) or 70% ethanol. All DNA was isolated using a Fermentas Life Sciences^{TM} genomic DNA purification kit according to the instructions of the manufacturer. All samples were amplified according to the method of Miller and Howard (2004) in the publication of their simple tandem repeat (STR) primer pairs. PCR amplifications of specimen DNA were carried out using 3% bovine serum albumin as an adjuvant and at a variety of DNA dilutions to account for the inhibiting effects of contaminants. Primer pairs were then labelled with fluorescent dyes and successful PCRs were repeated with labelled oligonucleotides.

Genotyping

STRs, labelled with recommended dyes (5' 6-FAM, 5' CAL Fluor Orange 560^{TM}), were genotyped on an ABI 3750XL automated sequencer and scored manually using STRand v. 2.2.30 (Locke *et al*. 2000).

Data quality and null alleles

The data were checked for null alleles and errors in scoring with Micro-checker (van Oosterhout *et al*. 2004). Null allele frequencies were calculated and adjusted with FreeNA and F_{ST}s were calculated, restricting calculations to observed allele sizes (excluding null alleles, ENA) (Chapuis and Estoup 2007; Weir 1996). From this a distance matrix and a neighbour-joining tree were calculated (Saitou and Nei 1987)**.**

Genetic diversity and Hardy-Weinberg equilibrium

Data were explored using GenAlex 6 (Peakall and Smouse 2006). Microsatellite analyser (Dieringer and Schlotterer 2003) and Genepop (Raymond and Rousset 1995) were used to ascertain levels of genetic diversity and deviations from Hardy-Weinberg equilibrium, respectively. F-Stat was used to measure linkage disequilibrium amongst populations (Goudet 1995). In order to adjust for unequal sample sizes the rarefaction approach as implemented in Adze (Szpiech *et al* 2008) was used to calculate allele richness values, in order to adjust for unequal sample sizes.

The final data set comprised 231 individuals collected from 19 localities in the western Indian Ocean (Fig. 4.1). Levels of heterozygosity, both expected (He) and observed (Ho), mean (Na) and effective (Ne) number of alleles per population and the Shannon-information index (I) were calculated (Tab. 4.1). Large numbers of loci failed to amplify across all populations and, in order to adjust for these large numbers of null alleles, we calculated adjusted F_{ST} values and population distances (Tab. 4.2). **Population genetic structure**

Meta-population structure was inferred from the data using Structure v2.2.4 according to the prescribed method, by applying the default settings without specifying populations to which specimens belonged (Pritchard *et al.* 2000). Five runs, each consisting of 100 000 burn-in iterations and 100 000 iterations, were computed for each value of k (the number of populations) from 2 to 22. The distribution of variance among and within these populations was assessed using AMOVA in Arlequin 3.1.1 (Excoffier *et al.* 2005) and compared to other population structures which might be expected on the basis of geographic and physiological parameters (life strategy, larval competency and settlement patterns) (Tab. 4.3). Missing data were ignored in generating distance matrices for AMOVA analyses in Arlequin.

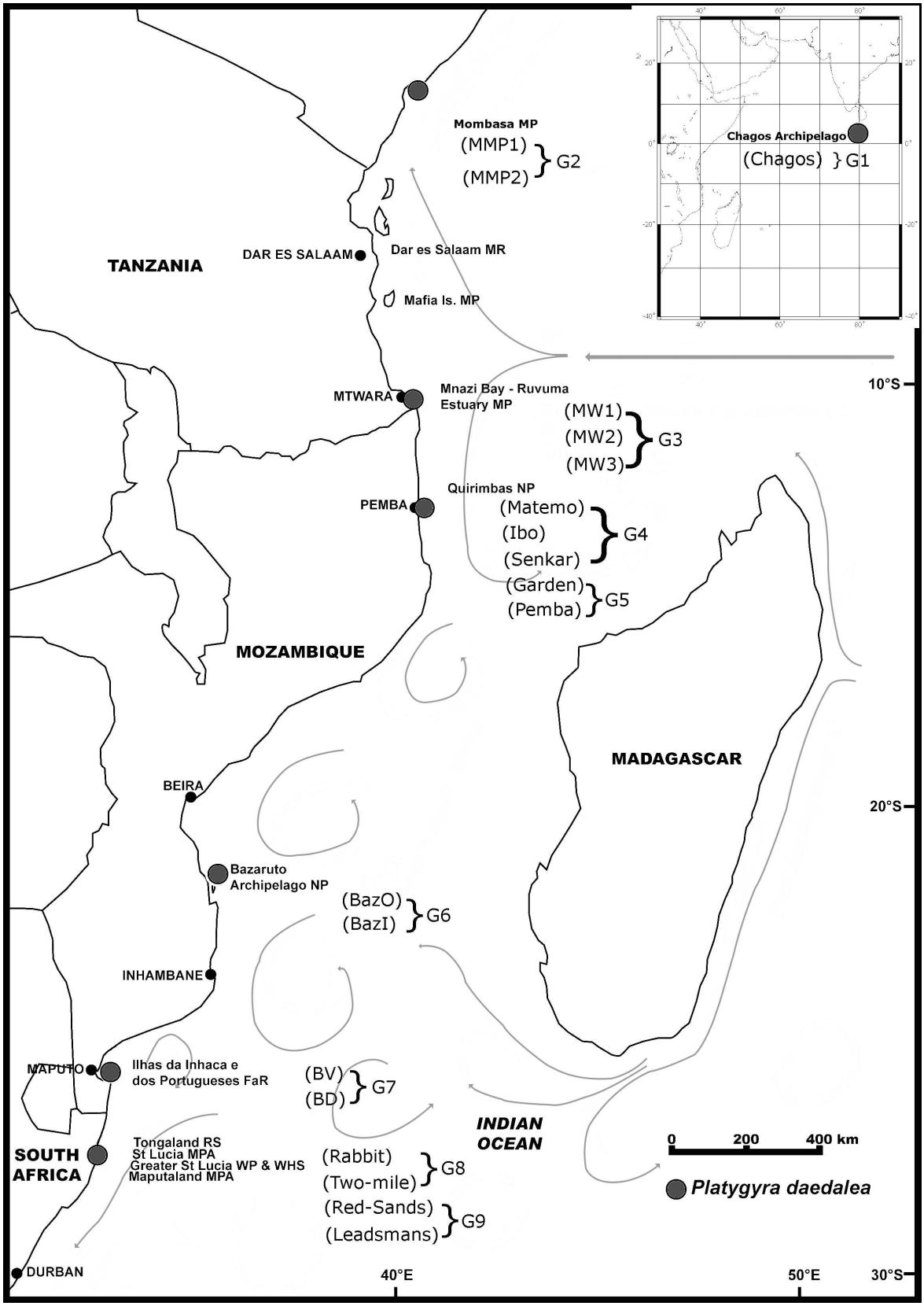

Figure 4.1 *Platygyra daedalea* sampling locations within the western Indian Ocean (in parentheses) and population groups used for analysis of molecular variance of five simple tandem repeat loci. Groups tested for variance were; G1= Chagos Archipelago, G2 = Mombasa Marine Park, G3 = Mnazi Bay, G4 = Quirimbas Archipelago, G5 = Pemba Bay, G6 = Bazaruto Archipelago, G7 = Inhaca Island, G8 = Rabbit Rock Reef and Two-mile Reef and G9 = Redsands Reef and Leadsman Shoal.

4.4 Results

Specimens for which there was too little information were assumed to have damaged template material and were removed from the data-set. Some populations contained very low numbers of individuals, as colonies of *Platygyra daedalea* were sometimes difficult to locate where they occurred in low abundance (such as at the Sencar Channel and Ibo Island sites). Specimens from low-density populations were included in analyses where appropriate, *e.g*. as individuals in composite populations (Fig 4.1).

Data quality

Although there were large amounts of missing data due to non-amplification in the original data-set, not all non-amplification conformed to expectations of null alleles or large allele dropout. Considering the large amounts of missing data, all specimens with a minimum of three successfully-amplified loci were considered as containing null alleles at loci that did not amplify (Tab. 4.1). Specimens with data missing for more than two loci were considered to contain damaged templates (due to considerable transit fatigue) and were discarded. The data were checked for the presence of clonemates and Ng:N was calculated; levels of sexual reproduction were inferred from this. None of the loci were found to be in linkage disequilibrium after standard Bonferroni correction.

Genetic diversity

Some indices of genetic diversity in *P. daedalea* (Tab. 4.1) showed a wide range, for example allelic richness (Na = 7.4 – 1.2, SE = 0.23) and effective number of alleles (Ne = 3.67 – 1.2, SE = 0.12); others showed lesser variability, for example Shannons index of diversity (I = 1.53 – 0.42, SE = 0.04). Expected heterozygosity of STR loci (He = 0.62, SE = 0.02) was lower than that observed (Ho = 0.8, SE = 0.3). Large numbers of null alleles were found in the sub-set of samples (this data-set) considered to be of high enough quality for effective data-analysis (Null = 0.18).

Distance-based analyses

In the neighbour joining analysis, populations grouped with those immediately adjacent to one another at scales of less than 100km (Fig. 4.2). There was no obvious grouping according to geo-political boundaries or arbitrary assignment to either southern or northern population groups (as in the AMOVA, Tab. 4.3).

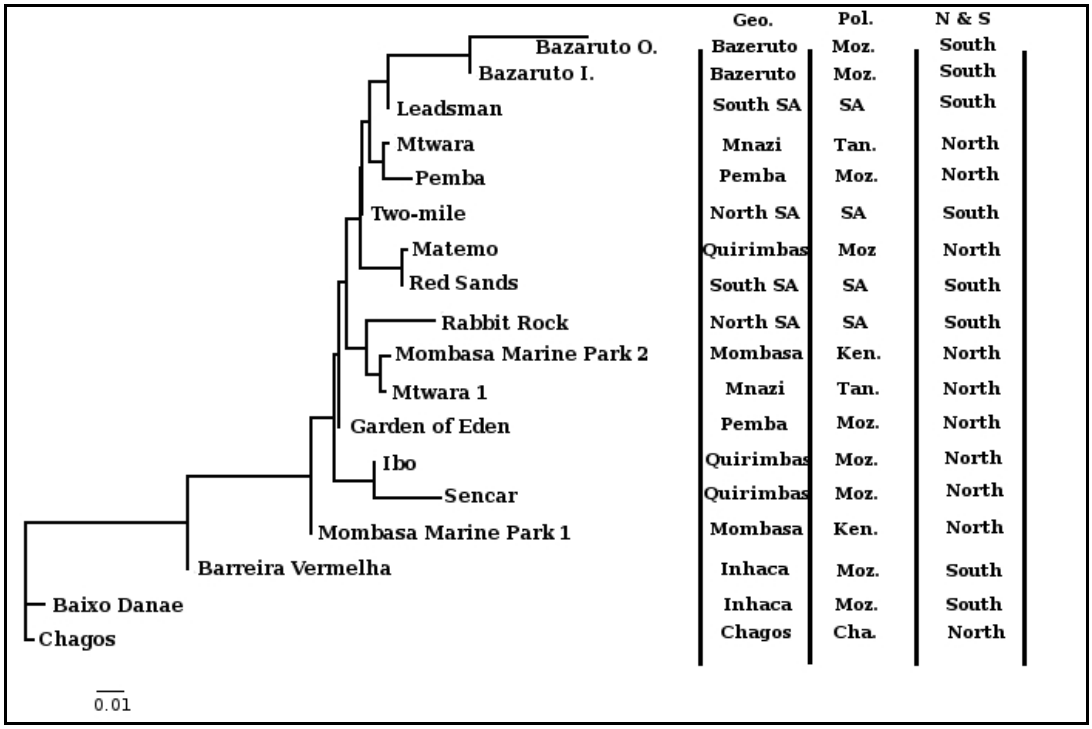

Figure 4.2 Neighbour-joining tree of population distances between *Platygyra daedalea* populations in the western Indian Ocean, calculated from the allele frequencies of microsatellite markers. Columns indicate population membership to geographic group of origin (Geo.), country of origin (Pol.) and northern or southern region (N & S).

Table 4.1 Genetic diversity indices for *Platygyra daedalea* from the western Indian Ocean based on five simple-tandem repeat (STR) loci. N = sample size, Null = null allele frequency, Na = allelic richness, Ne = effective number of alleles, I = Shannon diversity index, Ho = observed heterozygosity and He = expected heterozygosity.

Pop	N	Null	Na	SE(±)	Ne	SE(±)	I	SE(±)	Ho	SE(±)	He	SE(±)
Chagos	**5**	0.1	**2.2**	0.37	**1.87**	0.26	**0.63**	0.17	**0.7**	0.2	**0.41**	0.11
MMP1	**18**	0.24	**5.4**	0.87	**3.41**	0.38	**1.35**	0.14	**0.8**	0.08	**0.69**	0.04
MMP2	**26**	0.26	**6.2**	0.37	**3.48**	0.35	**1.4**	0.09	**0.91**	0.05	**0.7**	0.03
Mw1	**10**	0.27	**5.2**	0.8	**3.67**	0.37	**1.41**	0.13	**0.88**	0.07	**0.72**	0.03
Mw2	**7**	0.36	**4**	0.55	**3.22**	0.47	**1.21**	0.16	**0.95**	0.05	**0.66**	0.05
Mw3	**1**	0	**1.2**	0.49	**1.2**	0.49	**0.42**	0.17	**0.6**	0.25	**0.3**	0.12
Matemo	**6**	0.14	**3.4**	0.51	**3.07**	0.49	**1.11**	0.16	**0.82**	0.09	**0.64**	0.05
IboLHR	**2**	0.2	**2.2**	0.2	**2.13**	0.13	**0.76**	0.07	**1**	0	**0.53**	0.03
Sencar	**4**	0.06	**4**	0	**3.3**	0.25	**1.27**	0.05	**0.6**	0.1	**0.69**	0.03
Garden	**18**	0.18	**5.2**	0.66	**3.67**	0.55	**1.37**	0.15	**0.85**	0.06	**0.7**	0.05
Pemba	**12**	0.26	**4**	0.45	**2.87**	0.38	**1.15**	0.13	**0.78**	0.15	**0.63**	0.05
BazO	**22**	0.29	**5.6**	1.08	**3.24**	0.33	**1.32**	0.12	**0.75**	0.1	**0.68**	0.04
BazI	**6**	0.11	**4**	0.45	**3.36**	0.28	**1.27**	0.1	**0.81**	0.08	**0.69**	0.03
Bareira	**7**	0.28	**2.8**	0.37	**1.98**	0.27	**0.77**	0.13	**0.63**	0.19	**0.46**	0.07
Baixo	**6**	0.19	**2.4**	0.4	**1.99**	0.33	**0.69**	0.19	**0.67**	0.19	**0.43**	0.12
Rabbit	**15**	0.08	**4.8**	1.07	**3.23**	0.47	**1.25**	0.19	**0.86**	0.05	**0.66**	0.06
TMR	**18**	0.12	**7**	1.3	**4.1**	0.65	**1.53**	0.18	**0.91**	0.02	**0.73**	0.04
RedSnd	**12**	0.1	**4.8**	0.8	**3.5**	0.5	**1.33**	0.16	**0.81**	0.07	**0.69**	0.05
Leadman	**36**	0.19	**7.4**	1.21	**4**	0.55	**1.51**	0.17	**0.78**	0.06	**0.72**	0.06
Grand Mean and SE over Loci and Pops												
Average	**10**	0.18	**4.31**	0.23	**3.01**	0.12	**1.14**	0.04	**0.8**	0.03	**0.62**	0.02

Heterozygosity and Hardy-Weinberg equilibrium

With the exception of the Sencar channel population from northern Mozambique, which showed a heterozygote deficiency, all populations had negative inbreeding coefficient (F_{IS}) values indicating an excess of heterozygotes. Overall, 17 of the 95 loci (5 loci per population) deviated significantly from proportions expected under Hardy-Weinberg (HW) equilibrium conditions; 13 due to heterozygote excesses and four due to heterozygote deficits (Tab 4.1). In all, 10 populations deviated significantly ($p < 0.05$) from proportions expected under HW equilibrium. Populations from the central and southern reef complexes in South Africa showed no overall significant deviations from HW equilibria, although, except for the northernmost of the South African populations (Rabbit Rock), they were all characterized by negative F_{IS} values.

Northern populations (eight sites north of the Bazaruto Archipelago) were found to differ from proportions expected under HW equilibrium; seven displayed heterozygote excesses and one a heterozygote deficit. Among-population comparison of allele frequencies at the 5 STR loci indicated significant heterozygote excesses, and that two loci, Pd 31 and Pd 61, deviated significantly from expected HW proportions of hetero- and homozygotes (Tab. 4.2). Both loci were found to harbour an excess of heterozygotes in a global test of adherence to HW equilibrium proportions. The mean overall Ng (214):N (231) value was 0.93, an indication that most genotypes from this dataset were unique. In fact only four samples from population MMP2 were likely to be clonal and their genotype probability was relatively high ($p = 0.018$).

Population genetic structure

Based on analysis using the programme Structure, four populations ($K = 4$), which were largely independent of the locality of the source population, were identified as the most probable meta-populations of specimens collected in this study (Fig. 4.3).

Table 4.2 Fixation indices calculated for *Platygyra daedalea* in the western Indian Ocean based on analysis of five STR loci. Bold values indicate significant differences from assumptions of HW equilibrium. 95% confidence limits are indicated for overall F_{ST}.

	F_{IS}	F_{IT}	F_{ST}	F_{ST} (ENA)	N_m
Pd29-2	-0.192	-0.046	0.122	0.053	1.793
Pd31	**-0.297**	-0.191	0.081	0.022	2.832
Pd48	-0.293	-0.149	0.111	0.057	2.002
Pd61	**-0.289**	-0.168	0.094	0.035	2.406
Pd62	-0.298	-0.125	0.134	0.08	1.622
Overall	-0.274	-0.136	0.108	0.049 $_{(0.032- 0.064)}$	2.131

F_{IS} = inbreeding coefficient among individuals within populations F_{IT} = inbreeding coefficient among individuals among populations; F_{ST} = inbreeding coefficient among individuals among populations N_m = effective number of migrants.

Table 4.3 Components of variance from AMOVAs based on analysis of five STR loci of *Platygyra daedalea* from the western Indian Ocean. These are based on different potential population structures namely; (1) geographic groups (Fig. 4.1), (2) geopolitical (country) groups, (3) north/ south groups and (4) groups designated by Structure 2.2.4 (Pritchard *et al.* 2000).

Source of variation	F_{ST}	Component variance in %	p < 0.05
(1) Populations in 9 geographic groups (Fig. 4.1)	0.12		
Among groupings		6.32	*
Among populations within groupings		5.73	*
Within populations		87.96	*
(2) Populations grouped according to country (Fig. 4.2)	0.12		
Among groupings		3.21	*
Among populations within groupings		8.73	*
Within populations		88.06	*
(3) Populations in two geographic groups (North and South) (Fig. 4.2)	0.13		
Among groupings		2.58	*
Among populations within groupings		9.94	*
Within populations		87.48	*
(4) Populations in groups designated by Structure 2.2.4 (Fig. 4.3)	0.18		
Among groups		17.59	*
Within groups		82.41	*

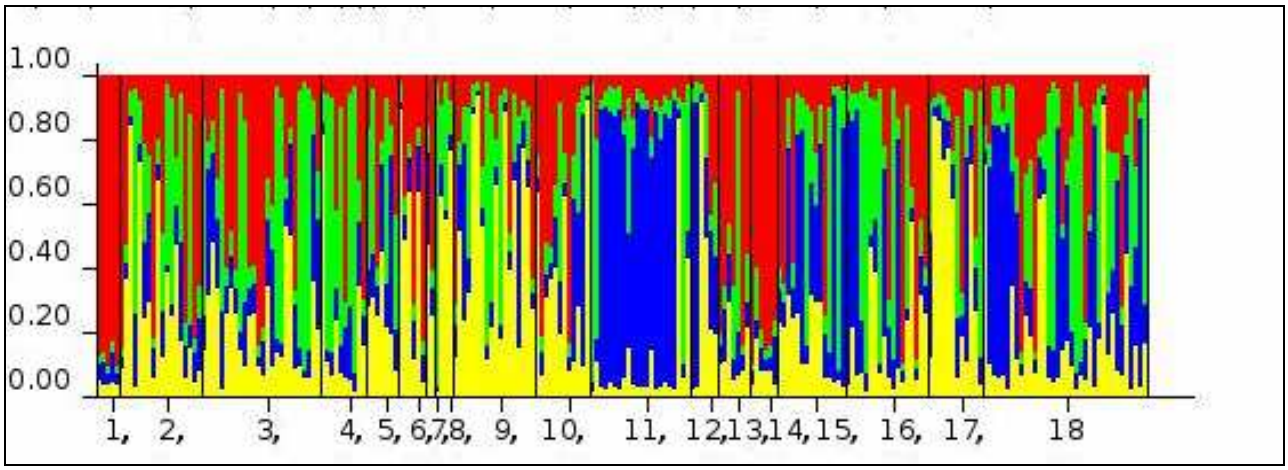

Figure 4.3 Structure v2.2.4 (Pritchard *et al.* 2004) plot based on analysis of five STR loci of *Platygyra daedalea* in the western Indian Ocean. The graph illustrates the likelihood (Y-axis) that corals from each sampling location (X-axis) belong to each of four metapopulations defined by Hardy-Weinberg Equilibria. The numbers 1-18 represent populations of corals arranged from north to south; 1 = Chagos Archipelago; 2 = Mombasa Marine Park 1; 3 = Mombasa Marine Park 2; 4 = Mtwara 1; 5 = Mtwara 2; 6 = Matemo; 7 = Ibo Island; 8 = Senkar Island; 9 = Garden of Eden Reef; 10 = Pemba Bay; 11 = Bazaruto Island 1; 12 = Bazaruto Island 2; 13 = Barreira Vermelha Inhaca Island; 14 = Baixo Danae Inhaca Island; 15 = Rabbit Rock; 16 = Two- mile Reef; 17 = Red Sands Reef; 18 = Leadsman Shoal.)

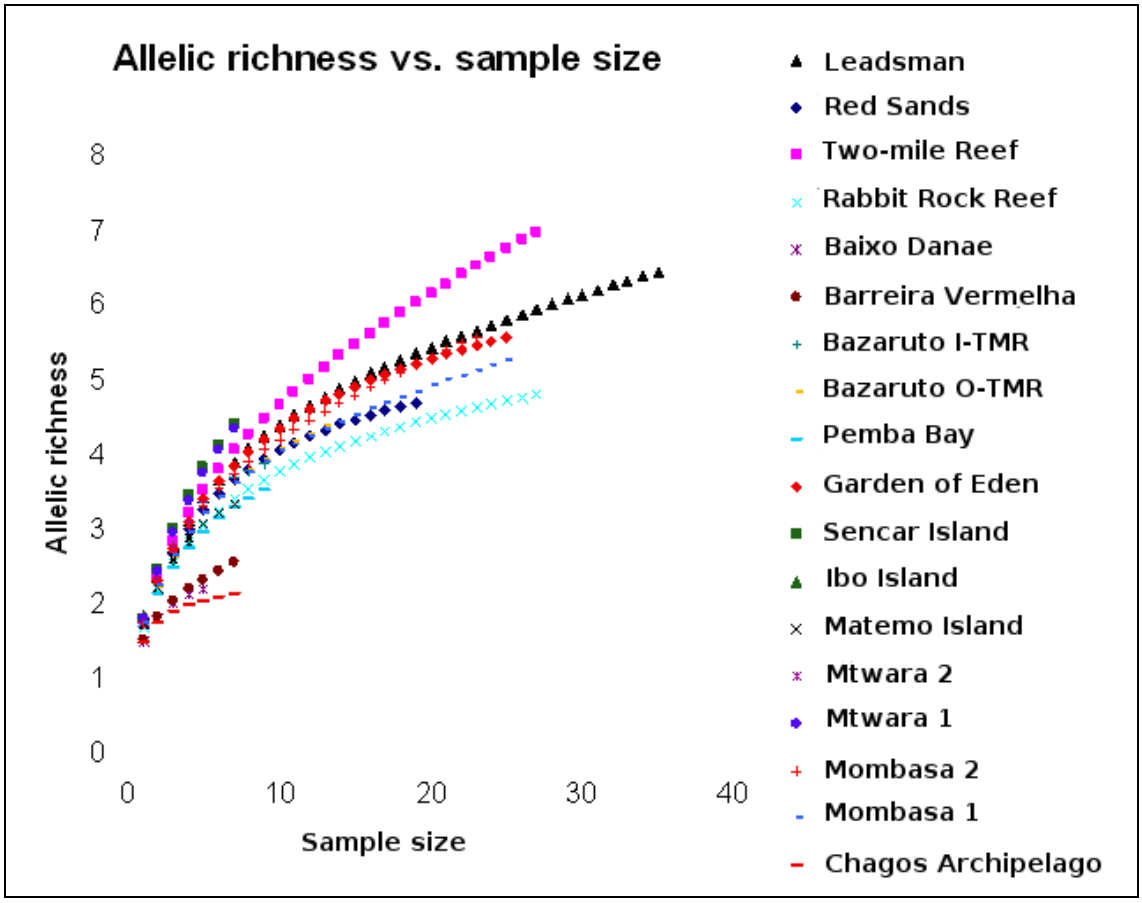

Figure 4.4 The relationship between allelic richness and sample size based on analysis of 5 STR loci in populations of *Platygyra daedalea* sampled in the western Indian Ocean.

4.5 Discussion

Genetic diversity

Levels of genetic diversity in this study of *Platygyra daedalea* were higher than those found in previous regional studies (Tab 1.1). Higher levels of heterozygosity at all loci compared to indices reported in the publication of the markers (Souter and Grahn 2008, Miller and Howard 2004) belie the fact that the STR primers used in this study were developed from specimens collected on the Great Barrier Reef. The elevated levels of genetic diversity may be attributed to parapatric evolutionary processes within the genus *Platygyra*. Evidence for high levels of paraphyly within the *Faviidae* has recently been reported by Huang *et al.* (2009). However, except for locus Pd29-2, all other loci conformed more closely to originally-published levels of heterozygosity (Miller and Howard 2004) than to those reported from a study further north in the western Indian Ocean (Souter and Grahn 2008). Interestingly, elevated values for heterozygosity concur with findings for SWIO zooxanthellar clade diversity (Macdonald *et al.* 2008, Starzak 2008).

Tests of Hardy-Weinberg (HW) equilibria indicate that loci in 10 of 19 populations of

Platygyra daedalea deviate from allele frequencies expected in natural populations and are therefore likely to have been subjected to evolutionary forces: selection, non-random mating, genetic drift or mutation. Most loci that were not in HW equilibrium deviated due to heterozygote excesses. F_{IS} values (inbreeding coefficients) were negative for most localities in this study, indicating either high levels of exchange between localised populations or saturation of allelic differences (Hellberg *et al.* 2002). Souter and Grahn (2008) obtained comparable results for lagoonal populations of *Platygyra daedalea*, and accounted for this by invoking the increased stress to which these populations were subjected. Plausible scenarios that could lead to the levels of heterozygosity observed in this study include small effective population sizes, self-incompatibility (SI) in sexual reproduction, or significant asexual contributions to the gene pool (Stoekel *et al.* 2006, Balloux *et al.* 2003) and sweepstakes reproductive success (SRS) (Hedgecock 1994, Flowers *et al*. 2002).

Miller and Babcock (1997) worked extensively on potential reproductive barriers in *Platygyra* and concluded that there was limited incompatibility between species, let alone within a species, thus SI was not considered as a plausible reason for the observed negative F_{IS} values. It is plausible that asexual reproduction could lead to the estimation of smaller effective population sizes from a given data-set. Balloux *et al.* (2003) report that variance of F_{IS} (the major component of variance in this project) amongst loci is an indication of the level of asexual reproduction. Hard corals are known to reproduce asexually and this data may be interpreted as stemming from a certain level of clonal propagation amongst the populations sampled. However, this conflicts with the overall Ng:N ratio of 0.93, this being roughly the minimum sexual contribution to the measured genotypic diversity. The proportion of asexual reproduction within a population that is also sexually reproducing is difficult to discern, thus caution should be exercised in inference of this parameter (Balloux *et al.* 2003). It should be noted that no evidence for asexual reproduction in *P. daedalea* has been observed in this study.

Small effective population sizes may contribute to heterozygote excesses as described by Potts (1984), since hard corals are organisms that may have extremely long generation times. Instead of organisms beyond a certain age threshold contributing very little to the gene pool in a particular reproductive cycle, old, longer-lived colonies in a region may contribute inordinately to successive generations. “Chronic evolutionary disturbance” and the failure to attain genetic equilibrium may have combined to generate high levels of intraspecific variation in scleractinian corals (Potts 1984). Furthermore, Mangubhai and Harrison (2006) found that *P. daedalea* in Kenya has a reproductive cycle wherein it may spawn biannually within a relatively concise time-frame, possibly leading to genetic contributions from even fewer individuals. Census population sizes may potentially be much larger than estimated effective population sizes.

Localised mating, wherein colonies within a localised area mate with each other but not with colonies from the greater population (or sub-population) may lead to "chaotic patchiness" (Hellberg *et al.* 2002). This scenario is characterised by high migration rate estimates combined with low estimates of effective population size (Ne), and fits patterns of poorly defined genetic structure observed in the present data (Tab. 4.3). This pattern of chaotic patchiness is echoed in studies of marine invertebrate dispersal in other taxa and has been termed 'sweepstakes recruitment success' (Hedgecock 1994, Flowers *et al*. 2002). The high levels of observed heterozygosity and low levels of allelic richness in samples from this study are tell-tale signs of production by a small number of adult organisms (Hedgecock *et al.* 2007). Island populations (Chagos Archipelago and Inhaca Island) in this study exhibit low levels of allelic diversity (Fig. 4.4); this is to be expected in terms of the chance recruitment of a particular 'cohort' of propagules, relative to other corals in the region. Genetic diversity indices in this study show that corals of the Chagos Archipelago are genetically more isolated than coral populations from the east African coastline (Tab. 4.1; Fig. 4.4), probably due to their relative geographic isolation. Inhaca Island coral populations may be expected to be similarly isolated as a result of their location amidst slow-moving current regimes and distance from comparatively-habitable substrata for corals in the north (Fig. 4.1). The data collected here indicates that the Inhaca Island populations are isolated from other nearby reefs, although they may seed South African reefs (south of Inhaca Island) with propagules (Chapter 3).

Allelic richness, calculated from the smallest population included in the study (Ibo Island), is comparable throughout most of the study area, with the exception of three island populations (Baixo Danae, Barreira Vermelha –both Inhaca Island and the Chagos Archipelago). Use of the rarefaction approach to standardizing sample size makes it clear that populations of *Platygyra daedalea* at Inhaca Island and in the Chagos Archipelago have relatively lower levels of allelic richness (Fig. 4.4). This is corroborated by values of the Shannon-Information Index which mirror the results of rarefaction of allelic richness indices (Tab. 4.1).

Connectivity

Within the complex of populations sampled, four populations were defined by the program Structure 2.2.4 irrespective of their geographic origin (Fig. 4.3). Structure 2.2.4 assumes linkage disequilibrium and Hardy-Weinberg equilibrium to assign colonies to a most probable population on the basis of their individual allele frequencies. The most likely number of four populations, designated by Structure v2.2.4, may in this case correspond with the original habitats from which specimens were sampled, namely island-fringing reefs, reef flats (0 – 10m), reef slopes and deep reefs (10 – 20m). The subsequent structuring of four populations within the data-set may be considered to be the uppermost level of hierarchical structure (Evanno *et al.* 2005). Sub-structure within this hierarchy may be indicative of patterns of topographic- and ocean current-mediated

levels of exchange between sub-populations (*i.e*. the connectivity within a designated population).

Ridgway *et al.* (2001, 2008) studied the population genetics of *Pocillopora verrucosa* using allozymes and microsatellites. The allozyme data indicated large-scale panmixia amongst reefal populations in South Africa, whilst STR data revealed that *Pocillopora verrucosa* from the Bazaruto Island Archipelago was only connected to a limited extent to populations in Southern African waters (F_{ST}. = 0.054). Local patterns of gene flow in *P. daedalea* showed that this southern Mozambique-Maputaland discontinuity in connectivity may be prevalent in other genera of hard corals with very different life-strategies. The South African populations of *P. daedalea* on Rabbit Rock deviated significantly from expected HW proportions due to an excess of heterozygotes and, as an anomaly, potentially indicates this to be a local discontinuity in genetic connectivity. However, South African populations of *P. daedalea* satisfy the expectations of Hardy-Weinberg equilibrium and thus certainly appear to be sexually reproductive populations.

Although the Indian Ocean was subject to some of the most severe of the bleaching episodes experienced globally during 1998, bleaching incidences in the SWIO were considerably less severe than those reported further north (Obura 2000). Lower levels of bleaching may be attributed to a number of factors, including the moderating-effect of the fast-flowing Agulhas Current on temperatures at South African reefs (Lutjeharms 2006). High levels of heterozygote excess and significant deviations of gene frequencies from HW equilibrium appear to characterise most northern populations of *Platygyra daedalea*, and may indicate selection due to exacerbated stressors in this region.

Cryptic speciation amongst the *Platygyra* complex

Evidence for cryptic speciation in *Platygyra* sp. in the western Indian Ocean is accumulating. Segregation of populations amongst micro-habitats has been shown in recent research on the Great Barrier Reef (Bongaerts *et al.* 2010). Although cryptic speciation was not reported by Souter and Grahn (2008), they found segregation of sampled populations between habitats. Their data indicated that island and reef-slope populations harboured more genetic diversity than lagoonal populations, and were thus a potential reservoir of the genetic diversity necessary for evolutionary adaptation. Importantly, as migration between populations was detected, it appears plausible that reef populations could serve as a reservoir for lagoonal communities and re-seed these after events involving mortality. Global F_{IS} values for sampled lagoonal communities indicated that they were characterised by heterozygote excesses at levels comparable to those found in the present study. Importantly, island populations sampled by Souter and Grahn (2008) were coastal islands and not subject to the levels of isolation that are represented by Inhaca Island or the Chagos Archipelago, but indicate similar trends in connectivity, albeit at a finer scale. Levels of genetic subdivision between island and lagoonal communities were lower in Souter and Grahn's

(2008) study (F_{ST} = not significant), than between geographic goups in this study (F_{ST} = 0.12, $p < 0.05$), even when considering all populations and accounting for null alleles (F_{ST} = 0.049).

Mangubhai *et al.* (2007) described distinct morphotypes of *Platygyra* sp., although genetic support, based on both microsatellite and ITS sequence data, was weak. In this study however, four populations of *P. daedalea* living in sympatry were found to be significantly differentiated from one another (Tab. 4.3), although this may be an artefact of SRS and attributable to patterns of recruitment success in this widespread species, this may also be a consequence of cryptic speciation. However, present data (F_{ST} = 0.18, $p < 0.001$) indicates significant structuring of allele frequencies amongst the four sub-populations designated by Structure v2.2.4 (Tab 4.3). Definition of groups on the basis of political borders along the east African coast resulted in an increase in the within-group variance (5.73% - 8.73 %) and a decrease in structuring among groups (F_{ST} = 0.12, $p < 0.05$). Although this may indicate that some of the variance amongst the four meta-populations identified in Structure 2.2.4 is geographically based, it clearly cannot all be attributed to groups defined by political borders.

Recent reports of high levels of cryptic speciation in *Platygyra* samples that were identified as single species using traditional taxonomy (Huang *et al.* 2009) brings into question the basis for species differentiation in this genus. These species appear neither to conform to molecular boundaries nor to exhibit significant reproductive boundaries (Mangubhai *et al.* 2007, Miller and Babcock 98). Since proposed by Veron (1995), the possibility of reticulate evolution amongst the Scleractinia has been considered by numerous authors (Diekman *et al.* 2001, Vollmer and Palumbi 2004, 2007, Richards *et al.* 2008), with conflicting conclusions in some instances. This study provides evidence for the existence of cryptic populations of *P. daedalea*, suggested by the four meta-populations defined by Structure v2.2.4 and supported both by high differentiation between groups as calculated by AMOVA (Tab. 4.3) which is also evident in the high levels of null alleles in the data (Tab. 4.1).

Comparison of diversity between MPAs, adjacent coral populations and amongst geo-political regions

Most of the variability within this data-set may be attributed to between-individual differences in allele frequencies, as within-population variances ranged from 87.48% to 88.06% (Tab. 4.3). Variation amongst groupings accounted for between 17.59% and 2.58% of the variance (Tab. 4.3). The lowest between-group variance (data not shown) arose from a comparison of protected with open areas; there were no overarching differences between gene frequencies of *P. daedalea* from protected and open areas. However, further sampling of unprotected areas should be undertaken before this can be regarded as conclusive evidence for homogeneity of protected and unprotected populations.

South African reef systems may be the best-protected on the East African coast in terms of legislation, but some are potentially heavily perturbed through diver damage. Reef systems to the north have recently been declared protected areas, but this may only be in name, with little real protection from anthropogenic extraction of resources or eutrophication (*e.g.* Bazaruto Archipelago National Park, Quirimbas National Park). The lack of variability amongst data grouped according to the legislated level of protection (MPAs vs open areas) reflects the lack of discernible genetic signal amongst these groupings. South African reef populations of *P. daedalea* satisfy Hardy-Weinberg equilibrium measures in general, which may be evidence of their relatively undisturbed habitats. Comparisons of allelic richness and HW equilibria between true accretive reef systems and marginal reefal communities of hard corals would be expected to reveal higher indices of richness in the highly diverse, accretive reef systems (Ridgway *et al.* 2008, Souter and Grahn 2008). The lack of real differences in allelic richness, aside from those between island and coastal communities, could be an indication that northern reef systems are degraded. As this study has been conducted over a geographic cline, selection may not be ruled out as the evolutionary force acting upon these populations.

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5. Marine Protected Area-based management of hard coral communities in the SWIO

5.1 Summary

Migration, population structure and genetic diversity analyses of *Acropora austera, Platygyra daedalea* and other scleractinian corals from the western Indian Ocean were integrated with information on coral biology (pelagic larval duration, reproductive and life strategy) and local oceanography in order to better understand regional coral connectivity. Simple tandem repeats were analysed for *P. daedalea*, and nuclear carbonic anhydrase 3/550 intron sequences for *A. austera*. Both hard corals showed levels of connectivity comparable to those found in previous research in the region. Although there was evidence for counter-current gene-flow (estimated using the coalescent) in both species, there was also support for current-mediated gene-flow. The mean number of individuals migrating between populations per generation was higher for *A. austera* (6.83 ± 3.3) than for *P. daedalea* (1.11 ± 0.3). It appears that *A. austera* emigrants from Inhaca Island may have colonised the more southerly marginal Central and Southern Reef Complexes in Maputaland, South Africa, which showed a net immigration of propagules. *P. daedalea*, however, showed net emigration from Maputaland reefs, increasing southward. The mean Nei's genetic distance between populations of *A. austera* was 1.5%, whilst that for *P. daedalea* was 17.4%. There was no significant correlation of interpopulation genetic distances shown by *A. austera* and *P. daedalea*. Analysis of population assignment indicated that both species were likely to comprise three populations in the region between the Bazaruto Archipelago, southern Mozambique, and Maputaland, South Africa. Fine-scale genetic structure indicated that at ecological time scales groups of northern and southern populations may be isolated and unable to provide each other with population subsidies. Both *A. austera* and *P. daedalea* showed a potential break in genetic connectivity in the region of Inhaca, between Bazaruto and Two-mile Reef in Maputaland. It is important for management authorities to incorporate this into their strategies for the long-term protection of these diverse species assemblages of hard corals.

5.2 Introduction

Coral reefs in the western Indian Ocean (WIO) are important centres of productivity and species diversity and are a valuable resource to local communities (Muthiga *et al.* 2008). Only a small proportion are managed (Wells *et al* 2007), even though they are considered amongst the most threatened coral reefs worldwide (Wilkinson 2008). In a regional assessment of the threat that

climate change poses to coral reefs, marginal high-latitude reefs (HLRs) were found to be most vulnerable to potential fluctuations in environmental parameters (McClanahan *et al.* 2007). These reef systems rarely feature in regional reviews as they are proportionally small and may not contribute substantially to regional diversity. However, HLRs in South Africa are unique and species-rich and constitute the southernmost hard coral communities in the WIO (Schleyer 2000). They are therefore of global importance (Wells *et al*. 2007). In this study we consider their relationship to more northerly coral reefs of the region and the threats that environmental and anthropogenic perturbation pose to their persistence.

South African reefs host 132 species of corals, both hard and soft (Schleyer 2000). This is the southern extent of the range of many of these species, although opportunistic 'weed' species are found much further south, albeit in low abundance (Celliers *et al* 2007). The scleractinian corals under consideration, *Platygyra daedalea*, *Pocillopora verrucosa* and *Acropora austera,* are responsible for reef building in warm waters. They are sessile as adult colonies and must disperse between adjacent reef complexes or even further, primarily as larvae in surface currents (van Oppen *et al*. 2008). In reality, most coral larvae that successfully recruit, recruit to local reefs and very few recruit successfully far from natal reefs (Sammarco and Andrews 1989; Underwood *et al.* 2007). South Africa's coral reefs are situated at the northerly extent of the Agulhas Current and, as such, are subject to temperature regulation by this warm, fast, poleward-flowing body of water (Fig. 5.1) (Lutjeharms 2006).

Southern Africa's coastal marine diversity has long been acknowledged as unusually rich, diminishing less from the equator polewards than in comparable northern hemisphere habitats (Gray 1997). Although marine protected areas (MPAs) have been established throughout the study area, their location has not been supported by adequate connectivity studies (Wells *et al.* 2007). Further, randomly designated MPAs do not necessarily benefit local populations (Crowder *et al.* 2000).

Protecting coral reefs in the long term is not as straightforward as protecting the most biodiverse habitats. Strategies to maintain community diversity in the face of catastrophic local extinction events such as bleaching or cyclone damage may be necessary. External sources of larval emigrants must be identified, characterised and managed appropriately in order that they may re-seed damaged reefs. This includes measuring levels of genetic relatedness or 'connectivity' between reefs. A primary goal in establishing marine protected areas is to "maintain essential ecological processes" (pg 19, Kelleher and Kenchington 1991). Connectivity between coral populations in reef systems is an essential ecological process and may be inferred from genetic data based on studies of adult populations.

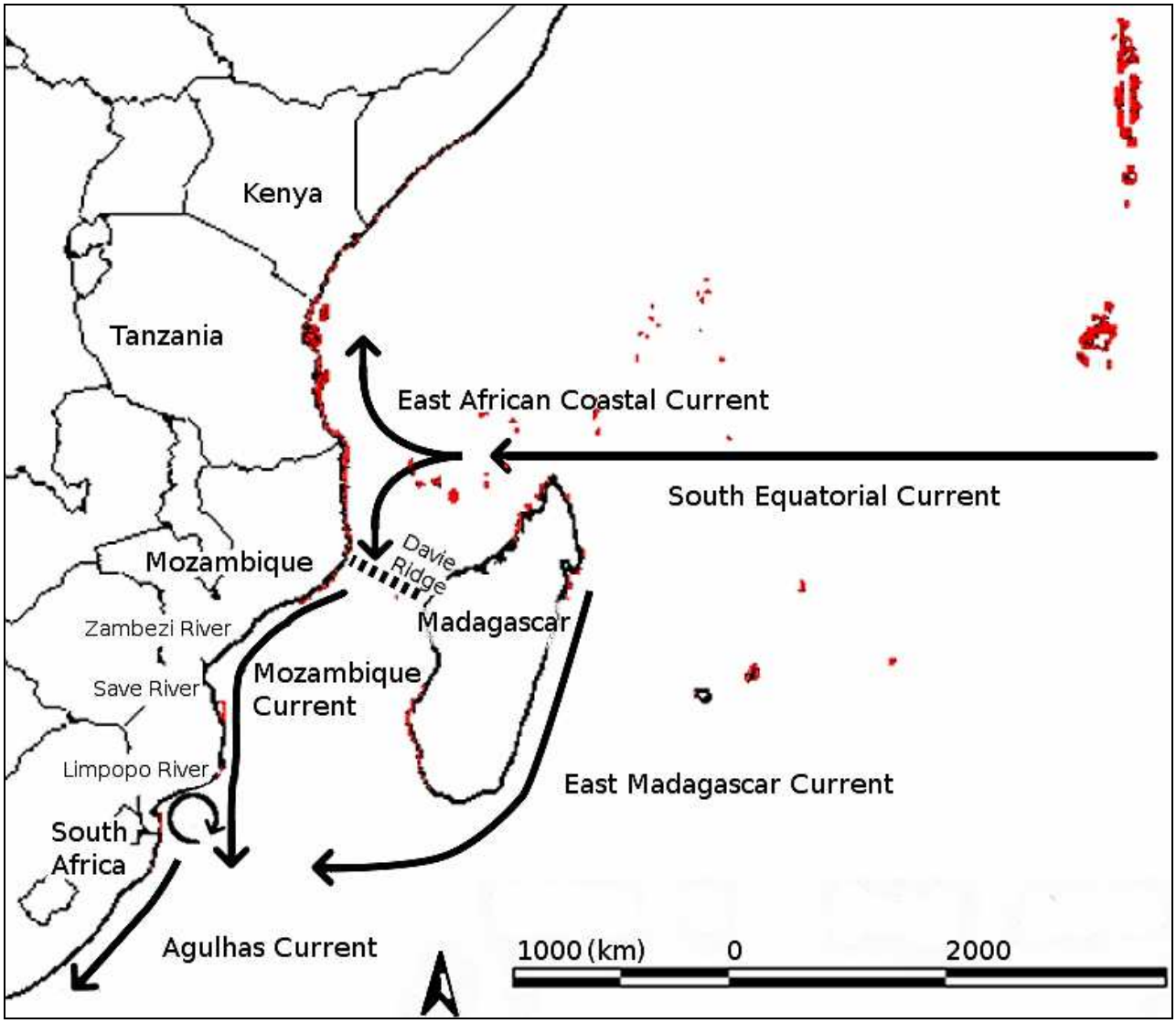


Figure 5.1 Map showing location of coral reefs and currents in the western Indian Ocean region. Predominant water movement patterns that may influence the dispersal of propagules between the coral reef populations that were studied are indicated by arrows (Quartly and Srokosz 2004). Coral reef in the region is indicated in red.

Connectivity has two contexts; these are evolutionary connectivity, whereby genetic diversity is maintained by limited gene flow among populations, and demographic connectivity, whereby a population is maintained by large-scale emigration from an external source (Shanks *et al.* 2003). In this study, we are primarily concerned with the former, as maintenance of genetic diversity is a gauge of resilience in sedentary marine invertebrates. Demographic connectivity may also be of interest in the event of a local extinction, as the levels of migration of propagules that support gene flow are much lower than those needed for the maintenance of population size. Demographic connectivity may be inferred from genetic data as levels of genetic homogeneity between populations are related to numbers of migrants per generation.

Allozyme studies revealed general panmixia amongst reef corals in Maputaland (Ridgway *et*

al. 2001). Further refinement in the use of molecular markers began to show the fine-scale genetic structure of reef coral populations both in Maputaland and globally (Magalon *et al*. 2005; Baums *et al.* 2005; Underwood *et al*. 2007; van Oppen *et al*. 2008; Ridgway *et al*. 2008; Souter and Grahn 2008). Factors influencing dispersal of propagules are now being studied in conjunction with research on reproductive strategies and patterns. The timing of spawning and recruitment rates of coral larvae have been determined for reefs in Kenya (Mangubhai and Harrison 2006), Tanzania (Nzali *et al.* 1998; Franklin *et al*. 1998) and South Africa (Schleyer *et al.* 1997; Glassom *et al*. 2006). Seasonal peaks in recruitment and significant differences in the genera dominating recruitment patterns have been found both regionally and locally. Studies of the effects of bleaching on reproductive effort (Ward *et al.* 2000) imply vulnerability to environmental perturbation during peak stages. Post-recruitment survival has not been investigated adequately on South Africa's reefs (but see Schleyer *et al*. 2008) which, due to their marginal nature, may show different patterns from those which characterise the region (Franklin *et al.* 1998). All of the coral genera included in this study (*Platygyra*, *Pocillopora* and *Acropora*) are widespread and might therefore be expected to have large dispersal distances (Pechenik *et al.* 1984).

Regional population genetic studies of scleractinian corals

Ridgway and Sampayo (2005) reviewed population genetic data for marine organisms in the western Indian Ocean and found knowledge of population genetic processes in the western Indian Ocean to be lacking, with few studies focusing on the Scleractinia (Ridgway *et al*. 2001). Since 2005, a series of studies have been initiated to explore the population genetics of hard corals. Reef corals in Maputaland were found to be panmictic (Ridgway *et al.* 2001). Reef corals closer to the equator were found to be somewhat isolated from one another (Tab. 5.1), although tropical populations of *Pocillopora damicornis* show trends similar to those observed in congeners much further south (Ridgway *et al*. 2001; Ridgway *et al*. 2008; Souter *et al.* 2009). Interestingly, higher levels of genetic discontinuity were observed in studies comparing sites in South Africa and Mozambique (Ridgway *et al.* 2008).

Oceanographic factors

Coral communities are found at Inhaca Island, the interface between the Agulhas Current and the Mozambican eddies (Fig. 5.1) which move south from the north of the Mozambique Channel (Lutjeharms 2006; Quartly *et al*. 2006). The South Equatorial Current bifurcates and feeds both the Mozambican Channel eddies (MCE) and the East African Coastal Current (EACC) (Swallow *et al.* 1991), which move south and north respectively. The MCE are mesoscale eddies which move slowly in a general southerly direction, with the result that the net flow of surface water from southern Tanzania to southern Mozambique is north to south. The distribution and dispersal of south-east African scleractinian corals should be considered within the context of

southerly water flow (Tab. 5.2). The pelagic larval dispersal potential of the scleractinians considered in this (*Platygyra daedalea, Acropora austera*) and other (*Platygyra daedalea, Pocillopora verrucosa*) regional population genetic studies were considered a plausible factor in determining the structure of their populations (Tab. 5.1), the further a coral is able to disperse the more homogeneous its population might be. This information, coupled with the oceanographic current regimes of the region, may allow the likely dispersal distance of propagules along the east African coast to be inferred (Tab. 5.2).

The present study area incorporates the western Indian Ocean but focuses primarily on the south-west Indian Ocean, in particular the south-east African coast. The major currents and water-movement patterns within this region are summarised by Lutjeharms (2006). What was formerly regarded as the Mozambique Current (calculated from shipping drift patterns) is now considered a series of gyres which move south at 5 $cm.s^{-1}$ (Tab. 5.2). This series of gyres gives way to the Agulhas Current between Maputo and Durban at around 28° S. The Agulhas is a fast-moving western boundary current which moves poleward along the continental shelf margin at average velocities >1.5 $m.s^{-1}$ (Tab. 5.2). The east African coastal current (EACC) is the most important current in the northern coastal region of the study area, and is purported to reach velocities in excess of 1 $m.s^{-1}$ (Tab. 5.2) (Swallow *et al.* 1991; Shankar *et al.* 2002). As the coral gametes examined in this study are buoyant or pelagic in nature (Miller and Babcock 1997), sea-surface vicariance must play an important role in the dispersal of these propagules prior to fertilization. The predominant current patterns are therefore likely to be responsible for dispersal of coral propagules over evolutionary timescales. Fine-scale oceanographic vicariance is ultimately responsible for the dispersal of most local propagules (Sammarco and Andrews 1989), but long-term trends of longer-distance dispersal may become apparent from region-wide sampling.

Table 5.1 Summary of studies on coral connectivity in the western Indian Ocean region**.** Pelagic larval duration (PLD) is inferred from experimental data (within genus) and is given for the time period by which 80% of propagules have settled.

Species	Scale (km)	Current velocity (ms^{-1})	PLD (hr)	Fixation index (F_{ST})	Region	Marker system	Reference
Pocillopora verrucosa	100	1.5	30	NS	South Africa	Allozymes	Ridgway *et al.* 2001
Pocillopora verrucosa	1000	0.05-1.5	30	0.054	Southern Mozambique, South Africa	STRs	Ridgway *et al.* 2008
Platygyra daedalea	1000	>1	66	NS	Kenya, Tanzania	STRs	Souter and Grahn 2008
Platygyra daedalea	2500	0.05-1.5	66	0.049	Kenya, Tanzania, Mozambique, South Africa	STRs	Chapter 4

Pelagic larval dispersal (PLD) is inferred from experimental data (within genus). Pv = *Pocillopora verrucosa (30 hr)* (Harii *et al.* 2002), Pd = *Platygyra daedalea (66 hr)* (Miller and Mundy 2003), , Ns = not significant.

Comparisons of the distribution of MPAs containing hard corals along the Tanzanian, Kenyan and South African coastlines and the likely PLDDs of three corals, *Platygyra daedalea*, *Acropora austera* and *Pocillopora verrucosa*, suggest that their dispersal between MPAs is plausible (Tab. 5.2). The connectivity of corals on reefs is reliant on the dispersal kernels generated by each population. These hypothetical dispersal kernels indicate that MPAs in the north of this region may not be connected to those in the south on account of the slow-moving waters in the Mozambique Channel (Tab. 5.2). The PLD of propagules in the Mozambique Channel ranges between 5km (*Pocillopora verrucosa*) and 26km (*Acropora austera*) whilst the average distance between MPAs is 321km (Tab. 5.2). The coral dispersal kernels calculated for reefs situated in the East African Coastal Current (Tanzania / Kenya) and the Agulhas Current (Maputaland) are much larger on average (288km (108km – 518km) and 432km (162km -778km) respectively, (Tab. 5.2)) than the mean distance between reefs in this region, allowing for demographic connectivity between reefs and MPAs. Thus MPA populations in the north of the study area may be well-connected to one another, as is also the case for those in the south, whereas those in the Mozambique Channel may have limited connectivity of evolutionary consequence only. The work of Ridgway et al. (2008) further demonstrated the likelihood of a break in connectivity between coral populations in Mozambique and South Africa (Tab. 5.1). Again, this may be a result of the predominantly slow-moving eddies that have resulted in gradual southward drift in the Mozambique Channel. Results from previous studies on the partitioning of genetic variance between populations support this notion (Tab. 5.1). This study may either confirm or negate the hypothesis that regional currents play a major role in the dispersal of coral propagules in the WIO.

Regional prevalence of coral reefs and MPAs

McClanahan *et al*. (2007) compared reefs along the east African coastline and found coral species diversity to be highest in southern Kenya and Tanzania, whereas Veron (2000) predicted southern Tanzania and northern Mozambique to be most diverse. Wells *et al.* (2007) demonstrated that the greatest area of east African coral reef occurs on the Tanzanian coast and in northern Mozambique. Further south there appear to be large gaps between hard coral reefs (Fig. 5.2). Such long expanses of coastline devoid of significantly-large coral reefs have been attributed to the riverine input from large deltas which dominate the nearshore environment. Low current velocities within the Mozambique Channel may be significant determinants of the distribution of hard corals amongst these unsuitable, high-sediment and nutrient-rich habitats.

Table 5.2 Summary of information on genetic structure, distance between MPAs and average current velocities, derived from studies on hard coral populations in the western Indian Ocean.

	Distance between MPAs	Current velocity ($m.s^{-1}$)	PLDD Pv (km)	PLDD Pd (km)	PLDD Aa (km)	F_{ST} from MPAs	Reference
Tanzania / Kenya	47	1	108	237.6	518.4	Ns	Souter and Grahn 2008
Mozambique	321	0.05	5.4	11.9	25.9	S	Chapters 3 and 4; Ridgway *et al.* 2008
Maputaland	80	1.5	162	356.4	777.6	Ns	Ridgway *et al.* 2001
Average	112		88	193	422		
Total coastline	3009.857						

Pelagic larval dispersal distance (PLDD) is inferred from experimental data (within genus). Pv = *Pocillopora verrucosa (30 hr)* (Harii *et al*. 2002), Pd = *Platygyra daedalea (66 hr)* (Miller and Mundy 2003), Aa = *Acropora austera (144 hr)* (Harrison and Wallace 1990). S = significant, Ns = not significant.

Coral reefs that are of interest in this study span the east African coast from Mombasa in Kenya to Maputaland in South Africa (Fig. 5.2). The coral rich coastline of Tanzania contains a large proportion of the accretive coral reefs of east Africa. Northern Mozambique is also rich in coral reefs, which occur from the northern border of Mozambique, the northern Quirimbas, to the northern extent of the swamp coast region, the Primeiras Archipelago. Moving south from the Equator, the Zambezi River delta constitutes the first of the large expanses of habitat which are inhospitable to reef corals. Beyond this, coastal coral reefs are next found in the Bazaruto Archipelago and southwards to Xai-Xai, whereupon more river–delta habitat provides another formidable barrier to further southward distribution in the form of the Limpopo and Komati River mouths.

Coral reef distribution is fragmented and hard coral diversity begins to attenuate along a latitudinal gradient south of Xai-Xai, although Inhaca Island has substantial hard coral diversity and Maputaland, South Africa, harbours high levels of both hard and soft coral diversity. South Africa's corals, however, do not form true accretive reefs and are the last point along the East African coast with truly diverse coral assemblages.

Few studies have estimated the level of local protection provided by MPAs within the study area (Wells *et al.* 2007). Roughly 5% of the reef habitat off Kenya, Tanzania and Mozambique is under some level of protection. The proportion of protected area is lower in Tanzania and Mozambique, where most of the regional coral reefs are situated (Wells *et al.* 2007). Additionally, the level of protection within these MPAs is questionable, as no-take areas are small and levels of anthropogenic extraction may be unmanaged (Francis *et al*. 2002; Wells *et al*. 2007). Neither the distance between these protected reef habitats, nor the distribution of suitable extant habitat (coral communities and reefs) appear to have been considered in the creation of MPAs along the east African coast. Both are important for understanding reef-coral connectivity, as these coral populations may be considered stepping-stones within vast habitat deserts.

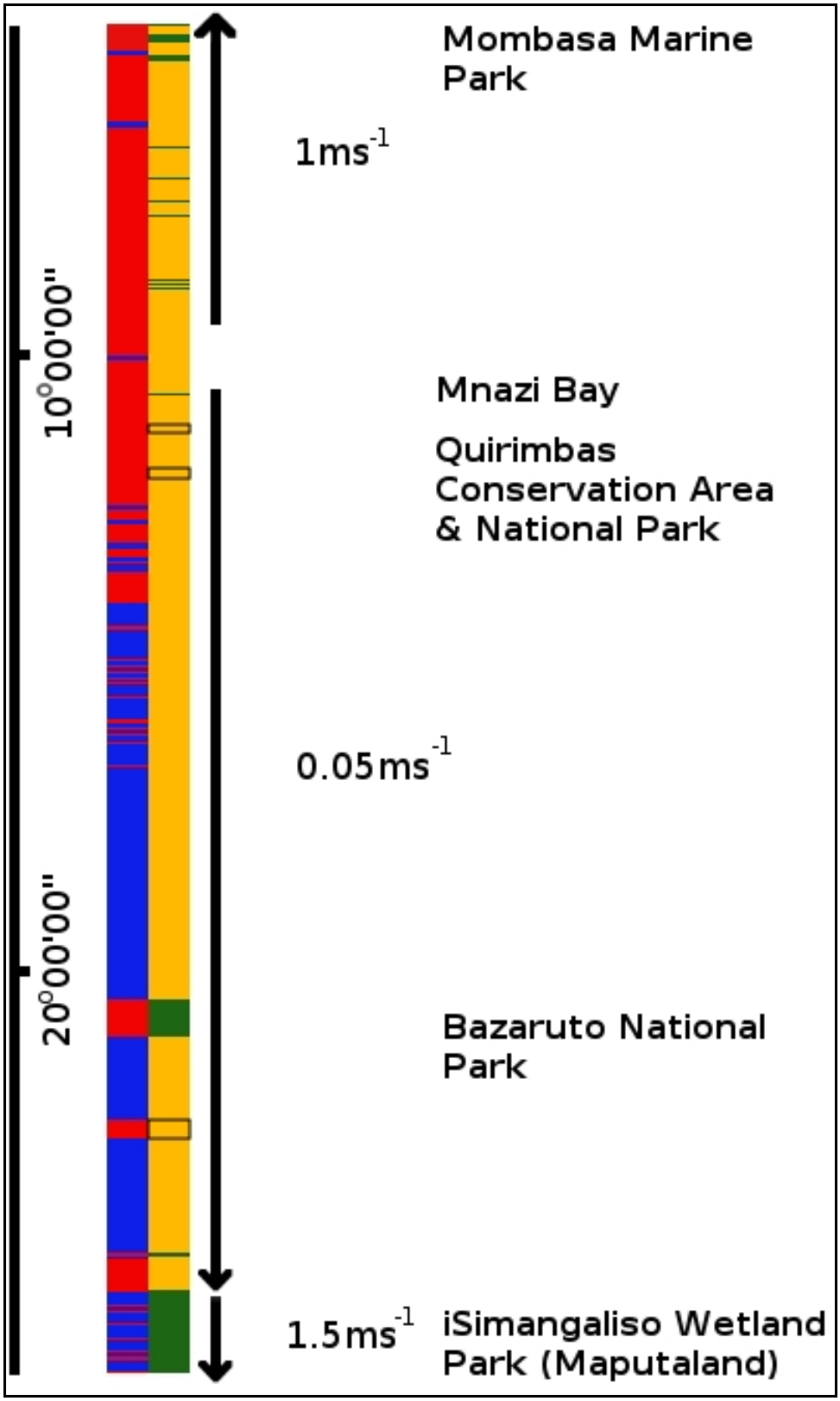


Figure 5.2 Linear representation of the location of coral reefs and marine protected areas (MPAs) along the east African coastline, from Mombasa Marine Park in the north to Maputaland in the south. Coral reef is represented in red, areas lacking reef in blue, MPA in green and unprotected regions in yellow. Open rectangles represent areas that are considered MPAs, but which have neither fisheries restrictions nor tangible policing.

Dispersal and recruitment

Reef-coral spawning and settlement patterns are relatively easy to observe in an experimental setting, but less so *in situ*. A number of studies have measured recruitment in East African waters and examined specimens for evidence of reproductive effort (Schleyer *et al.* 1997; Kruger and Schleyer 1998; Glassom *et al.* 2006; Nzali *et al.* 1998; Franklin *et al*. 1998; Masse` 2009). Although most spat are likely to recruit locally, some may travel long distances before recruiting (Babcock 1988, Wilson and Harrison 1998). Numerical modelling of recruitment patterns indicated that the local recruitment of larvae is likely (Black *et al.* 1990). Experimental data appear to indicate that most acroporids settle after planktonic development of 48 to 144 hrs (Harrison and Wallace 1990). At least 80% of the larvae recruit within 66 hrs in most *Platygyra* spp. (Miller and Mundy 2003) and within 30 hrs in *Pocillopora* spp. (Harii *et al*. 2002). Although it has been reported that *Pocillopora* spp. are brooding corals, they appear to be broadcast-spawners in South African waters (Kruger and Schleyer 1998; Masse` 2009). Such differences between regional lineages of hard corals serve as a reminder of possible paraphyly between populations separated by trans-oceanic distances (Richmond and Hunter 1990). The species selected for study, *Platygyra daedalea* and *Acropora austera*, represent two life history strategies (slow-growing, long-lived and fast-growing, short lived, respectively) amongst reef-building corals.

Coral larvae are considered to be passively-dispersed as their swimming velocity is negligible compared with the velocity of reefal water currents (Harrison and Wallace 1990). Variation in current velocities ($0.05ms^{-1}$ in the Mozambique Channel, over $1.5ms^{-1}$ in the Agulhas Current and $>1ms^{-1}$ in the EACC) creates the potential for differences in average dispersal between genera and regions along the East African coastline. The implication of these different patterns of sea-surface vicariance is that connectivity will depend on regional current velocities and specific larval recruitment patterns (Fig. 5.1).

The aim of this study is to apply population genetic data derived from local hard coral species, together with oceanographic and morphological/ life-history data to the management of local hard coral communities. The usefulness and long-term effectiveness of current MPAs in protecting these corals will thus be considered.

In order to accomplish this, all available genetic data for determining modes of connectivity between coral reefs and coral reef systems will be used. Data from these studies will be re-interpreted in light of the results of coalescent analyses of migration, other regional genetic studies, regional oceanic current patterns and the size and distribution of MPAs.

5.3 Materials and methods

Study area and sampling

The study area incorporates the shallow waters of the tropical east coast of Africa from Kenya in the north to Maputaland in South Africa and includes the Chagos Archipelago in the central Indian Ocean (Fig. 5.1). Samples were collected from throughout this study area and analysed using a variety of genetic markers (Tab. 5.3).

Estimates of migration between populations from *Acropora austera* scn intron sequences

The carbonic anhydrase 3/550 nuclear intron regions of 94 *Acropora austera* specimens drawn from six populations (Tab. 5.3) were PCR-amplified and sequenced. Migrate 3.0 (Beerli 1998; 2004; 2006; Beerli and Felsenstein 1999; 2001) was used to estimate migration per generation between designated populations for the *A. austera* dataset. Parameter space was searched for trees using the infinite allele model of nucleotide substitution, with prior assumptions for estimates of theta (Θ) and migration (M). Both Θ and M were estimated from trial runs of Migrate 3.0 and set to a uniform scale, $1x10^{-6}$ - 0.1 and 1 - 1000 respectively. A Bayesian approach was used to search for appropriate genealogies with 50 000 recorded iterations in increments of 20 steps; 1000 000 different parameter value combinations were sampled (burn-in, 10 000), with an exponential adaptive heating scheme and a swapping interval of 1 (Beerli 2006). The results of these analyses are presented as a table (Tab. 5.4) which shows the migration both ways between each pair of populations, and a histogram (Fig. 5.3) which shows the mean migration into and out of each population.

Estimates of migration between populations from *Platygyra daedalea* microsatellites (STRs)

Microsatellites were amplified from 231 samples of *Platygyra daedalea* drawn from 19 populations along the east African coast (Tab. 5.3) using 5 STR primer pairs as described. Immigration rates for *Platygyra daedalea* were calculated in Migrate 3.0 (Beerli 1998; 2004; 2006; Beerli and Felsenstein 1999; 2001). A Bayesian approach was used to search for appropriate genealogies with 10 000 recorded iterations in increments of 100 steps; 1 000 000 different parameter value combinations were sampled (burn-in, 20 000), with an exponential adaptive heating scheme and a swapping interval of 1 (Beerli 2006). Exponential priors for both theta and migration values were used. This was replicated for five independent runs with a different random seed for each run; confidence intervals were different from 0 in all estimates. The results of these analyses are presented as a table (Tab. 5.5) which shows the migration both ways between each pair of populations, and a histogram (Fig. 5.4) which shows the mean migration into and out of each population.

For both *Acropora austera* and *Platygyra daedalea*, a histogram was constructed showing net mean migration (mean immigration into minus mean emigration from a population) for

comparable groups of reefs in the SWIO (Fig. 5.5)

Congruence of genetic distance and structure between populations of *A. austera* and *P. daedalea*

Inter-population genetic distance matrices were calculated using Nei's (1972) population distance measure for both *Acropora austera* carbonic anhydrase 3/550 intron sequences and *Platygyra daedalea* STR loci; these matrices included only populations from which both genera were sampled. The resulting matrices were compared and tested for correlation. Population structure in the two data-sets was compared for the populations for which data is directly comparable (from Bazaruto to Maputaland). Structure 2.2.4 (Prichard *et al*. 2000) was used to infer the number of likely populations (K) from data comparable between data-sets (Tab. 5.3), by applying the default settings without specifying populations to which specimens belonged (Pritchard *et al.* 2000). Five runs, each consisting of 1100 000 iterations including a burn-in of 100 000, were computed for each value of K (the number of populations) from 2 to 10. The method of Evanno *et al.* (2005) was used to find the most likely number of populations.

5.4 Results

Migration between populations

Analyses showed both immigration into and emigration from all populations (Tab. 5.4, Fig. 5.3). The mean number of migrants per generation between populations of *A. austera* was 6.83 (2.79-11.3) (Tab. 5.4). The Inhaca Island population appeared to represent a break-point between net immigration to the north and the south, and had a contrasting net emigration rate (1.5) (Figs. 5.3 and 5.5). The populations with the highest mean number of immigrants per generation were those at Two-mile (10.01) and Bazaruto (8.46), whilst those with the lowest mean number of immigrants per generation were Red Sands Reef (3) and Inhaca Island (4.24).

Migration between populations of *Platygyra daedalea* is summarised in Table 5.5 and Figures 5.4 and 5.5. The mean number of migrants per generation between populations of *P. daedalea* was 1.11 (0.21 – 1.73). Immigration and emigration rates were very similar for the more northerly populations (Bazaruto, Inhaca and Rabbit Rock). The northern (Mozambican) population at Bazaruto showed a net immigration of propagules, whilst all populations to the south of this showed a net emigration of propagules, which was most pronounced in the CRC and SRC (Fig. 5.4; Fig. 5.5). The populations with the highest mean number of immigrants per generation were those at Pemba (1.47) and the Quirimbas (1.19), whilst those with the lowest mean number of immigrants per generation were located at the Southern Reef Complex (0.83) and Rabbit Rock (1.04).

Table 5.3 List of samples, locations and co-ordinates for this study of genetic connectivity of reef-coral populations in the western Indian Ocean.

	***Platygyra daedalea* (STR)**	***Platygyra daedalea* (ITS)**	***Acropora austera***	**South**	**East**
Pop	**N**		**N**		
Chagos	5			6°26’58”	71°18’25”
Mombasa Marine Park 1	18			4°02’53”	39° 43’07”
Mombasa Marine Park 2	26			4°03’54”	39° 42’04”
Mtwara Bay 1	10	5		10°16’30”	40° 23’35”
Mtwara Bay 2	7			10°18’18”	40° 23’31”
Mtwara Bay 3	1			10°15’53”	40° 23’04”
Matemo Island	6			12°11’49”	40° 33’26”
Ibo Island	2			12°18’29”	40°36’10”
Sencar Island	4			12°29’19”	40°37’19”
Garden of Eden Reef	18			12°57’28”	40°33’54”
Pemba Bay	12	6		12°54’39”	40°30’02”
Bazaruto Island Outer	22		14	21°48’24”	35°30’15”
Bazaruto Island Inner	6			21°48’27”	35°30’15”
Inhaca Bareira Vermelha	7	6	19	25°55’46”	32°55’30”
Inhaca Baixo Danae	6			26°01’54”	32°52’37”
Rabbit Rock	15		12	27°04’44”	32°51’08”
Nine-mile Reef		2		27°24’43”	32°43’40”
Two-mile Reef	18	3	18	27°31’29”	32°41’18”
Red Sands Reef	12		13	27°43’57”	32°38‘31”
Leadsman Shoal	36		18	27°54’45”	32°35’56”

Table 5.4 Number of migrants per generation (Nm) from source populations (left) into sink populations (right), for *Acropora austera* on the east African coastline. Results are based on analysis of the 3/550 intron from the nuclear carbonic anhydrase gene. Confidence intervals (97.5%) are given in parentheses.

	Bazaruto	Inhaca	Rabbit Rock	Two-mile	Red Sands	Leadsman
Bazaruto		4.46 (3.88-5.05)	4.5 (3.53-5.26)	11.3 (9.71-12.85)	2.94 (2.47-3.36)	7.79 (6.41-9.39)
Inhaca	8.83 (7.03-10.79)		7.25 (6.49-7.92)	7.85 (6.66-8.83)	1.98 (1.65-2.24)	7.03 (5.66-8.27)
Rabbit Rock	9.65 (8.21-10.79)	3.47 (2.5-4.1)		11.06 (8.51-12.77)	3.48 (3.11-3.84)	8.79 (7.38-10.13)
Two-mile	7.65 (6.04-9.1)	4.09 (3.7-5.15)	5.97 (4.55-7.77)		2.81 (2.47-3.14)	8.72 (7.75-9.76)
Red Sands	9 (7.42-10.49)	5.59 (5.05-6.43)	6.02 (5.16-6.8)	9.87 (8.75-10.92)		9.96 (8.87-11.03)
Leadsman	10.6 (8.41-11.87)	3.59 (3.09-4.07)	6.92 (5.77-8.02)	9.99 (8.19-11.88)	3.81 (3.17-4.34)	

Genetic distance analyses

Distances between populations ranged from 0.00 to 0.037 for *Acropora austera* and from 0.00 to 0.36 for *Platygyra daedalea* (Table 5.6). The mean Nei's genetic distance between populations of *A. austera* was 1.5%, whilst that for *P. daedalea* was 17.4%. The *P. daedalea* populations separated by the greatest genetic distance were Inhaca Island and Bazaruto Island (0.361), whereas for *A. austera* they were Inhaca Island and Rabbit Rock Reef (0.037). For both corals, the populations at Leadsman Shoal and Red Sands Reef were the most similar populations, with no measurable genetic distance between them (Tab. 5.6), which is likely to reflect their close proximity at the south of the study range. No correlation was observed in genetic distances (Nei 1972) between comparable populations of *A. austera* and *P. daedalea* ($R^2 = 0.1$ $p = 0.2$).

Structure in populations

For both the *Acropora austera* carbonic-anhydrase 3/550 intron and the *Platygyra daedalea* STR loci, Structure 2.2.4 assigned the data to three populations (Fig. 5.6). Although admixture is suggested in both bar plots, as suggested by more than one population represented in a single individual (as represented in a bar), the data for *P. daedalea* (Fig. 5.6a) suggests much higher levels of admixture than that for *A. austera* (Fig. 5.6b). The *A. austera* bar plot (Fig. 5.6b) indicates that there is little admixture amongst the three assigned populations. For both marker systems there appears to be a discontinuity in the region of the Mozambique-South Africa junction, between southern Mozambique and Maputaland. For the *P. daedalea* STR data this occurs between the Bazaruto Archipelago and Inhaca Island, whereas the discontinuity

appears in the region of Rabbit Rock for the *A. austera* data (Fig. 5.6).

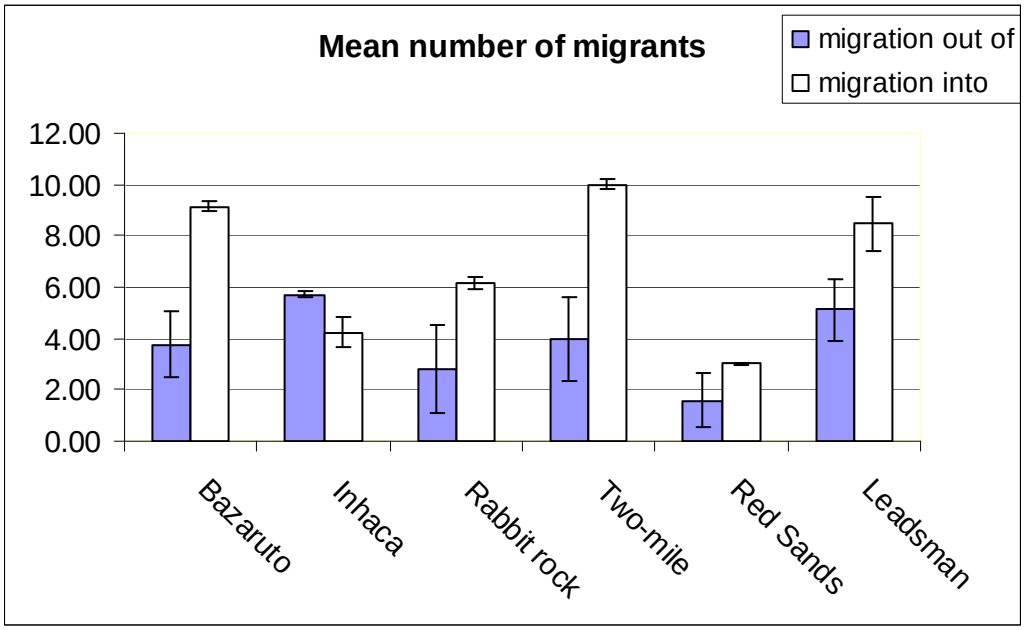


Figure 5.3 Mean number of migrants per generation between populations of *Acropora austera* on the south east African coast, based on analsyis of carbonic anyhdrase 3/550 nuclear intron sequences. The bars represent the standard deviation of each measurement.

Table 5.5 *Platygyra daedalea* migration from source populations (left) into sink populations (right) in the western Indian Ocean, as estimated from 5 STR loci. Values are numbers of migrants between populations per generation. Confidence intervals (97.5%) are given in parentheses.

	Chagos	Mombasa	Quirimbas	Pemba	Bazaruto	Inhaca	Rabbit Rock	Central reef complex	Southern reef complex
Chagos		0.93 (0.61-1.25)	0.56 (0.4--0.74)	1.69 (1.27-2.14)	0.91 (0.69-1.08)	1.01 (0.74-1.3)	1.28 (1.02-1.61)	0.74 (0.53-0.94)	0.58 (0.39-0.77)
Mombasa	0.21 (0.20-0.24)		1.37 (0.73-1.84)	1.73 (1.4-2.01)	1.46 (1.22-1.68)	1.13 (0.71-1.43)	0.9 (0.61-1.13)	1.39 (1.19-1.66)	1.4 (1.11-1.71)
Quirimbas	1.26 (0.94-1.58)	0.98 (0.73-1.22)		1.24 (0.76-1.66)	1.36 (1.1-1.68)	1.06 (0.84-1.29)	0.75 (0.45-0.97)	1.21 (1.07-1.63)	0.79 (0.58-1.08)
Pemba	1.13 (0.87-1.38)	1.14 (0.94-1.4)	1.25 (0.98-1.52)		0.82 (0.6-1)	0.68 (0.69-1.02)	1.25 (0.9-1.66)	0.82 (0.59-1.04)	0.52 (0.34-0.69)
Bazaruto	1.39 (1.15-1.65)	1.38 (1.12-1.63)	1.43 (0.93-1.75)	1.47 (1.05-1.84)		0.65 (0.33-0.99)	1.02 (0.75-1.24)	0.81 (0.54-1.11)	0.94 (0.75-1.16)
Inhaca	1.05 (0.81-1.27)	1.09 (0.89-1.3)	1.4 (1.15-1.64)	1.06 (1.05-1.84)	1.35 (1.06-1.67)		0.83 (0.22-0.91)	1.29 (1.01-1.63)	0.88 (0.71-1.02)
Rabbit Rock	1.55 (1.21-1.85)	0.67 (0.28-1.01)	0.97 (0.73-1.19)	1.67 (1.18-2.23)	1.23 (0.75-1.55)	1.16 (0.82-1.48)		0.73 (0.49-1.08)	0.68 (0.31-0.89)
Central reef complex	1 (0.74-1.28)	1.31 (0.96-1.76)	1.4 (1.13-1.75)	1.19 (0.82-1.57)	1.07 (0.49-0.92)	1.41 (0.94-1.85)	1.19 (0.88-1.45)		0.85 (0.63-1.09)
Southern reef complex	1.06 (0.61-1.6	1.32 (1.08-1.56)	1.15 (0.7-1.2)	1.72 (1.32-2.06)	1.27 (0.99-1.5)	1.26 (0.94-1.53)	1.07 (0.72-1.36)	1.59 (1.11-1.94)	

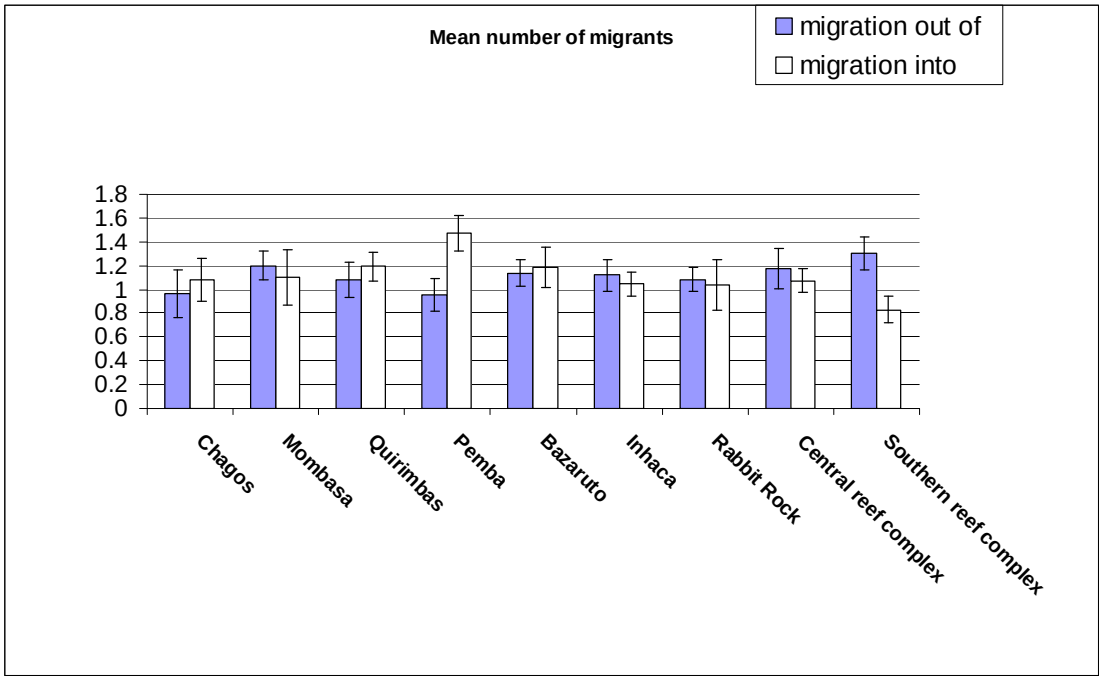


Figure 5.4 Mean number of migrants per generation between populations of *Platygyra daedalea* on the south east African coast, based on analysis of STR data. The bars represent the standard deviation of each measurement.

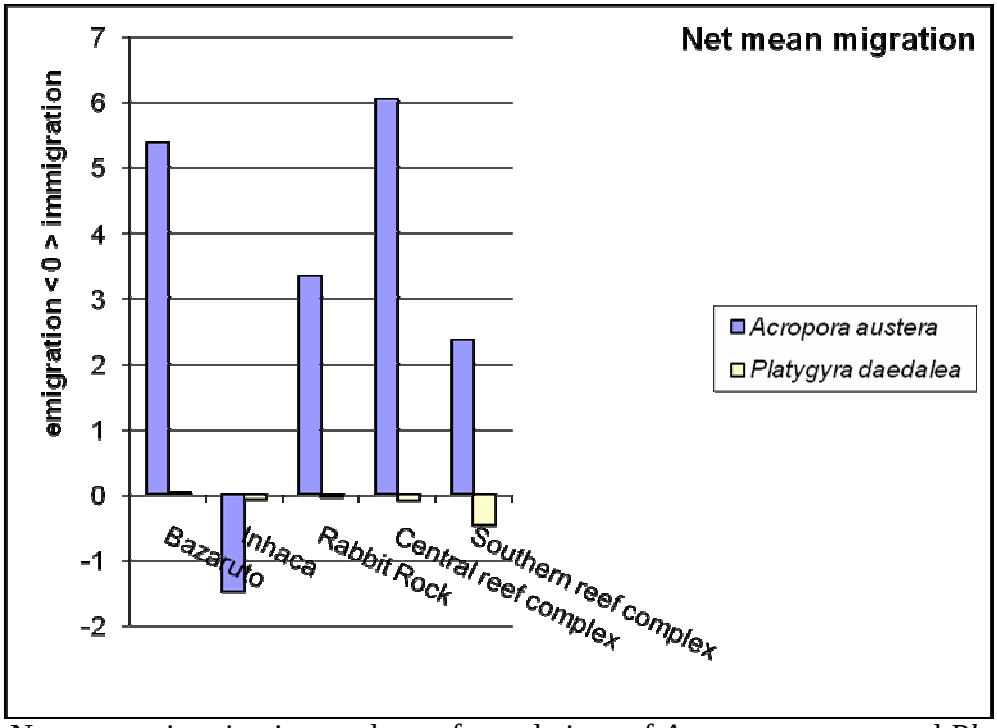
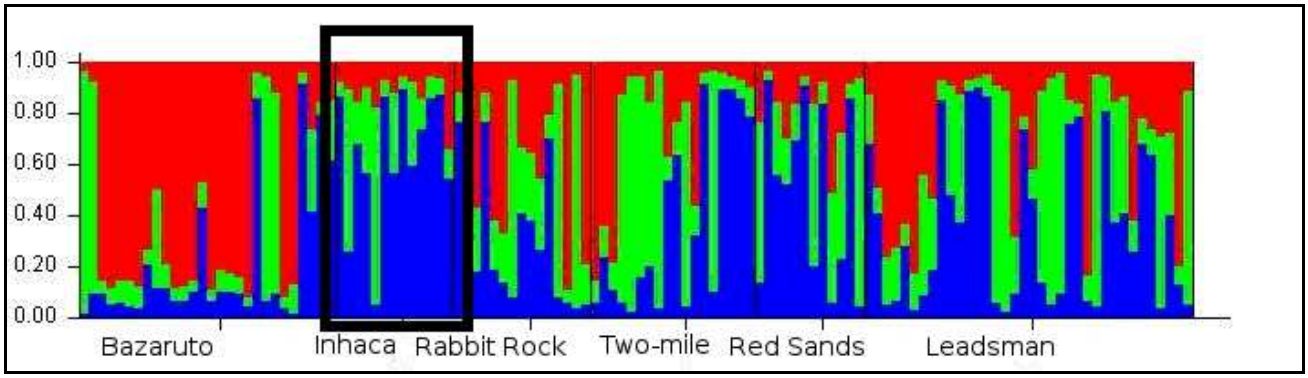


Figure 5.5 Net mean migration into and out of populations of *Acropora austera* and *Platygyra daedalea* in the south-western Indian Ocean.

Table 5.6 Nei's genetic distances between sites from Bazaruto Island, central Mozambique, to Maputaland, South Africa. *Acropora austera* (3/550 intron from the carbonic anhydrase gene) distances below the diagonal and *Platygyra daedalea* (5 STR loci) distances above the diagonal**.**

	Bazaruto	**Inhaca**	**Rabbit Rock**	**Two-mile**	**Red Sands**	**Leadsman**
Bazaruto	-	0.361	0.183	0.136	0.233	0.097
Inhaca	0.008	-	0.31	0.23	0.24	0.17
Rabbit Rock	0.027	0.037	-	0.11	0.25	0.09
Two-mile	0.003	0.016	0.019	-	0.14	0.06
Red Sands	0.005	0.022	0.022	0.004	-	0
Leadsman	0.006	0.023	0.022	0.004	0	-

a)



b)

Figure 5.6 a) Population structure inferred from a sub-set of *Platygyra deadalea* STR loci from chapter 4 with K = 3 (K = number of inferred populations). b) Population structure inferred from the 3/550 intron of the carbonic-anhydrase gene from *Acropora austera* samples from chapter 3 with K = 3. Populations included in this analysis were from reefs at Bazaruto Island, Inhaca Island, Rabbit Rock Reef, Two-mile Reef, Red Sands Reef and Leadsman Shoal. The black rectangle indicates a unique population situated between northern and southern groups.

5.5 Discussion

Studies of hard coral population genetics on the East African coast have given an indication of the extent of linkages amongst populations that are separated at various scales by a variety of barriers. Predominant oceanographic regimes, although uninformative about the fine-scale

distribution of reef-coral propagules, are likely to be important determinants of the evolutionary patterns of coral distribution amongst regional reef systems. In addition, geologically-related sea-level changes may prove important in understanding genetic relationships between contemporary coral reefs.

Analyses of the population structure of *Acropora austera* and *Platygyra daedalea* using the programme Structure (Pritchard *et al*. 2000) assigned both datasets to three populations (Fig. 5.4). The higher mean genetic distances between *P. daedalea* than between *A. austera* populations are likely to reflect a greater rate of fixation of mutations in the STR loci of *P. daedalea* than in the 3/550 intron of *A. austera*. The *P. daedalea* populations separated by the greatest genetic distance were those at Inhaca Island and Bazaruto Island (0.361), with different populations (predicted by Structure) appearing to predominate at these two sites (Fig. 5.6a). Similarly, different Structure-predicted populations (Fig. 5.6b) predominated at the genetically most-distant *A. austera* study sites, located at Inhaca Island and Rabbit Rock Reef. For both corals, Leadsman Shoal and Red Sands Reef were the most similar populations, with no measurable genetic distance between them (Tab. 5.5), which is likely to reflect their close proximity at the south of the study range. No correlation was observed in genetic distances (Nei 1972) between comparable populations of *A. austera* and *P. daedalea*. Although genetic distances between populations of *A. austera* and *P. daedalea* are not directly comparable, their relative relationships might be expected to be.

The Structure 2.2.4-generated bar-plots (Fig. 5.6) indicate breaks between populations of both *Acropora austera* and *Platygyra daedalea* in the region of Inhaca Island and Rabbit Rock Reef. The population genetic structure inferred from the *A. austera* 3/550 carbonic-anhydrase intron sequences is similar to that from the *P. daedalea* populations, as each comprise three populations (K=3) and show a discontinuity in connectivity between northern and southern population groups. Populations in the north of the study region (Mozambique Channel), where the currents take the form of relatively slowly-moving gyres, are expected to be more genetically-structured than those located in the more southerly and fast-flowing Agulhas current, as propagules are not expected to be transported as far before settling and there is more suitable habitat available for recruitment (Fig. 5.2; Tab. 5.2). A break in connectivity between these and more southerly populations located in the Agulhas Current, which have larger hypothesised dispersal kernels, is not therefore unexpected, and has implications for management of the coral reefs of the region. In the case of *A. austera* this is supported by higher diversity in Mozambique (see Chapter 3), where monophyletic clades of unique haplotypes occur, and poorly-resolved structuring of genetically less-diverse (Chapter 3, Tab. 3.1) populations in the south. Although the Structure 2.2.4 algorithm is not ideal for analysing single-locus sequence data (Pritchard *et al*. 2000), and illustrates the genetic structure of the carbonic anhydrase 3/550 intron rather than the population structure of *A.*

austera, it is nevertheless an informative measure.

Counter-current gene flow – a reflection of past geological events / disequilibrium amongst populations?

For both *A. austera* and *P. daedalea*, the coalescent was used to infer migration between populations included in this study; Migrate (Beerli 2008) was used to analyse the carbonic-anhydrase 3/550 intron data from *A. austera* (Tab. 5.3) as well as for the STR data from *P. daedalea* (Tab. 5.4). In both species coalescent analyses indicated some gene-flow counter (from south to north) to the direction expected (north to south) considering prevalent oceanographic regimes. However, these inferences of migration rates (Tab. 5.4; Tab. 5.5) are based on the present distribution of hard coral populations, which has been governed by historical, geological and evolutionary processes. Geological processes likely to have influenced the distribution of hard coral in the study area are sea-level change, a narrowing of the Mozambique Channel (Rabinowitz and Woods 2006) or even a possible land bridge between Africa and Madagascar (McCall 1997). Despite the projected historical prevalence of the Agulhas Current (Gordon 1973), such changes undoubtedly influenced evolutionary processes in the hard coral inhabiting this region (Benzie 1999). The Davie Ridge runs east-west between northern Mozambique and western Madagascar and is a significant feature of the Mozambique Channel (Fig. 5.1). It was much nearer to the sea-surface in times of lower sea levels, and although it may not have been a land bridge, it was almost certainly a significant barrier to the southerly flow of oceanic water from Tanzania and northern Mozambique. North of the Davie Ridge, populations were probably established by propagules transported by the South Equatorial Current (SEC). It is also possible that hard coral propagules reached southern Mozambique and Maputaland by way of the East Madagascan Current.

Thus, the contemporary distribution of hard coral south of the Davie Ridge may have been established by a means other than the Mozambique Current. Therefore, although the counter-current gene-flow generated by coalescent models seems counter-intuitive, this may reflect patterns of dispersal over geological time periods. Additionally, genetic equilibrium amongst populations is assumed for these models (of evolutionary relationships) to accurately reflect gene-flow patterns. It takes thousands of generations for genetic equilibrium to be effectively established in corals, as they are reported to have slowly-evolving genomes and asymmetrical generations (Potts 1984; Shearer *et al.* 2002). Corals conform very poorly to the discrete generation patterns shown by most ’model‘ organisms that were used in designing genetic relatedness/ differentiation algorithms (Potts 1984). In summary, the unexpected patterns of counter-current gene flow predicted using Migrate may be the result of evolutionary and geological processes, rather than a snapshot of present-day relationships between reefs.

Comparisons between regional population genetic studies

Both studies of genetic differentiation that were restricted to a single oceanographic regime (Tab. 5.2, Fig. 5.7a) found evidence for a single panmictic regional population (*Pocillopora verrucosa*, Ridgway *et al*. 2001; *Platygyra daedalea*, Souter and Grahn 2008). The EACC and Agulhas Current are both fast-moving, with velocities in excess of $1ms^{-1}$ and $1.5ms^{-1}$ respectively and have the potential to move propagules between reefs separated by as much as 800 km. However unlikely this may seem, considering habitat available for recruitment within both of these ocean currents, the potential for successful recruitment by the widespread dispersal of propagules is evident in the apparently-panmictic populations of these two corals at opposite ends of the life-strategy spectrum.

Those studies that included the area encompassing the Mozambique Current regime all exhibited significant differences from the null hypothesis of panmixia, with F_{ST} values of between 0.049 and 0.113 (Fig. 5.7) (Ridgway *et al*. 2008). This may be expected as water in the Mozambique Channel moves slowly in southerly-drifting gyres (Lutjeharms 2006) and thus propagules may not be transported very far before they recruit to appropriate habitats. This is reflected in this thesis by significant genetic structuring of populations when measured over large scales incorporating the Mozambique Channel (Fig. 5.7b). One might expect the Pocilloporidae to be the most structured of hard coral populations, as they are generally considered brooders of their larvae, with consequently shorter PLDs (Shanks *et al*. 2003). However, in South African high-latitude coral-communities, research indicates that *Pocillopora verrucosa* and *P. damicornis* are broadcast spawners (Kruger and Schleyer 1998; Masse` 2009) and are therefore likely to exhibit a different PLD from their eastern Pacific con-specifics (Sammarco and Andrews 1989).

Populations of hard corals along the coastline on which this study is based are subject, on average, to unidirectional current flow. This has important consequences for the structure of downstream populations (Wares and Pringle 2008). In this model, the source population (*i.e*. Chagos Archipelago, Tanzania and possibly East Madagascar) is upstream (Fig. 5.2). Contained within the dispersal kernel of that upstream population is the genetic diversity that might be fixed within the downstream meta-population (Quirimbas, Pemba and the Primeiras / Segundas or eastern Madagascar). Novel diversity arising in downstream populations at the limit of the southerly range of scleractinia may be lost to genetic drift with only novel upstream alleles contributing to overall levels of genetic diversity. Further, faster moving currents may lead to the reduction of effective population sizes (Wares and Pringle 2008). In this context southern African populations of hard corals bear a resemblance to sinks of genetic diversity (Crowder *et al.* 2000), losing new genotypes to inhospitable downstream habitats.

Patterns of immigration and emigration in *Acropora austera* and *Platygyra daedalea*

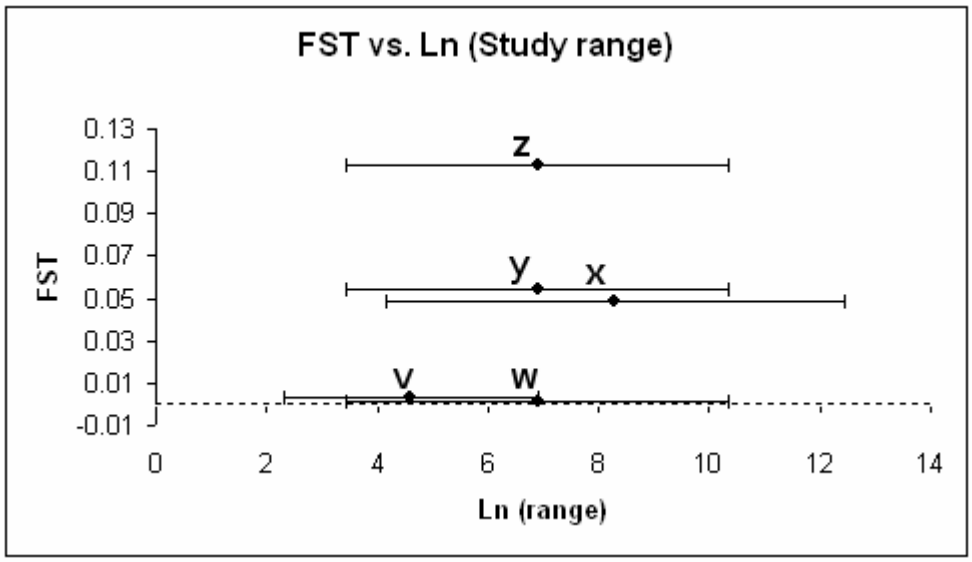
There were significant amount of both immigration into and emigration from all populations of *Acropora austera* and *Platygra daedalea* which formed part of this study. With one exception (Inhaca Island), all *A. austera* populations showed a net immigration of propagules. In contrast, *P. daedalea* populations, except Bazaruto, showed net emigration, although this was slight. The mean number of migrants per generation was 6.1-fold higher (6.83 *vs*. 1.11) for *A. austera* than for *P. daedalea*. Overall, differences between *P. daedalea* and *A. austera* migration rates might be expected considering that Acroporids are likely to spend more time in their pelagic stage than the Faviids (Tab. 5.2), and thus be dispersed further on average (reflected in higher migration rates amongst populations of *A. austera*).

In the Mozambique Channel at Bazaruto, populations of both *P. daedalea* and *A. austera* show higher rates of immigration than emigration, which may partly be related to their exposure to the slower-moving eddies of the Mozambique Channel (Figs 5.3, 5.4 , 5.5), and which may allow the accumulation of genetic diversity. This is consistent with the higher levels of genetic diversity found in *A. austera* in the north of the study area (see Chapter 3).

Further south, *A. austera* and *P. daedalea* show different relative prevalence of immigration and emigration. There is a net emigration of *Acropora austera* propagules from Inhaca Island; this may be a source of migrants entering reefs to the south, such as Rabbit Rock and reefs in the Central and Southern Reef complexes, which show net immigration (Figs 5.3, 5.5). Inhaca Island is located within Maputo Bay, around the region of origin of the fast-flowing Agulhas current, which is likely to transport propagules southwards from Inhaca, thus allowing them to seed Maputaland reefs.

North-south and south-north gene-flow in *Platygyra daedalea* appears to occur at similar levels in most northern study populations (Bazaruto to Rabbit Rock), although towards the south, the Central and Southern Reef Complexes show increasing net emigration of propagules, which are likely to be lost as the inhospitable habitat to their south is not suitable for the establishment of reef-corals (Tab. 5.4; Figs. 5.4 and 5.5).

a)



b)

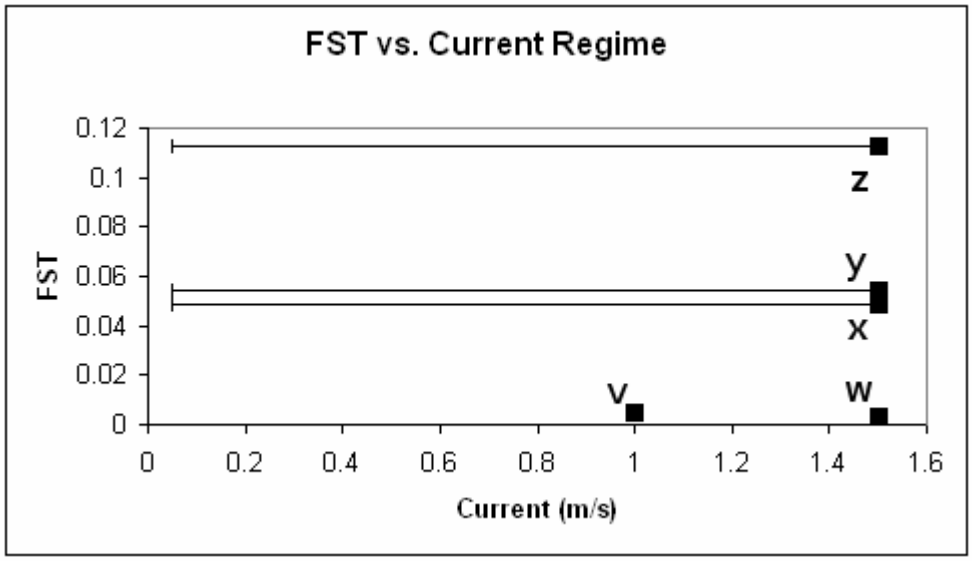


Figure 5.7 Graphs illustrating results of a variety of genetic studies on corals from the south west Indian Ocean (Souter and Grahn 2008; Ridgway *et al*. 2001; 2008; Chapters 3 & 4). In a) F_{ST} values are compared with the range (log n values of ranges in km) over which those values were measured whilst in (b) F_{ST} values derived from studies of hard coral are compared with current speed in different ocean current regimes in the western Indian Ocean. v – Souter and Grahn 2008; w – Ridgway *et al*. 2001; x – Chapter 4; y – Ridgway *et al*. 2008 and z – Chapter 3.

Such loss of novel alleles is possibly reflected in *Acropora austera* populations (Chapter 3), where northern populations were found to harbour more genetic diversity than southern populations. This was not, however, the case for *Platygyra daedalea* (Chapter 4), where allelic diversity did not appear to be higher in northern populations. This may be explained by the degradation of northern reef systems by bleaching and anthropogenic perturbations, combined with

the existence of larger populations on the marginal Maputaland reef systems (pers. obs.). On closer analysis of predicted dispersal kernels (Tab. 5.2), it is apparent that different regions in the study area are subject to different current directions and speeds. This may help to explain why the genetic diversity of populations in the Mozambique Channel do not reflect the model of loss of diversity in downstream populations. However, populations situated downstream of one another within the fast moving current regime of the Agulhas Current do appear to harbour lower levels of allelic richness (*i.e.* Leadsman Shoal < Red Sands Reef < Two-mile Reef; Chapter 4 – Fig. 4.4).

The SEC meets the African coastline and diverges in a northerly direction in the form of the EACC (> $1ms^{-1}$) and into slow-moving eddies (< $0.05ms^{-1}$) that run south in the Mozambique Channel (Fig. 5.1). These have a profound influence on the genetic structure of local hard coral populations at Mombasa Marine Park, Mnazi Bay, the Quirimbas Archipelago and Pemba Bay (Fig. 5.2, Tab. 5.1). The Agulhas current (> $1.5ms^{-1}$) begins at the south of the Mozambique Channel in the region of Maputaland, which is at the southernmost limit of hard coral communities (Swallow *et al*. 1991; Lutjeharms 2006).

The dispersal range of coral propagules may be extended in regions of higher current velocity, where genetic structure might be less discernable, as is evident in Ridgway *et al*. (2001) and Chapter 3. Genetic structure amongst populations at Two-mile Reef, Red Sands Reef and Leadsman Shoal is similar in composition in both *Platygyra daedalea* and *Acropora austera* (Fig. 5.7) indicating panmixia amongst these reefs. In lower-velocity current systems, however, it is more likely that populations will display 'genetic structure' in the form of fixed allelic differences, as was found for *A. austera* populations (see Chapter 3). This is also contingent on larval dispersal strategy, and corals with long pelagic larval durations may be expected to display less structure than those with fast-recruiting, crawling or reef-seeking larvae (Ridgway *et al* 2001; Goffredo *et al.* 2004). Differences in genetic diversity and structure between *P. daedalea* and *A. austera* in the SWIO seem best explained by life history differences than merely pelagic larval dispersal kernels. Analyses using Structure 2.2.4-analyses indicate that populations of *P. daedalea* exhibit more admixture (Fig. 5.6a) than those of *A. austera* (Fig. 5.6b), an indication that the former constitute a single large panmictic population. This must of course be considered in light of the use of different markers for the two corals, potentially with different evolutionary rates. This is in contrast to expectations based purely on PLD, and the success of *P. daedalea* in the marginal habitat of Maputaland attests to its ability to adapt to a broader suite of environmental conditions than *A. austera*.

It is also important to account for the effects of sweepstakes recruitment success (SRS), wherein a few genotypes are randomly selected for because of chance success in recruitment (Hedgecock 1994). Thus, F_{ST} values summarised in this study may be somewhat inflated due to the

successful chance recruitment of propagules from a limited number of individuals upstream (Tab. 5.1, Fig. 5.5). Evidence for SRS in studies of *Platygyra daedalea* on the coral reefs of east Africa is clear in high observed heterozygosity (Ho=0.80 ±0.03), a tell-tale signature of this effect (Hedgecock *et al.* 2007).

Comparable F_{ST} values (0.054 and 0.113 respectively) were observed by Ridgway *et al*. (2008) and for *A. austera* (Chapter 3, this study), in studies of coral population genetics spanning the Bazaruto Archipelago and reefs in Maputaland. These are an indication of fine-scale genetic structure when considering the sample sizes of the studies from which these data were drawn. However gene flow estimates calculated in Migrate (Beerli 2006) indicated that populations were probably exchanging more than an effective individual per generation, thus effectively homogenising them (Tab. 5.5). Further, as sample sizes were low in the above studies, F_{ST} values must be considered with caution (Waples 1998). The resilience of reef coral populations, as they are currently composed, should be of concern to management authorities. Practically, managers should consider that stress to upstream populations could significantly reduce the number of propagules that might reach downstream populations. Thus, trans-political barrier co-operation is necessary for the long term management of coral reefs in South Africa.

Oceanographic transport of propagules

Many studies of population genetics fail to consider the oceanographic regimes of their study areas because it is assumed that fine-scale oceanographic dynamics dictate the dispersal of propagules. Recent evidence supports the notion that most reef-coral propagules recruit locally (Underwood *et al*. 2007; Souter *et al*. 2009). However, patterns that are exhibited by reef-coral populations over larger scales may be expected to bear some of the signature of the predominant oceanographic current system.

Populations in the far south of the region are influenced by the fast-flowing Agulhas Current, which has seemingly dictated population genetic structure through the rapid unidirectional dispersal of propagules (Wares and Pringle 2008). Although genetic markers are a proxy measure of dispersal, they are a measure of successful recruitment and survival to an age where the organism is able to contribute gametes to subsequent generations of corals, an index to which studies in other taxa aspire (Pineda *et al*. 2007). It was evident from the accumulated data of several population genetic studies (Tab. 5.2) and available oceanographic data (Lutjeharms 2006; Quartly *et al*. 2006) that the Mozambique Channel region may present a barrier to passively-dispersing coral larval propagules (see Chapter 3 for evidence of a southern Mozambique / South Africa barrier). Estimated larval dispersal kernels for both *Acropora austera* and *Platygyra daedalea* are significantly shorter than the average distance between MPAs in the Mozambique Channel region. Further, it is in this region of the Mozambique Channel (Quirimbas, Pemba and Bazaruto) that the

rates of immigration are higher than rates of emigration (Fig. 5.4; Fig. 5.5). The northern part of the Mozambique Channel harbours the most diverse region of east African coral reef (Veron 2000), the southern coast of Tanzania and northern coast of Mozambique (Fig. 5.1). These regions are a potential source for many more propagules than the relatively depauperate regions directly to the south, although the barrier presented by the Mozambique Channel may limit connectivity with Maputaland reefs, particularly at demographic levels. This barrier may be the consequence of a number of different factors, including the lack of availability of suitable habitat, terrigenous freshwater input, subsequent higher nutrient levels and ultimately, slow coastal water movement (Riegl 1996; Lutjeharms 2006; Jones *et al*. 2007). However, Acroporid patterns of migration rates (Tab. 5.4) appear to indicate the regular transmission of propagules between Bazaruto and both Inhaca Island and Maputaland (Fig. 5.3, Fig. 5.5).

The East Madagascar Current (EMC) presents a possible conduit for the transmission of propagules from the SEC to the SWIO. The EMC rounds the southern tip of Madagascar and flows south-west towards the East African coastline in the form of large eddies. There has been debate about the subsequent direction of this surface current, with some studies finding evidence for the retroflection of the EMC (Lutjeharms 2006) and others finding evidence for westerly movement of eddies from the EMC across the southern mouth of the Mozambican Channel (Quartly *et al*. 2006) (Fig. 5.1). These anti-cyclonic eddies may form a significant part of the source of the Agulhas current. This surface water, although slow-moving, presents a possible route for coral larvae to travel from the eastern coast of Madagascar to southern Africa. However, as eastern Madagascan coral communities exist only in the north-eastern coastal waters, this would require that propagules be transported from north-east Madagascar to Maputaland in slow-moving oligotrophic waters which are potentially as significant a barrier to dispersal as the large expanses of unsuitable habitat on the east African coast in the Mozambique Channel area. Estimates of migration have indicated that inter-population counter-current gene-flow does occur in both *Platygyra daedalea* and *Acropora austera* in the study region (Tab. 5.4; Tab. 5.5). This provides some support for the hypothesis that coral propagules may be dispersed from the east coast of Madagascar, around the southern tip of Madagascar and then northward into the Mozambique Channel. Further support for this hypothesis of gene-flow from the southern tip of Madagascar to the east African coast is however, necessary.

Habitat and MPA scales of separation

The Reefbase (www.reefbase.com) online geographic information system (GIS) was used to estimate scales of separation between MPAs and suitable habitat along the east African coastline (Fig. 5.2). These rough estimates and averages may be considered as preliminary pending formal GIS study. The data is consistent with estimated values from recent publications and

communications from experts concerning newly-established MPAs (Perreira pers. comm.; Wells *et al*. 2007).

There are large gaps between coral communities from the equator south to the marginal communities of Maputaland, South Africa. These gaps arise as suitable habitat for larval recruitment attenuates and riverine input increases where a number of large river deltas (Zambezi, Rovuma, Limpopo, Komati and Savé) dominate the coastline. The low-velocity eddy systems that meander poleward down the Mozambican Channel south of Mnazi Bay in Tanzania (Quartly and Srokosz 2004) do not transport propagules efficiently between reefs in this area of poor habitat (Fig. 5.2). This is reflected on evolutionary scales in our findings of stepwise attenuation of coral diversity from reefs in southern Mozambique toward the marginal reefs of Maputaland (Chapter 3), likened by Riegl (1996) to differences between Kenyan and Somali reefs in the northern WIO.

Kenya protects approximately 9% of its coral reef habitat, Tanzania 4% and Mozambique 2% (Wells *et al.* 2007) whereas all of South Africa's coral reefs lie within MPAs and over 50% are strictly no-take zones (iSimangaliso Wetland Park Authority 2008). Kenya has 630 km^2 of coral reef, Tanzania 3500 km^2, Mozambique 2000 km^2 and South Africa <50 km^2. Thus, Tanzania protects the largest area of coral reef on the east African coastline (Wells *et al*. 2007; Schleyer and Celliers 2005). Certainly, the management burden in the highly-populated coastal regions of Kenya, Tanzania and Mozambique is heavier with a larger proportion of artisanal fishers reliant on coral reef resources for their survival than in South Africa (van der Elst *et al*. 2005). These gaps between suitable habitat and between MPAs must be considered in light of the dispersal kernels of hard corals, measured levels of population subdivision (F_{ST}) and the adequacy of regional MPAs in ensuring the continued persistence of coral reefs.

Pelagic dispersal of larvae within the east African coastal oceanographic environment

Pelagic larval dispersal distances (PLD) for the three hard corals (*Platygyra daedalea*, *Acropora austera* and *Pocillopora verrucosa*) examined in this study were estimated from recent studies of larval settlement (Tab. 5.1) (Harrison and Wallace 1990; Harii *et al*. 2002; Miller and Mundy 2003), taking regional oceanography into account (Tab. 5.2). The PLDs are large as a result of the simplistic approach taken, but 60% of the variation in dispersal distance of pelagic larvae may be explained by duration in the pelagos (Shanks *et al*. 2003). Most larvae released in spawning events are predicted to recruit to natal reefs (Black *et al*. 1990). In addition, a large number have been recorded recruiting near natal reefs (Sammarco and Andrews 1989) and genetic data has indicated that local populations of brooding corals are self-seeded (Underwood *et al*. 2007). Indeed, it is likely that even propagules with longer PLD ranges are recruiting to natal reefs, although a few individuals may travel further, between reef systems (Magalon *et al*. 2005; Jones *et al*. 2007; van Oppen *et al*. 2008).

The values obtained in this study are inferred from experimental observations of settlement rates and represent values obtained for 80% settlement of propagules (Harrison and Wallace 1990; Harii *et al*. 2002; Miller and Mundy 2003). These values have yet to be assessed under natural conditions, but it appears that, except for *Pocillopora* spp., PLD is sufficient to maintain genetic, if not demographic, connectivity between populations (Tab. 5.2). On average it appears that pocilloporids may not have large enough PLDs for routine inter-MPA dispersal (Tab. 5.2), although it has been shown that they may be able to spend extended periods in the plankton prior to recruitment (Richmond 1987). Nonetheless, distances that the larvae of *Platygyra* spp. and *Acropora* spp. might disperse in average current velocities in this study (203km and 442km, respectively), are sufficient to span average distances between MPAs in the region (129km) (Tab. 5.2) (this is supported by estimates of migration between populations).

The patterns of migration revealed for *P. daedalea* and *A. austera* (Tab. 5.4; Fig. 5.3; Tab. 5.5; Fig. 5.4; Fig. 5.5) may be related to oceanic current regimes (Fig. 5.1) and predicted larval dispersal kernels (Tab 5.1; Tab. 5.2). In the *Platygyra*, both of the faster moving current regimes (the EACC and Agulhas Current populations; Mombasa, Rabbit Rock, the CRC and SRC) were dominated by higher rates of emigration than immigration (Fig. 5.4), whilst the intermediate Mozambique Channel region was characterised by higher levels of immigration in both species, with much reduced larval dispersal kernels (Tab. 5.2) as a result of slower-moving water. Thus regions with faster-moving currents appear to lose more propagules than they gain in the *Platygyra*. This result seems to be independent of available habitat, with Mombasa Marine Park (more available habitat suitable for recruitment) and the Southern Reef Complex in Maputaland (limited available habitat for recruitment) similarly displaying net emigration (Fig. 5.5).

Pocilloporids and acroporids are widely recognised as having significantly shorter PLDs than estimated here (Shanks *et al*. 2003; Underwood *et al.* 2007; Kinlan and Gaines 2003). This is a reflection of the disparity between experimental estimates of settlement (Harii *et al*. 2002; Richmond 1987; Harrison and Wallace 1986) and natural realization of dispersal determined in studies of coral population genetics (Underwood *et al*. 2007; Magalon *et al*. 2005; Baums *et al*. 2005). In light of the evidence presented here for connectivity between MPAs along the east African coastline, two levels of connection ought perhaps to be considered: demographic and evolutionary. Demographic connectivity allows for populations that are subject to mass-mortality to be re-seeded by nearby connected reefal populations. In the case of catastrophic events, such as the recent mass-bleaching episodes, evolutionary connectivity to reefs from regions unlikely to have been affected by the same events need to be considered (reefs further away than that encompassed by a single dispersal event). Thus reef-systems may maintain high levels of diversity from occasional migrants from far-off reefs.

Implications for regional MPA management

Kenyan and Tanzanian MPAs are large enough that, if their protection is enforced and existing no-take areas are extended within already-managed areas, these important sources of regional diversity may be adequately protected. The central region of the east African coast (south Kenya, Tanzania and northern Mozambique) is the centre of regional coral diversity, with species diversity showing typical attenuation from the equator southwards (Gray 1997). Considering the lower levels of genetic diversity in the south of the study area (Chapter 3), these populations may be sinks for regional genetic diversity and may therefore be considered a lower priority for protection (Wares and Pringle 2008; Crowder *et al*. 2000).

High heterozygosity of island populations throughout the study area (Chapter 4; Souter and Grahn 2008) is an indication that these populations may have been established by chance recruitment of a few individuals (Hedgecock 1994). Southern populations of reef corals, particularly in the marginal region encompassed by this study, also bear this signature of high heterozygosity and thus may not be connected to the extent that the low levels of genetic differentiation indicate. Thus Maputaland reefs may not be connected at demographic levels to southern Mozambican reefs; rather, they may be reliant on reefs in southern Mozambique alone for occasional migrants in order to maintain the observed high levels of genetic diversity. This is corroborated by the data on migration (Tab. 5.5).

Implications for marginal MPA management

Maputaland reefs are subject to high velocity ocean water movement and are situated close to the continental shelf along which the current flows. These corals are likely to have wide dispersal kernels, and are therefore less likely to recruit locally (i.e. within the protected area in which they are situated) than corals in regions with slower surface currents. Also, as indicated in this chapter, migration rates on Maputaland reefs differ between species with different life strategies and PLDs (Fig. 5.3; Fig. 5.4, Fig. 5.5). Thus Maputaland reefs may be losing many propagules to inhospitable habitats downstream of the last significant communities of corals on the east African coast. High-latitude MPAs need to be larger if they are to protect the same levels of biodiversity as occur in smaller areas at lower latitudes (Laurel and Bradbury 2006; Kelly and Eernisse 2007). It is very important for the demographic maintenance of local communities that MPAs are large enough to accommodate self-recruitment (Botsford *et al*. 2003). The relatively small size of South African reef systems may limit their ability to harbour viable populations of corals with large dispersal kernels (Tab. 5.2, Fig. 5.5). However, as all of South Africa's reefs are subject to some level of management and protection, the limiting factor is the available habitat within the MPAs suitable for the recruitment of coral larvae (Schleyer and Celliers 2005; Jones *et al*. 2007). If the dispersal predictions for hard corals from studies in other regions apply (Underwood *et al*. 2007; Kinlan and

Gaines 2003; Shanks *et al*. 2003), the reefs of Maputaland are likely to be large enough to contain kernels of 1-20km dispersers and 100km dispersers within the series of reefs in the iSimangaliso Wetland Park.

It is likely that many coral recruits in this region may be lost to local populations as they are carried further south to unsuitable habitat by the Agulhas Current. However, considering the relatively high local diversity, we surmise that local oceanography dictates higher levels of retention than we account for here and this has been shown elsewhere in theory and through study (Black *et al*. 1990; Underwood *et al*. 2007; van Oppen *et al*. 2008). The southern reef complex (SRC) may be considered a sink region, reliant on self-seeding and propagules from the northerly central reef complex (CRC) to maintain genetic diversity on evolutionary scales. Considering this inequality in exchange of propagules, it is possible that these southern marginal reefs are slowly being leached of diversity (Fig. 5.3; Fig. 5.4; Fig. 5.5).

The current management practice regarding utilization of these diverse reefs is of concern (Ridgway *et al.* 2001; 2008; Chapter 3 and 4). The CRC and specifically Two-mile Reef (TMR) are currently the most heavily-dived of the Maputaland reefs, owing to their accessibility and pricing by dive operators (Schleyer and Tomalin 2000; Schleyer 2000). In Chapters 3 and 4, it was reported that TMR harbours more diversity than other Maputaland reefs that were included in the study. TMR is a large reef situated at the southernmost extent of the CRC. It is likely to be a repository of recruits from more northerly reefs and a source of propagules for reefs in the SRC. Protecting a certain proportion of this habitat from the effects of intense SCUBA diving is probably essential to sustain downstream reefs. Recent studies of the faunistic component of this habitat indicate large-scale shifts in complement which may be attributed to higher utilization of this habitat (Schleyer and Tomalin 2000; Schleyer 2000; Floros, pers. comm.), and may have wide-ranging effects on its health.

Climate change

As sea-surface temperatures (SSTs) rise it is expected that pelagic larvae will begin to settle earlier and dispersal kernels will begin to shrink (Munday *et al.* 2009). The tropics may not be seriously affected as reef populations are situated relatively close to one another. There may, however, be dire consequences for isolated populations at higher latitudes, which will become more isolated from nearby viable source populations. A consequence of loss of demographic subsidy will be an attenuation of population densities and, if not sufficiently self-seeding, they will eventually dwindle below densities necessary for sexual cross-fertilization (Allee effect) (Levitan and McGovern 2005). Genetic isolation may result in drift within isolated communities, further loss of diversity, population collapse and diminished capacity to respond to increasing environmental stress associated with climate change (increasing SSTs, dissolved CO_2) (Kleypas *et al*. 1999; Hansen *et al*.

2006).

In models of population connectivity under fragmented habitat conditions, as dispersal kernels extend beyond the extent of suitable habitats, so the chances of successful recruitment become proportionally lower (Jones *et al*. 2007). If a suitable habitat falls within the margins of likely recruitment, only the proportion of that habitat within the kernel may contribute to success. Thus, it is likely that the stepwise spread of scleractinian corals to marginal habitats (Ridgway *et al*. 2008), has probably progressed over evolutionary time periods as a series of fortuitous long-distance dispersal events. Thus populations that have been maintained in the recent past (3000 ybp) at current sea-levels (Ramsay 1996) under ideal climatic conditions are unlikely to persist in the face of anthropogenic perturbations, including climate change. Such a reduction in upstream coral communities capable of seeding those further to the south makes protection of self-seeding and self-sustaining units in the CRC more important. For this reason, diving, fishing and associated recreational activities on the reefs of the CRC should be carefully monitored and regulated and representative components of the reefs should be closed to these activities.

5.6 References

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6. Summary and synthesis

6.1 General

The data from this study of genetic variation and connectivity of populations of *Acropora austera* and *Platgyra daedalea* in the western Indian Ocean are collated here and used to infer dispersal patterns from evolutionary evidence. These patterns will enable effective management decisions to be taken about the creation and management of MPAs in the region. This is one of the first studies of genetic connectivity of the south east African coastline including southern Mozambique and the marginal reefs of Maputaland, South Africa. One of the principal findings was a disjunction in demographic genetic connectivity between populations of both *Platygyra daedalea* and *Acropora austera* southern Mozambique and South Africa. Both corals displayed similar patterns of migration, with a net loss of propagules from Maputaland reefs and net gain of propagules in Mozambican reef systems.

Chapter 2 is the report of a pilot study in which the carbonic anhydrase 3/550 nuclear intron of *Acropora austera* and the nuclear ribosomal internal transcribed spacer (ITS) region of *Platygyra daedalea* DNA were amplified to obtain preliminary data on genetic variability between populations and species and to assess the suitability of these markers for use in a wider study. Phylogenetic analyses of *A. austera* 3/550 intron sequences identified one well-supported clade containing haplotypes unique to the northern (Mozambican) part of the study area, but little structure in the southern regions, which contained no unique haplotypes. There appeared to be regular genetic exchange between *A. austera* populations in south east-African coastal waters. This method of analysis was applied to a more extensive sample of *A. austera* (Chapter 3). *P. daedalea* appeared to be an open population (Hellberg *et al*. 2002) in the SWIO with little evidence of structuring, as phylogenetic analyses failed to identify any well-supported clades, even with respect to Asian *Platygyra* outgroups. ITS sequencing was not carried out with a larger dataset as this region appeared to yield inconclusive results, possibly because of potential confounding factors related to interpretation of data from this repeated DNA region. Instead, population genetic variation in *Platygyra daedalea* was investigated with microsatellite markers (Chapter 4).

Chapter 3**:** *Acropora austera* populations were sampled from six localities on the south east African coastline (Bazaruto Archipelago in the north to the southern limit at Leadsman Shoal in Maputaland, South Africa) (Fig. 3.1). The hypervariable carbonic anhydrase 3/550 intron marker was sequenced in order to ascertain levels of genetic variability and connectivity within and between reefal populations. Phylogenetic analyses indicated the presence of a clade of haplotypes

exclusive to the Mozambican populations at Inhaca Island and the Bazaruto Archipelago. Indices of migration and levels of haplotype sharing suggested significant connectivity between populations in South Africa and at the Bazaruto Archipelago (Fig. 3.2). Measures of population sub-division, however, indicated a significant amount of fixation of allele frequencies amongst populations. Although fine, this level of differentiation in a marker from the nuclear genome of a hard coral indicates that, on evolutionary scales, there is a level of isolation between *A. austera* populations in Southern Mozambique and those in Maputaland, South Africa. The population at Rabbit Rock was found to be significantly isolated compared with the more connected populations at the Bazaruto Archipelago, Inhaca Island, Two-mile Reef, Red Sands Reef and Leadsman Shoal. High levels of heterozygosity in the Rabbit Rock population indicated that it might constitute a zone of hybridization amongst congeners from the genus *Acropora*.

Chapter 4: *Platygyra daedalea* was collected from coral reefs along the east African coast from Mombasa Marine Park in Kenya (North) to Maputaland in South Africa (South). Simple-sequence repeat markers from five independent loci in the nuclear genome were used to assess differentiation between populations of *P. daedalea*. Large numbers of null alleles were encountered across loci; these may be attributed to parapatric evolution in this genus. Populations in this study conformed closely to expectations of a pan-mictic metapopulation. However, evidence points to the existence of a bio-geographic barrier between southern Mozambique and Maputaland, South Africa (Fig. 4.3). Exceptions to Hardy-Weinberg equilibrium in 9 of 19 populations indicated that sweepstakes reproduction success may contribute to the structuring of populations in this region. Indices of allelic richness demonstrate that islands in this region harbour low diversity, and that Inhaca Island, although coastal, may be isolated from local gene flow. Further, southern populations show similar levels of allelic richness to those shown by populations from tropical east Africa. It appears that diversity in northern populations, which might have been expected to be higher than that of southern populations, may have been diminished by exposure to environmental and anthropogenic stress not experienced by southern populations.

Chapter 5: Patterns of connectivity amongst both *Acropora austera* and *Platygyra daedalea* were examined. The 3/550 intron of the carbonic anhydrase gene was used for *A. austera* analyses, whilst microsatellites were used for *P. daedalea* analyses. Both hard corals showed levels of connectivity similar to those established by previous research in the region. Data on both *A. austera* and *P. daedalea* both showed a potential break in connectivity between southern Mozambique and South Africa, in the vicinity of Inhaca Island and the northern reef complex in Maputaland. It is important for management authorities to incorporate such information into their strategies for the long-term protection of these diverse species assemblages of hard corals, which are important for maintenance of diversity as they provide the complex substrata which form the habitat of many

coral reef-based organisms.

Analyses of the *Acropora austera* and *Platygyra daedalea* datasets revealed both counter-current and current-driven geneflow (Tab. 5.3, 5.4). The existence of counter-current gene flow was an unexpected finding; this may be due to northward transport of propagules in stochastic wind-driven movement of surface water in the Mozambique Channel, or due to longshore drift counter to the north-to-south flow of the prevailing western boundary current, the Agulhas. Additionally, this may reflect gene flow on evolutionary scales, when currents in the region may have been different owing to vicariance caused by changes in sea-level over geological time scales. It is also possible that genetic equilibrium has not yet been established and that the mutation rate is higher than the real rate of exchange of migrants between populations, leading to inflated estimates of migration rates (Hellberg *et al.* 2002). Future research should help to establish the validity of this assumption, although the issue may be confounded by reticulate evolution (Veron 1995; Diekmann *et al*. 2001; van Oppen *et al*. 2001), possible hybridisation (Richards *et al*. 2008) and paraphyly of coral lineages (Huang *et al.* 2009).

Hard corals in the western Indian Ocean form important habitats for a diverse complement of flora and fauna (Veron 2000). Hard coral propagules disperse passively in ocean currents and have spread throughout the habitable substrata available to them bounded by the narrow physical parameters that they require for survival. Local communities rely heavily on the resources that coral reefs provide for subsistence and income. Unfortunately, due to population expansion in local and riverine catchment areas, coral reefs are subject to increasing levels of exploitation and eutrophication. This exploitation and perturbation, coupled with climatic change, is threatening the ecological integrity of these systems. Management of these reefs must therefore be based upon observations of the current trends of regional reef health.

East Africa was reported to have lost an effective 15% of its coral reefs during the 1998, 2002 and 2005 bleaching events (Wilkinson 2008). Specifically, 35% of the regional reefs are considered to be at low risk to loss and the remaining 50% are considered threatened or critically-threatened (Wilkinson 2008). Thus the role of MPAs in this region may be vital in ensuring that these reefs are not degraded to a point at which they are evolutionarily no longer viable. The emphasis of this study has been to ascertain the extent to which reef corals, particularly in the SWIO, are connected to one another. If connected reef populations are protected along the length of the reef-harbouring coast, reefs at any point in the continuum may be reseeded if affected by catastrophic mortality.

It appears that East African coral populations that are separated by large expanses of slow-moving water and inhospitable habitat are ecologically isolated from one another and may not, therefore, subsidise each other with enough propagules to maintain population demographic

equilibrium (whereby populations display comparable size-class distributions). It is even possible that hard-corals with longer PLDs may be constrained by these factors to recruiting to their local reefs (Steneck *et al*. 2009).

6.2 East Africa (Kenya, Tanzania and northern Mozambique)

Coral reefs in the tropics have been subjected to the highest levels of bleaching world-wide, with significant loss of hard coral cover in the last fifty years (Bruno and Selig 2007). Tropical reefs in the WIO were most damaged in the recent series of bleaching events that spread along the East African coast (Wilkinson (Ed.) 2000; 2008). In general, hard corals showed high levels of connectivity amongst reefs in Kenya, Tanzania and northern Mozambique (Chapter 3 and 4). There was, however, a measure of disjunction between communities from different habitats (Fig. 4.3; Chapter 4).

The disjunction between coastal and island coral communities is an indication that they are not related to the extent that they may be considered truly panmictic (Chapter 4). Fine scale differences in population structure (F_{ST}) amongst marine species with pelagic larval dispersal is an indication of ecological isolation between populations (Waples 1998). Levels of population differentiation between island and coastal populations, as measured by F_{ST}, indicate that island populations may be be isolated from coastal populations at demographic scales. Thus, populations may be linked at evolutionary but not ecological scales (Chapter 4). East African hard corals may not, therefore, be expected to recover from catastrophic bleaching events over ecological time-scales, this may lead to a deleterious shift in levels of heterogeneity of coral populations, as occurred in the Caribbean (Baums *et al*. 2006). The increased incidence of anthropogenic perturbation of coral reefs is likely to increase the susceptibility of corals to mass-mortality events, which will have an adverse effect on levels of heterogeneity on affected reefs.

6.3 South East Africa (southern Mozambique and South Africa)

Acropora austera and *Platygyra daedalea*, two hard corals with different life histories, displayed similar patterns of connectivity in south east African coastal water. Both exhibited a similar pattern of disjunction between southern Mozambique and Maputaland, South Africa. They both displayed large-scale panmixia at evolutionary scales, although fine-scale structure was detected amongst these population groups. Such fine-scale structure is evidence of demographic discontinuity between population groups and may contribute to the large-scale loss of regional coral reefs. The loss of a portion of the continuum of connectivity will increase the likelihood that downstream reef coral populations will be isolated and at risk to ecological extinction (Steneck *et al*. 2009). This is exacerbated by the paucity of available substrata for coral recruitment in this

marginal environment. It is important to consider the level of ecological isolation (demographic connectivity) of reef-coral populations when formulating management strategies.

Maputaland coral populations, located in the extreme south of the study area, are connected to one another at demographic scales (Ridgway *et al.* 2001; Tab. 5.3; Tab 5.4). It may be inferred that they are connected in a stepwise fashion, with gene-flow facilitated by the north to south flow of the Agulhas, a fast western boundary current. Downstream populations must be considered reliant on northerly populations for demographic subsidies, whilst a large proportion of the propagules produced by the southern populations may be lost as they are carried to inhospitable habitats even further south by the fast-moving waters of the current (1.5 $m.s^{-1}$). South to north gene-flow also occurs as measured in the analyses of migration using the coalescent (Chapter 5), and may be mediated by coastal longshore drift and wind drift (Heydorn *et al.* 1978).

South-east African coral reefs are unique in their species composition and richness, containing tropical, sub-tropical and endemic species. In order to manage these systems and retain genetic diversity it is recommended that highly diverse reefs (see Celliers and Schleyer 2008) in each reef complex be zoned as no-take reserves. This is especially important for reefs subjected to high levels of recreational diving, as reduction of diver-mediated influence on vertebrate communities which contribute to reef health (Bellwood *et al.* 2006) may be an important consideration in their protection (Schleyer and Tomalin 2000; Floros pers. comm.).

6.4 Molecular marker efficacy

We tested a number of molecular marker systems for the estimation of heterogeneity between populations. As previous research indicated that mtDNA in the scleractinia was too slowly-evolving to use for estimations of among-population variation (Shearer *et al.* 2002), faster evolving regions of the nuclear DNA, such as introns, spacers and microsatellites were used to elucidate patterns of genetic structuring (Tab. 1.1 and refs. therein). The ITS region of the ribosomal DNA of *Platygyra daedalea* was highly variable and did not reveal any structuring amongst populations even across oceanic scales. Its suitability for studies of the scleractinia and the Acroporidae in particular (van Oppen, 2001; 2002) has been questioned on the basis of its unknown patterns of inheritance and poorly-understood modes of evolution (Vollmer and Palumbi 2002, van Oppen *et al.* 2000) (Chapter 2). Therefore microsatellite (STR) markers developed by Miller and Howard (2004) were used in preference to ITS sequences to estimate genetic diversity and structure among populations of *P. daedalea*. For *Acropora austera,* a novel region, the hypervariable single-copy carbonic anhydrase 3/550 nuclear intron was sequenced using primers which were made available by their developer Tyrone Ridgway (pers.comm.). This is one of the first studies of genetic variation based on these as-yet-unpublished primers.

6.5 Future research direction

Research into the fine-scale distribution of genetic variation amongst reef corals will help researchers to understand the within-reef relationships of hard corals, including estimations of within-reef clonality, self-recruitment, the likely resilience of reef corals and their need for demographic subsidies from elsewhere. For good resolution of inter-reef connectivity, the positions of corals should be spatially auto-correlated.

It is important that a variety of marker systems be developed for the little-studied soft coral taxa that form a significant part of South African reefs. This will facilitate identification of cryptic species amongst the common genera and investigations of local soft coral connectivity and dynamics. This in turn will lead to a better understanding of the diversity of soft corals on Maputaland reefs.

6.6 Communication of implications to MPA managers and local subsistence communities

Ultimately, the responsibility for the protection of southern African reef corals must be met by management authorities. New ideas about the management of any resource are often met with resistance; a solution for this may be to engage with resource users, such as diving operators at Sodwana Bay (Maputaland, South Africa) and local communities in Mozambique reliant on fisheries for protein. The long-term consequences of non-compliance, such as decreases in fisheries yield and reef coral abundance, and a consequent decline in financial security, should be explained. If the consequences of new management regimes are not accepted by local communities who subsist on the natural resources being protected, compliance may be expected to be minimal.

However, perhaps the most pervasive damage being inflicted on coral reefs around the world is through non-localised anthropogenic pollution of the environment (Lough 2008). Deforestation leading to eutrophication of once-oligotrophic waters, poor wastewater treatment from large settlements, global warming and ocean acidification from the massive amounts of climate-changing pollutants released into the atmosphere are synergistically changing the environmental conditions which support these fragile systems (De'ath *et al.* 2009; Lough 2008, Hansen *et al.* 2006; Kleypas *et al.* 1999). Detailed evidence for the demise of coral reefs is accumulating (Bruno and Selig 2007, Hoegh-Guldberg *et al*. 2007) and the onus rests on our generation to make decisions that will surely change the future of these biodiverse systems.

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7 Appendix 1

Allele frequencies and sample sizes of *Platygyra daedalea* simple tandem repeat data from the Indian Ocean.

Allele Frequencies and Sample Size by Populations

Locus	Allele/n	Chagos	Mombasa	Quirimbas	Pemba	Bazaruto	Inhaca	Rabbit Rock	Central reef complex	Southern reef complex
Locus1	**N**	4	41	14	12	20	15	10	29	42
	178	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036
	187	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036
	199	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000
	202	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.017	0.024
	205	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.048
	208	0.500	0.463	0.321	0.417	0.375	0.333	0.450	0.379	0.286
	211	0.000	0.012	0.036	0.167	0.275	0.167	0.000	0.017	0.107
	214	0.000	0.244	0.321	0.042	0.175	0.267	0.000	0.431	0.179
	217	0.500	0.220	0.107	0.292	0.150	0.233	0.550	0.138	0.286
	220	0.000	0.000	0.107	0.083	0.000	0.000	0.000	0.017	0.000
	223	0.000	0.037	0.071	0.000	0.000	0.000	0.000	0.000	0.000
	241	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Locus2	**N**	5	27	9	9	26	27	12	31	43
	1	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000
	149	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	170	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.016	0.000
	173	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012
	194	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.012
	197	0.000	0.000	0.000	0.000	0.058	0.000	0.042	0.016	0.000
	200	0.000	0.074	0.000	0.278	0.096	0.000	0.000	0.032	0.023
	203	0.500	0.278	0.222	0.167	0.250	0.148	0.417	0.258	0.198
	206	0.000	0.130	0.222	0.167	0.135	0.222	0.000	0.210	0.256
	209	0.100	0.037	0.167	0.056	0.019	0.259	0.042	0.113	0.186
	212	0.400	0.389	0.333	0.278	0.385	0.315	0.375	0.258	0.209
	215	0.000	0.056	0.056	0.056	0.058	0.037	0.000	0.065	0.058
	218	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.032	0.035
	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012
Locus3	**N**	5	37	13	12	26	12	13	29	38
	193	0.000	0.365	0.308	0.417	0.365	0.292	0.000	0.328	0.276
	199	0.000	0.095	0.000	0.083	0.096	0.083	0.077	0.086	0.092
	205	1.000	0.514	0.538	0.417	0.538	0.542	0.923	0.517	0.632
	211	0.000	0.014	0.154	0.083	0.000	0.083	0.000	0.034	0.000
	217	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.034	0.000
Locus4	**N**	5	29	10	10	25	28	9	31	42
	126	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.016	0.012
	130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.012
	134	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.048	0.012
	138	0.000	0.017	0.050	0.000	0.080	0.036	0.000	0.048	0.036
	142	0.100	0.293	0.150	0.300	0.340	0.339	0.556	0.306	0.321
	146	0.400	0.241	0.300	0.200	0.260	0.125	0.167	0.226	0.250
	150	0.000	0.155	0.100	0.300	0.220	0.071	0.000	0.065	0.095
	154	0.500	0.241	0.250	0.100	0.100	0.393	0.278	0.210	0.214
	158	0.000	0.017	0.050	0.000	0.000	0.018	0.000	0.016	0.036
	162	0.000	0.000	0.050	0.000	0.000	0.018	0.000	0.016	0.012

		166	0.000	0.017	0.050	0.000	0.000	0.000	0.000	0.032	0.000
Locus5	**N**		4	37	18	12	27	28	10	32	47
		252	0.000	0.014	0.000	0.000	0.019	0.000	0.000	0.000	0.000
		254	0.000	0.081	0.000	0.042	0.037	0.018	0.000	0.000	0.000
		256	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.016	0.011
		258	0.000	0.014	0.028	0.000	0.000	0.000	0.000	0.000	0.011
		260	0.250	0.311	0.333	0.250	0.352	0.268	0.150	0.391	0.372
		262	0.000	0.014	0.056	0.125	0.037	0.125	0.000	0.031	0.170
		264	0.000	0.122	0.083	0.042	0.074	0.018	0.000	0.000	0.000
		266	0.000	0.014	0.000	0.000	0.000	0.018	0.050	0.016	0.000
		268	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000
		270	0.000	0.027	0.028	0.000	0.037	0.446	0.000	0.141	0.085
		272	0.750	0.392	0.417	0.542	0.444	0.071	0.750	0.375	0.330
		274	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000
		276	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.016	0.000
		278	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000
		282	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000
		284	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021
		288	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000