ECOLOGICAL GENETIC CONNECTIVITY BETWEEN AND WITHIN SOUTHEAST AFRICAN MARGINAL CORAL REEFS

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Submitted in fulfilment of the academic requirements for the degree of

Doctor of Philosophy

in the Oceanographic Research Institute, School of Life Sciences

College of Agriculture, Engineering & Science

University of KwaZulu-Natal

South Africa

March 2014

As the candidate's supervisors we have/have not approved this thesis/dissertation for submission.

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ABSTRACT

Marine protected areas (MPAs) have been established along the East African coast to protect coral communities from human and natural disturbance. Their success is dependent on the degree to which resource populations are self-seeding or otherwise connected. Estimates of contemporary gene flow on or between south-east African reefs are thus required to reveal the interdependence of the South African coral communities and those to the north. Accordingly, the ecologically relevant (1 or 2 generations) connectivity of two broadcast-spawning corals, *Acropora austera* and *Platygyra daedalea*, was assessed on reefs in the region, from the Chagos Archipelago to Bazaruto Island in Mozambique and Sodwana Bay in South Africa, using hyper-variable genetic markers. Analysis of genetic diversity and differentiation provided evidence for the existence of four discrete genetic populations of *A. austera* and five of *P. daedalea* in the sampled area. Higher genetic diversity was found on northern South African reefs (Nine-mile Reef and Rabbit Rock) and migration patterns inferred from assignment tests suggested that, at ecological time scales, South African reefs are disconnected from those in Mozambique and might originate from a source of gene flow that was not sampled. The analysis of fine-scale genetic connectivity conducted on Two-mile Reef (TMR) demonstrated the existence of significant spatial genetic structure at the reefal scale that might be related to the non-random dispersal of coral larvae, putatively explaining the genetic discontinuity observed in the region. Altogether, the results are consistent with the isolation observed in other studies using less variable markers, and support the hypothesis that there is demographic discontinuity between the coral populations along the south-east African coast. More importantly, *Acropora austera* and *P. daedalea* represent different life strategies in the South African reef communities yet manifested similar genetic patterns, suggesting that these corals are responding similarly to forces that are driving genetic connectivity in the region. For management purposes, the genetically distinct populations identified at each of the spatial scales analysed in this study may correspond to management units, or evolutionarily significant units. Furthermore, since some reefs appear to act as "landing-sites" for migrants (Nine-mile Reef) and there is evidence of significant within-reef genetic structure (TMR), an adaptive management framework would be the best option for the MPA in the region.

PREFACE

The work described in this dissertation was carried out at the Oceanographic Research Institute (ORI), which is affiliated to the School of Life Sciences at the University of KwaZulu-Natal, Westville, from June 2009 to September 2012, under the supervision of Professor Michael H. Schleyer and under co-supervision of Dr. Angus H. H. Macdonald.

The 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 acknowledge in the text.

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DECLARATION 1 – PLAGIARISM

I, Phanor H. Montoya-Maya, declare that:

The research reported in this thesis, except where otherwise indicated, is my original research.

This thesis has not been submitted for any degree or examination at any other university.

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March 2014

DECLARATION 2 – PUBLICATIONS

Detail of contribution to publications that form part and/or include research presented in this thesis.

Publication 1

Montoya-Maya, P. H., Schleyer, M. H. & Macdonald, A. H. H. (In press). Cross-amplification and characterization of microsatellite loci in *Acropora austera* from the south-western Indian Ocean. Accepted for publication in Genetics and Molecular Research.

Author contributions:

PH Montoya-Maya conducted field and laboratory work, analysed data and was lead author. Prof. MH Schleyer assisted in authoring the paper, contributed funding for experimental work and contributed legwork in sampling material. Dr. AHH Macdonald assisted in authorship, experimental work and contributed sampling material.

Publication 2

Montoya-Maya, P. H., Macdonald, A. H. H. & Schleyer, M. H. (In prep). The ecological genetic connectivity of *Acropora austera* from South African marginal reefs.

Author contributions:

PH Montoya-Maya conducted field and laboratory work, analysed data and was lead author. Prof. MH Schleyer assisted in authoring the paper, contributed funding for experimental work and contributed legwork in sampling material. Dr. AHH Macdonald assisted in authorship, experimental work and contributed sampling material.

Publication 3

Montoya-Maya, P. H., Macdonald, A. H. H. & Schleyer, M. H. (In prep). The ecological genetic connectivity of *Platygyra daedalea* from South African marginal reefs.

Author contributions:

PH Montoya-Maya conducted field and laboratory work, analysed data and was lead author. Prof. MH Schleyer assisted in authoring the paper, contributed funding for experimental work and

contributed legwork in sampling material. Dr. AHH Macdonald assisted in authorship, experimental work and contributed sampling material.

Publication 4

Montoya-Maya, P. H., Macdonald, A. H. H. & Schleyer, M. H. (In prep). Populations of hard corals in South African marginal reefs are rather closed.

Author contributions:

PH Montoya-Maya conducted field and laboratory work, analysed data and was lead author. Prof. MH Schleyer assisted in authoring the paper, contributed funding for experimental work and contributed legwork in sampling material. Dr. AHH Macdonald assisted in authorship, experimental work and contributed sampling material.

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Phanor H. Montoya-Maya

Mach 2014

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ACKNOWLEDGEMENTS

I am grateful to the South African Association for Marine Biological Research (SAAMBR) and the Western Indian Ocean Marine Association (WIOMSA) MASMA programme (grant MASMACC201006) for funding the project that generated the data for this research.

I am greatly indebted with the SAAMBR for providing me with the studentship to pursue this research. A South African National Research Foundation (NRF) PhD grant through the Oceanographic Research Institute (ORI) – University of KwaZulu-Natal supported me throughout the duration of my PhD for which I am also grateful.

I thank the iSimangaliso Wetland Park authorities and Ezemvelo KZN Wildlife for permission to sample within the park. Thanks are also due to the Dr Angus Macdonald for permission to use samples he previously collected as part of my study.

I thank my supervisors Michael Schleyer and Angus Macdonald for their advice and sharing their expertise; for their helpful comments and suggestions. I am particularly indebted to Mike for not only providing guidance and advice through these but also for being so understanding of demands of my personal life. I will also like to thank for giving me the opportunity to SCUBA dive in Sodwana Bay as part of my field work. I thank Angus for introducing me to coral genetics and for teaching me molecular laboratory techniques.

This study would be impossible without the assistance of those who helped with field sampling. For their assistance, I would like to extend my sincere thanks to Justin Hart, Ashley Grimmer, Stuart Laing, Chris Wilkinson, Jade Maggs, Lola Masse, Mathieu Seré and Michael Schleyer from ORI.

For help during the laboratory phase, I would like to thank Christiaan Labuschagne and Jaco Broere from Inqaba Biotechnical Industries (Pty) Ltd for their instruction in applicable techniques. I also thank Debora Sweby from SASRI and Inqaba Biotec for the opportunity to work in their laboratories. Thanks are also due to the personnel from the Technical Department at SAAMBR for building, twice, a genetics lab at ORI so that I could process my samples. Thanks are due to Justin Hart and Jessica Escobar-Porras for their help with DNA extractions and PCR amplification.

Thanks are also due to the Coral Team at ORI Justin Hart, Ashley Grimmer, Stuart Laing, Lola Masse, Mathieu Seré and Camilla Floros, for their contribution through various discussions

and helpful suggestions. I special thanks is extended to Dennis Rouillard for generating some of the maps and figures.

I also thank the administration and support staff of SAAMBR, in particular Alison Moor, Ramini Naidoo, Maureen Landers, Desmond Hayes, Denis Browne, Ann Kunz, Paul Zammit, Jessica Pillay and Grace Samuriwo for assistance and advice with all admin related issues. You definitively made my PhD much easier to handle.

I thank all my friends and family for their understanding and support. At some points, it felt as if we all were on this together! I specially thank my Colombian family for all their support throughout my studies and for enduring my decision to study away from home; I know it has not been easy but we all agree it was worthy.

I thank my South African family and friends for their encouragement and constant support in so many ways. Special thanks are due to my South Coast family and friends, the last moments of this PhD were very difficult and you all were really caring, and some went well out of their way to get me going.

Finally, to my wife Jessica, for all her love, support, endless patience and full understanding right the way through. Cosita, I am deeply grateful to have you by my side. When I doubted myself, you were there to give me confidence and strength. I will do my best to be during your PhD what you were to me during mine. Te amo!

CHAPTER 1

GENERAL INTRODUCTION

Strategies needed to preserve coral reefs have to be scientifically rigorous and adaptable when these ecosystems are most threatened. Destructive fishing practices and overfishing, coastal development, pollution, unsustainable tourism activities, and global climate change are leading to irreversible degradation of most coral reefs worldwide (Hughes et al. 2007; Jackson 2008; Veron et al. 2009). Marine protected areas (MPAs) and MPA networks have been established globally in an effort to protect coral reefs from these disturbances (e.g. Schleyer & Celliers 2005; Miller & Ayre 2008b; Selig & Bruno 2010; McClanahan et al. 2006). The inclusion of scientific information on reef biodiversity, reef processes, oceanography, fisheries and socioeconomics in the design and planning of MPAs has been pivotal to their success in the recovery of fish stocks and a reduction in the loss of corals (e.g. Fogarty & Murawski 2005; Mumby & Harborne 2010; Selig & Bruno 2010; Cinner et al. 2012). Furthermore, this information can also provide a foundation for adaptive management to render MPAs even more effective (Pomeroy et al. 2004; Beeden et al. 2012; Cinner et al. 2012; Kruger & MacFadyen 2011; Tompkins & Adger 2004).

Almany et al. (2009) and Sale et al. (2010) identified the need to address connectivity within MPAs, MPA networks and their associated reefs to ensure their effectiveness as marine conservation tools. Connectivity, simply defined as the linking of populations through the exchange of propagules (e.g. adults or gametes) of a species, contributes to gene flow, species persistence and population growth. Sale et al. (2010) considered two kinds of population connectivity: evolutionary (genetic) connectivity, referring to "*the amount of gene flow between populations over a timescale of several generations"*; and demographic (ecological) connectivity, referring to "*the exchange of individuals among local populations that can influence population demographics and dynamics"*. Although genetic variation in populations tends to fall within the scope of evolutionary connectivity (Lowe et al. 2004), it can contribute to an understanding of demographic connectivity when tools employed in its assessment measure the effective exchange of propagules between populations leading to gene flow (Broquet & Petit 2009), which is currently referred to as "*ecological genetic connectivity"*, "*ecologically relevant gene flow"* or "*ecological relevant dispersal"* (Underwood et al. 2009; Noreen 2010).

Numerous studies have examined the evolutionary genetic connectivity of coral species in reefs worldwide (Baums et al. 2005; Vollmer & Palumbi 2006; Nakajima et al. 2012; Macdonald et al. 2010), providing important insights into barriers to connectivity as well as gene flow

variability across reproductive modes and spatial scales (Severance & Karl 2006; Miller & Ayre 2008a; Vollmer & Palumbi 2006; Nishikawa 2008). However, studies assessing the magnitude of demographic connectivity and ecological genetic connectivity are still uncommon (but see Underwood et al. 2007; Ledoux et al. 2010). Particularly, this is because in situ tracking of coral propagules (i.e. gametes and larvae) is difficult (Thorrold et al. 2002; Botsford et al. 2009) and the screening of large numbers of species-specific highly polymorphic genetic markers is still limited to a handful of coral species (e.g. Shearer & Coffroth 2004; van Oppen et al. 2006; Wang et al. 2008).

1.1 The importance of knowledge on reef connectivity within and between MPAs and MPA networks

Connectivity is essential for reef resilience (see Mumby & Hastings 2007; Botsford et al. 2009). Jones et al. (2009) list two extremes of the connectivity spectrum: closed populations, where there is no connectivity, and open populations, where there is high connectivity. In this context, closed or weakly connected populations may be at higher risk of extinction since their ability to recover from localized damage may be restricted because of high levels of self-recruitment; open populations tend to be more resilient as replenishment is enhanced by recruitment from neighbouring populations (Jones et al. 2009; Botsford et al. 2009; Almany et al. 2009). Furthermore, open, well-connected populations may adapt more rapidly to a changing environment (Munday et al. 2009; Munday et al. 2008) through the exchange of favourable genotypes (Jones et al. 2008; Vollmer & Kline 2008; Silverstein et al. 2012). Although reef resilience requires knowledge of ecosystem processes and a thorough understanding of reef dynamics (Hughes et al. 2005), it is evident that elucidating reef connectivity can also contribute significantly to coral reef resilience.

Information on reef connectivity is useful in the design and management of protected areas. Planes et al. (2009) assessed the connectivity of populations of the orange clownfish (*Amphiprion percula*), in a network of marine reserves proposed for Kimbe Bay, Papua New Guinea. Their findings confirmed that the proposed MPA network would sustain resident populations, both by local replenishment and through larval dispersal from other reserves. Furthermore, the study identified new areas that needed protection as they were important sinks of fish larvae and were not included in the initially proposed MPA network. Similarly, Christie et al. (2010) confirmed through parentage analysis of the coral reef fish *Zebrasoma flavescens* that the network of nine MPAs along the Kohala-Kona coast, Hawaii, was connected and seeded non-protected neighbouring sites. Connectivity assessments have provided evidence of within-reef localized coral dispersal, leading to high levels of self-recruitment (e.g. Underwood et al. 2007;

Miller & Ayre 2008a) and that the degree of connectivity may vary over time (e.g. Berumen et al. 2012). Therefore, the size, shape and location of protected areas should ideally reflect the connectivity between diverse habitats used by various species and life stages (Palumbi 2003a; Almany et al. 2009).

Fortunately, the importance of connectivity in ecosystem resilience has been recognised at the management level. Wilkinson et al. (Wilkinson et al. 2003) suggested that, in assessing and monitoring the connectivity of populations of coral reef organisms, managers can identify the recovery potential of reefs from small- and large-scale disturbance; the provision of new coral recruits by adjacent healthy populations to repair reefs damaged by coral bleaching; and the adequate protection of areas naturally resistant or resilient to coral bleaching as well as fish spawning aggregation sites. In this way, managers can incorporate adaptive management into protected areas, improving their effectiveness and enhancing their conservation goals and objectives (Pomeroy et al. 2004; Tompkins & Adger 2004). This may be accomplished by implementing large-scale networks of connected MPAs which extend beyond political borders. Governments along the south-east African coast have realized this and are making efforts to establish transboundary networks of MPAs (WWF Eastern African Marine Ecoregion 2004; Guerreiro et al. 2010; Guerreiro et al. 2011). However, information on the evolutionary and ecological genetic connectivity between MPAs need to be elucidated and, to date, are poorly understood in the region.

1.2 Ecological genetic connectivity is more pertinent to reef managers

Although both evolutionary (genetic) and demographic (ecological) connectivity contribute to coral reef resilience, ecological genetic connectivity is more relevant to managers. Ecological genetic connectivity of coral reef populations is influenced by factors (e.g. recruitment, mortality, genetic fitness and larval retention) affected by disturbances the managers are trying to protect them from (e.g. bleaching and excessive diving). For instance, the ability of a reef to retain imported or locally produced larvae is reduced by habitat degradation, caused by activities such as dynamite fishing and excessive diving, as these result in a reduction of suitable habitat for settlement and recruitment (Jones et al. 2009; Botsford et al. 2009). Similarly, the size, location and zonation of protected areas require knowledge of the dispersal kernel of a species, i.e. the probability that larvae released from a particular location will disperse to and successfully settle at other locations, if suitable habitat is available (Largier 2003). Therefore, reef areas known to be vulnerable to bleaching or disease would need to be “covered” by the dispersal kernel of the coral species to ensure that they can be replenished from protected areas (Botsford et al. 2009; Almany et al. 2009). Finally, through the assessment of ecological genetic connectivity, managers can

identify and protect fish spawning aggregation/nursery sites (Wilson & Ferguson 2002) and measure the spillover effect of MPAs (Fogarty & Murawski 2005), particularly when assignment methods are used which use genetic information to ascertain population membership of individuals or groups of individuals (Manel et al. 2005).

Evidence that coral larvae do not necessarily disperse like passive particles and that climate change may affect connectivity adds to the relevance of ecological genetic connectivity studies for managers. According to laboratory experiments, larvae of the corals *Montastraea annularis* and *M. faveolata* can alternate their swimming pattern, velocity and depth (Pizarro et al. 2008). Mason et al. (2011) found that *Porites astreoides* and *Acropora palmata* larvae settle preferentially on red surfaces. Considering this, it can be expected that, in able to explore suitable substrata for settlement, coral larvae "choose" either to settle close to natal colonies or farther from them, resulting in coral communities with different degrees of connectivity based on species-specific requirements. On the other hand, Munday et al. (2009) predict that population connectivity is affected by climate change. They suggest that warmer waters affect the swimming abilities of marine organisms and ocean acidification will reduce coral calcification, causing habitat loss and fragmentation, both consequences having deleterious effects on population connectivity (Munday et al. 2009). Managers thus need to assess the ecological genetic connectivity of coral reef populations and integrate this into management planning if they are to ensure the effectiveness of MPAs.

1.3 Measuring ecological genetic connectivity

Gene flow or genetic connectivity can be estimated by several methods (Hedgecock et al. 2007). Traditionally, estimates of gene flow between populations were obtained from F -statistics, but this practice has come under criticism because they are based on simplified models of population structure with simplistic assumptions (e.g. constant population size, genetic equilibrium) (see Whitlock & Mccauley 1999). Fortunately, recent advances involving Bayesian theory in population genetics has permitted the estimation of recent migration rates under more realistic assumptions (e.g. Wilson & Rannala 2003). Bayesian clustering analysis can identify subdivision in a sample and assign individuals to clusters based on their probability of membership. Thus, gene flow between clusters can be inferred when one individual sampled at cluster has a higher probability of belonging to another cluster. This method relies heavily on theoretical models of population structure and estimates gene flow over many generations. In contrast, assignment methods and approaches based on isolation by distance can estimate gene flow dynamics among reefs over ecologically relevant time spatial and temporal scales.

Assignment tests attempt to assign unknown individuals to their population of origin, based on the multilocus genotype of an individual and the expected probabilities of that genotype occurring in each of the potential sources. This method assumes that all potential source populations are defined in advance, sampled randomly, and are in Hardy Weinberg or linkage equilibrium (Manel et al. 2005). Since this method can link individuals/propagules to their natal reefs, they are very relevant to management agencies during the configuration and linkage of MPAs (Miller & Ayre 2008b; Botsford et al. 2009; Jones et al. 2009). Correspondingly, methods based on isolation by distance theory (e.g. spatial autocorrelation analysis, linear regression analysis) analyse the relationships between genetic and geographic data; a positive relationship is often interpreted as a sign that gene flow between individuals or populations or dispersal is limited by distance. These methods have the advantage of being less affected by evolutionary or rare events when used over small spatial scales (Pinsky et al. 2010). More importantly, when information on effective population density is available these methods provide estimates of dispersal over the past few generations and indirect estimates of dispersal parameters (e.g. neighbourhood size) (Hardy & Vekemans 2002). Although Bayesian clustering methods have been applied in connectivity studies of coral species from south-east African reefs (e.g. Ridgway et al. 2008; Macdonald et al. 2010), spatial autocorrelation analysis and assignment test have yet to be applied in studies from this region. Furthermore, South-east African reefs provide a suitable opportunity for the use of these methods as they are small in size, separated by known distances and arranged almost in a straight line from north to south.

Assignment methods and spatial autocorrelation analysis require high numbers of polymorphic markers (Manel et al. 2003; Manel et al. 2005; Hedgecock et al. 2007). Microsatellites, i.e. short tandem repeats of nucleotides, are the genetic marker of choice for the use of these methods as they are co-dominant, highly polymorphic, species-specific and offer adequate genetic resolution (Gerber et al. 2000; Gupta & Varshney 2000; Jones & Ardren 2003). However, isolation of new microsatellite markers is expensive, time-consuming, and requires previous genome information on the target species. Fortunately, successful microsatellite cross-species amplification has been reported in the literature (Selkoe & Toonen 2006; Nakajima et al. 2009), and expressed sequence tags and whole-genome shotgun sequences that have become available are alternative ways to obtain microsatellites markers (Wang et al. 2008), overcoming these drawbacks. Correspondingly, nuclear introns of conserved single-copy genes have been found to be hyper variable allowing their use in multilocus analysis of genetic connectivity (e.g. Palumbi & Baker 1994; Berrebi et al. 2006; Flot et al. 2008; Macdonald et al. 2010; Macdonald, Schleyer, et al. 2008). When genetic information on the species of interest is limited, nuclear introns can still be used by implementing a technique called EPIC-PCR. In this variation of the

polymerase chain reaction (PCR), introns are amplified with exon-primed intron-crossing (EPIC) primers which are designed in the flanking exons of the gene of interest. By following this approach, cloning can be avoided and cross-species amplification becomes easier because the exon sequences flanking the introns are well conserved across species (Palumbi 2005; Gupta & Varshney 2000; Bierne et al. 2000).

1.4 South-east African coral communities and their connectivity

South-east African coral communities are small, marginal reefs that lack most of the geomorphological traits of typical reefs, yet they provide services (e.g. recreation) and resources (e.g. fishing) similar to their tropical counterparts. The reefs can be classified as patch reefs, with corals growing as a thin veneer on a rocky base formed from fossilized Pleistocene coastal sand dunes, and not from biogenic accretion (Riegl et al. 1995; Ramsay 1996). The reefs run parallel to the coastline, 1-2 km offshore. They are small, relatively flat, having shallow drop-offs and gullies (Riegl et al. 1995). Their marginal nature is attributable to low mean annual and seasonal temperatures and a low aragonite saturation state characteristic of high latitudes which are not optimal for coral growth (Kleypas et al. 1999). Despite this, the south-east African reefs are very diverse (Pereira et al. 2003; Schleyer & Celliers 2003a) and are nodes for nature-based tourism such as scuba diving and recreational fishing (Pereira et al. 2003; Schleyer & Celliers 2005). The reefs are also important for artisanal fisheries, especially in Mozambique, contributing substantially to the livelihoods of coastal communities (Pereira et al. 2003). Although these marginal reefs are important to the economies of Mozambique and South Africa, the interdependence of the coral communities in terms of genetic exchange and survival remains unclear.

Most studies on genetic connectivity in populations of south-east African reef organisms have addressed evolutionary gene flow; this being evident when the type of markers used in genetic studies from the WIO are examined. Ridgway and Sampayo (2005) found 31 genetic references for the region of which 11 were on reef organisms. The majority (10) of the latter addressed biogeography, phylogeography and evolution of various species but all used low mutation-rate markers (e.g. allozymes and mitochondrial DNA (mtDNA)). An updated literature search yielded 27 additional studies on population genetics in the region (Table 1). Although the results revealed an increase in genetic research, they are still biased towards information on evolutionary connectivity rather than ecological genetic connectivity since only seven studies (26% of the 27 studies in the region) used microsatellite markers, the marker of choice in studies on dispersal, population origin and parentage analysis (Sunnucks 2000), information needed to assess ecological genetic connectivity (Selkoe & Toonen 2006; Saenz-Agudelo et al. 2009).

Table 1 Web of Knowledge search results for scientific articles on "Western Indian Ocean" and "population genetics" between 2005 and 2011. Forty results were obtained; results on terrestrial organisms, marker development, phylogeny, and taxonomy were excluded, leaving the 28 listed below.

Phylum	Species	Molecular marker	Reference
Dinoflagellata	*Symbiodinium* spp.	ITS 2	Macdonald et al. (2008)
Dinoflagellata	*Symbiodinium* spp.	ITS, Microsatellite	LaJeunesse et al. (2010)
Cnidaria	***Acropora austera***	**Nuclear CAH 3-550 intron**	**Macdonald et al. (2010)**
Cnidaria	***A. tenuis***	**COI, Nuclear CAH 3-550 intron**	**Chiazzari et al. (2013)**
Cnidaria	***Platygyra daedalea***	**4 Microsatellites*, ITS****	***Souter & Grahn (2008), **Macdonald et al. (2008)**
Cnidaria	***Pocillopora damicornis***	**6 Microsatellites**	**Souter et al.(2009)**
Cnidaria	***Pocillopora verrucosa***	**4 Microsatellites**	**Ridgway et al. (2008)**
Arthopoda	*Aristeus antenantus*	16S rDNA and COI	Fernandez et al. (2011)
Arthopoda	*Neosarmatium meinerti*	mtDNA CytB	Ragionieri et al. (2010)
Arthopoda	*Scylla serrata*	COI	Fratini et al. (2010)
Arthopoda	*Uca annulipes*	COI	Silva et al. (2010)
Arthopoda	*Upogebia africana*	COI	Teske et al. (2008)
Echinodermata	*Acanthaster planci*	COI, mtDNA control region and 16S	Vobgler et al. (2012)
Echinodermata	*A. planci*	7 Microsatellites	Yasuda et al. (2009)
Echinodermata	*Tripneustes* spp.	COI	Lessios et al. (2003)
Urochordata	*Styela plicata*	COI, ANT nuclear gene	Pineda et al. (2011)
Chordata	*Centropyge* spp.	mtDNA control region	Bowen et al. (2006)
Chordata	*Cephalopholis argus*	mtDNA CytB, 2 nuclear introns	Gaither et al. (2011)
Chordata	*Carcharodon carcharias*	mtDNA control region	Gubili et al. (2011)
Chordata	*Dasyatis brevicaudata*	mtDNA control region	Le Port and Lavery (2011)
Chordata	*Epinephelus coioides*	4 Microsatellites	Antoro et al. (2006)
Chordata	*Latimeria latimeria*	mtDNA and d-loop	Nikaido et al (2011)
Chordata	*Lutjanus fulviflamma*	AFLP	Dorenbosch et al. (2006)
Chordata	*L. kasmira, L. fulvus*	mtDNA CytB, 2 nuclear intron	Gaither et al. (2010)
Chordata	*L. kasmira*	COI, 8 microsatellites	Muths et al. (2012)
Chordata	*Myripristis berndti*	mtDNA CytB	Craig et al. (2007)
Chordata	***M. berndti***	**mtDNA CytB, 8 microsatellites**	**Muths et al. (2011)**
Chordata	*Naso* spp.	mtDNA control region	Horne et al. (2008)
Chordata	*Pristipomoides filamentosus*	mtDNA CytB, 11 microsatellites	Gaither et al. (2011)
Chordata	*Scarus ghobba*	mtDNA control region	Visram et al. (2010)
Chordata	*Sicyopterus lagocephalus*	Nuclear ILP	Hoareau et al. (2007)
Chordata	*Sousa chinensis*	mtDNA control region	Mendez et al. (2011)
Chordata	*Stegostoma fasciatum*	4 mtDNA, 11 microsatellites	Dudgeon et al. (2009)
Chordata	*Thunnus albacares*	mtDNA control region	Wu et al. (2010)

ITS: internal transcribed spacer; CAH: carbonic anhydrase; rDNA: Ribosomal DNA; mtDNA: Mitochondrial DNA; COI: cytochrome oxidase I; CytB: Cytochrome B AFLP: Amplified fragment length polymorphism; ILP: Intron-length polymorphism. In bold are studies that found restricted connectivity between localities.

Marine protected areas should, primarily, preserve the genetic integrity of marine resources. The success of such MPAs is dependent on the availability of background information describing how populations are linked, or connected. Although the available information on genetic connectivity between protected reefs along the south-east African coast provides evidence that South African marginal reefs are connected to northern reefs at evolutionary time scales (Table 2), more information is needed to reveal the true dependence of the South African coral communities on those to the north. In particular, information is needed as to whether the systems are structured, with high levels of self-recruitment, since this will affect their recovery from large-scale damage and may cause it to be gradual and localized. The latter is of global importance in view of increasing coral mortality from climate change-related bleaching events. More importantly, evidence of weak ecological genetic connectivity, the absence of its consideration in MPA and MPA network design and management in the region, and the potential effects of climate change on coral reef organisms demand a revision of the MPA management approach. The importance of informed management decisions cannot be underestimated if we are to succeed in preserving our marine genetic resources, given the environmental and anthropogenic challenges these systems are facing.

Table 2 Available estimates of gene flow between reefs from Mozambique (MOZ) and South Africa (SA). *Nm* = effective number of migrants per generation.

Species	Marker	Locations	Scale (km)	F_{ST}	Nm	Source
Acropora austera	intron	MOZ/SA	800	0.180*	63.2a	Macdonald (2010)
Platygyra daedalea	ITS	MOZ/SA	800	0.090	1.31b	Macdonald et al. (2008)
Pocillopora verrucosa	Allozymes	SA	70	<0.039	-	Ridgway et al. (2001)
	Msat	MOZ/SA	1000	0.054*	44b	Ridgway et al. (2008)

a: estimated by coalescent methods; b: estimated from F_{ST} = 1(1+4Nm); Msat : Microsatellite; ITS: Internal Transcriber Spacer; * Statistically significant.

1.5 South-east African MPA planning failed to incorporate information on connectivity

Mozambique and South Africa are signatories to several international conventions and protocols that advocate the implementation of MPAs as a tool for marine conservation and fisheries. In so doing, these governments committed to the implementation of an ecologically representative, effectively managed MPA network by 2012 (Wells et al. 2007; Guerreiro et al. 2011; Guerreiro et al. 2010). Accordingly, several MPAs were established along the south-east African coast in

southern Mozambique and South Africa, to protect marginal coral reefs from human and natural disturbances. Overall, five MPAs in Mozambique protect 4.4% of its continental shelf (WWF Eastern African Marine Ecoregion 2004), while all of the South African coral-inhabited reefs are protected in the iSimangaliso Wetland Park (iWP); a World Heritage Site located in northern KwaZulu-Natal, that incorporates two contiguous MPAs, viz., the St Lucia and Maputuland Marine Reserves (Tunley 2009). The iSWP and the Mozambican Ponta do Ouro Partial Marine Reserve constitute a continuous series of protected reefs, forming a transboundary MPA network.

Information on connectivity is frequently ignored in the design and management of MPAs intended to protect coral reefs (Jones et al. 2009). As marine larvae tend to be passive particles or weak swimmers, population connectivity via larval dispersal by oceanic processes is considered a norm on coral reefs (Largier 2003; Sponaugle et al. 2002). Coral communities along the south-east African coast are swept by cyclonic and anticyclonic eddies formed in the Mozambique Channel and off southern Madagascar which propagate southward, and give rise to the southward flowing Agulhas Current (Lutjeharms 2006). Considering this, the connection of MPAs along the south-east African coast through the southward dispersal of gametes and larvae mediated by regional oceanographic conditions can be assumed. Indeed, connectivity was assumed during the design phase of the MPAs along the south-east African coast (von der Heyden 2009; POPMMP 2009), despite the limited research on population connectivity of most reef organisms in the region (Ridgway & Sampayo 2005; WWF Eastern African Marine Ecoregion 2004). Similarly, the management planning of the South African MPAs failed to include recent information on population connectivity (von der Heyden 2009) and currently assumes that the northernmost reefs will provide sufficiently dispersed larvae to maintain the diversity of the southern reefs (Ridgway et al. 2008). Consequently, the designation of no-take/sanctuaries i.e. no fishing or diving allowed, and restricted use areas appears to have been arbitrary, based largely on community structure and stakeholder needs (e.g. Schleyer & Celliers 2005; von der Heyden 2009; POPMMP 2009).

1.6 Ecological genetic connectivity assessment is needed in South African MPAs

The importance of ecological genetic connectivity between reef populations within south-east African MPAs is emerging. Its importance gained momentum after Ridgway and Sampayo (2005) drew attention to the paucity of population genetic studies on the Western Indian Ocean (WIO) and von der Heyden (2009) articulated reasons why information of this nature, if integrated in management planning, can improve the effectiveness of South African MPAs. For instance, genetic connectivity assessments can identify areas that are functionally connected (i.e. there is effective gene dispersal between their populations) which larval surveys and fish tagging studies alone cannot (von der Heyden 2009). Conversely, as we have gained an understanding of larval

motility and dispersal (e.g. Jones et al. 2005; Planes et al. 2009; Pizarro et al. 2008; Mason et al. 2011), regional oceanographic features (e.g. Sabarros et al. 2009; Kolasinski et al. 2012), and the potential effects of climate change on reefs (e.g. Hughes et al. 2007; Schleyer et al. 2008), the need for studies on reef connectivity in the region is emphasized. Despite the recognized significance of estimates of ecological genetic connectivity to the effectiveness of MPAs (see Ridgway et al. 2008; Macdonald et al. 2010; von der Heyden 2009), these are yet to be determined for south-east African MPAs. It will be particularly important to estimate the dependence of South African reefs on transboundary reefs further afield and the implications of this to the resilience potential of the coral communities.

The following study was thus initiated to provide much needed information on contemporary genetic connectivity in south-east African MPAs and fill in the gaps left by previous studies. Scleractinian corals are sessile organisms and are the major framework builders of coral reefs. Consequently, they are important candidates for connectivity assessments as the inferences made in this regard will translate into precise estimates of effective reef connectivity (Broquet & Petit 2009; Cowen & Sponaugle 2009). Accordingly, the ecological genetic connectivity of two scleractinian species, the branching coral *Acropora austera* and the brain coral *Platygyra daedalea*, was assessed on protected reefs along the south-east African coast. Recently, both corals have been the subject of population genetic studies in the South West Indian Ocean, offering baseline information on their evolutionary genetic connectivity in the region. The species are common on south-east African reefs but have different life histories and contrasting susceptibility to environmental stress; *A. austera* is a fast-growing bleaching-susceptible coral while *P. daeaalea* is a slow-growing bleaching-resistant species (Baird & Marshall 2002, Celliers & Schleyer 2002; MH Schleyer and PH Montoya-Maya, pers. obs.). Both are broadcast spawning species which suggests they have a great dispersal potential (Harrison & Wallace 1990), particularly along the south-east African coastline given the influence of the Agulhas Current. Considering this, the study began from the research hypothesis that south-east African reef subpopulations of the two species comprise single large populations showing significant levels of contemporary gene flow at all spatial scales i.e. between-reef and within-reef populations.

1.7 Research objectives

To test the above hypothesis, the following research objectives were proposed:

- To assess genetic diversity and variation in *A. austera* and *P. daedalea* populations within and between south-east African reefs using multiple, highly polymorphic molecular markers.

- To estimate levels of gene flow in *A. austera* and *P. daedalea* populations within and between reefs.
- To investigate within-reef patterns of spatial and temporal genetic connectivity in populations of *A. austera* and *P. daedalea*.
- To explore the implications of the estimated genetic connectivity of *A. austera* and *P. daedalea* for the effective management of south-east African MPAs.

1.8 Thesis outline

The dissertation comprises six chapters and six appendices. This general introduction (Chapter 1) provides the state of knowledge, rationale and contextualization for this study. The materials and methods for the research are detailed in Chapter 2. Two chapters prepared for publication (Chapter 3 and Chapter 4) investigate ecological genetic connectivity in the two species. A concluding chapter (Chapter 5) presents a general discussion and assesses the implications of the findings in the context of MPA management in South Africa and the region. Eight appendices conclude the thesis, providing detailed descriptions of the processes required to obtain the demographic and genetic information for the study.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study species

The two species of hard coral chosen for this study are widespread, found in the Indian and Pacific Oceans, and common on south-east African reefs. *Acropora austera* is a reef-building, fast-growing coral with a high population turnover (Carroll et al. 2005; Schleyer et al. 2008) and is regarded as opportunistic among reef corals (Macdonald et al. 2010). Colonies are hermaphroditic and are known to broadcast-spawn egg-sperm bundles (Carroll et al. 2005; Masse et al. unpub. data). Characteristics of the larval stage in *A. austera* are unknown. However, from studies on the larvae of other *Acropora* species, a median (50% mortality) survival time of <25 days and a pelagic larval duration of up to 209 days can be expected (Graham et al. 2008). Although found in a wide range of habitats on South African reefs, the colonies are more abundant in shallow areas (10-15 m) exposed to wave action (Celliers & Schleyer 2001) and, in particular, were dominant in the reef-crest of Five-mile Reef (FMR) in the iSimangaliso Wetland Park (Celliers & Schleyer 2007). *Acropora austera* is listed as a near-threatened species by the IUCN (Aeby et al. 2008); it is known to be very susceptible to coral bleaching (Celliers & Schleyer 2002; MHH Schleyer and PH Montoya-Maya pers. obs.); and, it is subject to predation from the crown-of-thorns starfish (Schleyer, unpub. data).

Platygyra daedalea has a massive growth form and is a slow-growing, long-lived species. It is a simultaneous hermaphrodite that spawns positively buoyant egg–sperm bundles and does not self-fertilize (Miller & Babcock 1997; Mangubhai & Harrison 2008). This coral species has a pelagic larval duration of 124 days as estimated from aquarium experiments (Nozawa & Harrison 2002; Souter & Grahn 2008), although it can settle as quickly as 2 days after fertilization (Miller & Mundy 2003). Asexual reproduction through fission or fragmentation has not been documented in *P. daedalea*, although numerous replicates of genotypes (i.e. putative clones) have been found in close proximity to each other having a low probability of being derived from sexual reproduction (Miller & Ayre 2008a). *Platygyra daedalea* is listed as of least concern by the IUCN (DeVantier et al. 2008) which is attributed to its high resistance to natural threats like bleaching (Baird & Marshall 2002; Celliers & Schleyer 2002).

2.2 Study area, sample collection and field sampling

The major oceanographic features in the South West Indian Ocean are the South Equatorial Current (SEC), East African Coastal Current (EACC), Mozambique Channel Eddies (MCE), East Madagascar Current (EMC) and the Agulhas Current (AC) systems. The South Equatorial Current bifurcates and feeds both the MCE and the EACC, which splits both south and north (Figure 1). The poleward Agulhas Current appears to originate between 25° and 30° S where waters from the Mozambique Current and the East Madagascar Current meet (Lutjeharms et al. 2012; Lutjeharms 2006). The current speeds in this region vary between 0.05 m/s for the MCE and 2 m/s for the AC and can be found within 31 km of the coast (Bryden et al. 2005), which suggests that it has a high potential for larval dispersal and reef connectivity. Together with wind patterns, these currents and their flow anomalies (e.g. eddies in the AC) have been related to the presence of high primary productivity and plankton transport in the Mozambique-South Africa region (Bryden et al. 2005; Roberts 2006; Morris 2009; Lutjeharms et al. 2012). Therefore, coral samples of the two species were collected from several locations in the region to assess the large-scale genetic connectivity of reefs (i.e. between reef gene flow) in the south-western Indian Ocean.

Accordingly, samples from 135 colonies of *A. austera* were collected from the iSimangaliso Wetland Park (iSWP), Inhaca Island, Bazaruto Archipelago and Reunion Island reef systems; and 182 samples of *P. daedalea* were collected from the iSWP, Inhaca Island, Bazaruto Archipelago, Pemba Bay, Mtwara Marine Park (Tanzania), Mombasa Marine Park (Kenya) and Chagos Archipelago reef systems (Figure 1). Although this last locality cannot be considered part of east Africa, it was included as an out-group. Geographic distances between sampled reefs ranged from 2 km, between Two-mile and Five-mile reefs (FMR), iSWP, to 11 000 km, between Leadsman Shoal and the Chagos Archipelago. Variations in species density on each reef yielded uneven sample sizes; in this regard, it is important to mention that *A. austera* was notoriously scarce at FMR which is surprising as this species was once a dominant component of the coral community on this reef (Celliers & Schleyer 2007). The samples comprised approximately 2 cm fragments of large colonies separated at least 2 m apart and loose fragments of colonies were avoided so that fragmented colonies produced from nearby colonies were not collected.

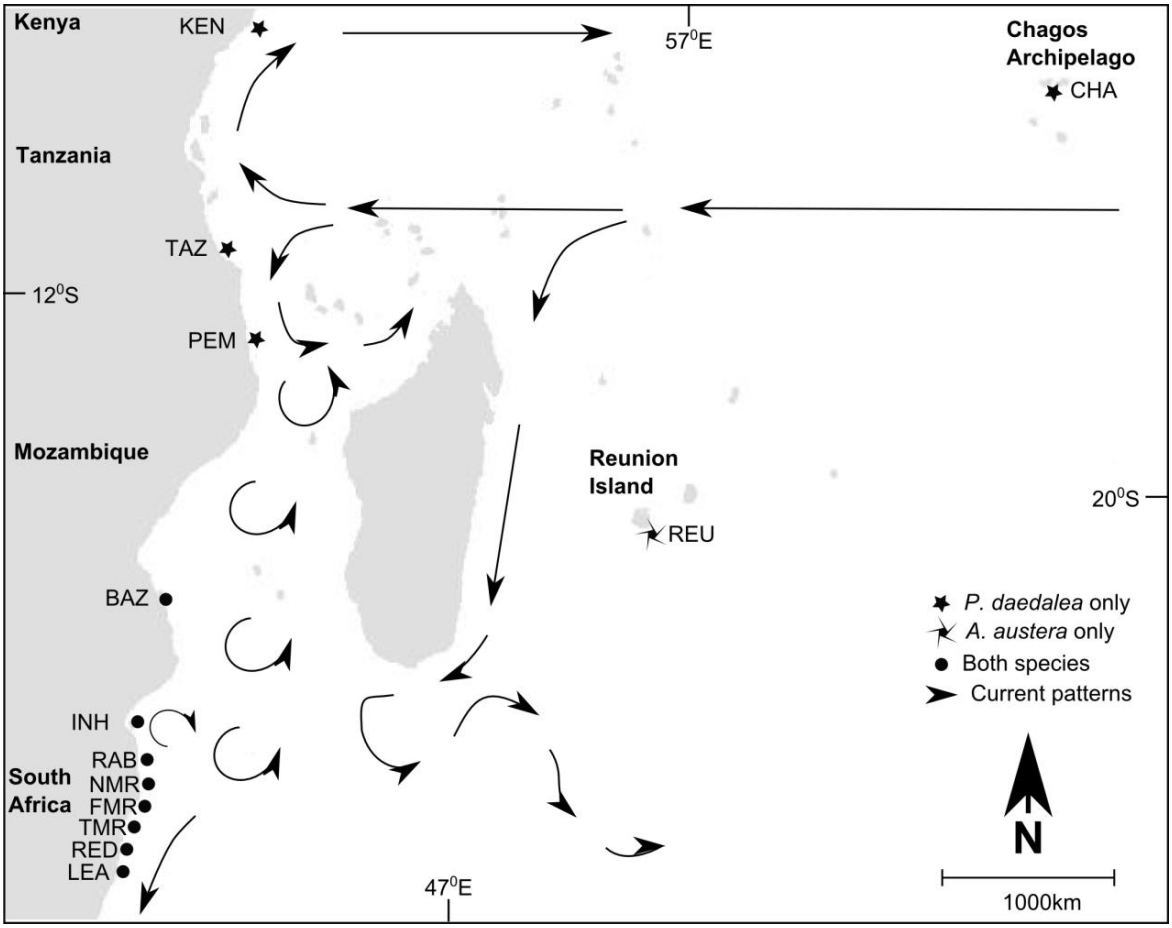

Figure 1 The map depicts the location of sampling reefs along the south-east coast of Africa. The arrows illustrate the predominant water circulation in the South West Indian Ocean. BAZ = Bazaruto Archipelago, CHA = Chagos Archipelago, FMR = Five-mile Reef, INH = Inhaca Island, KEN = Kenya, LEA = Leadsman Shoal, NMR = Nine-mile Reef, PEM = Pemba Bay, RAB = Rabbit Rock, RED = Red Sands Reef, REU = Reunion Island, TAN = Tanzania, TMR = Two-mile Reef.

Two-mile Reef (Figure 2) is the largest reef and is the reef most frequented by divers in the iWP in South Africa (Schleyer & Tomalin 2000; Schleyer et al. 2008). It is a fossilized sand dune running parallel to the coastline in a slight north to south direction with shallow, high-energy areas along the longitudinal axis of the reef (Riegl et al. 1995; Ramsay 1996). The core coral community on this reef is very diverse, found at a depth between 10 – 16 m and has an estimated area of 1.12 km^2 (Schleyer et al. 2008). Conversely, the reef appears to support coral populations with high levels of genetic diversity, suggested by high levels of allelic richness, haplotype diversity and mean observed heterozygosity in *Pocillopora verrucosa* (Ridgway et al. 2008; Ridgway et al. 2001), *A. austera* (Macdonald et al. 2010) and *P. daedalea* (Macdonald et al. 2008). Considering this, TMR was chosen to address fine-scale genetic connectivity (i.e. within-reef dispersal) of coral species on south-east African reefs. Therefore, an additional 159 samples of *A. austera* and 176 samples of *P. daedalea* were respectively collected from individual colonies of at eight sites on

TMR between July 2009 and February 2011. Sampling sites were selected on TMR that were representative of the entire reef area (Figure 2). The distance between sites varied between 200 m and 1200 m apart from each other and each sampling site varied marginally in depth and topography. Random colonies that were separated by ≥2 m were sampled at each site and their exact location (i.e. x and y coordinates) was recorded along a 10 x 4 m belt transect. Global positioning system (GPS) coordinates were recorded at the beginning and end of each transect. The maximum colony diameter (*d1*) and the diameter at right angles to this (*d2*) were measured to the nearest cm. The transect length varied in each case to sample a similar number of colonies of each species at each site; thus the minimum area sampled was 180 m^2 and the maximum 512 m^2. Note that variations in density of the target species on each reef were encountered.

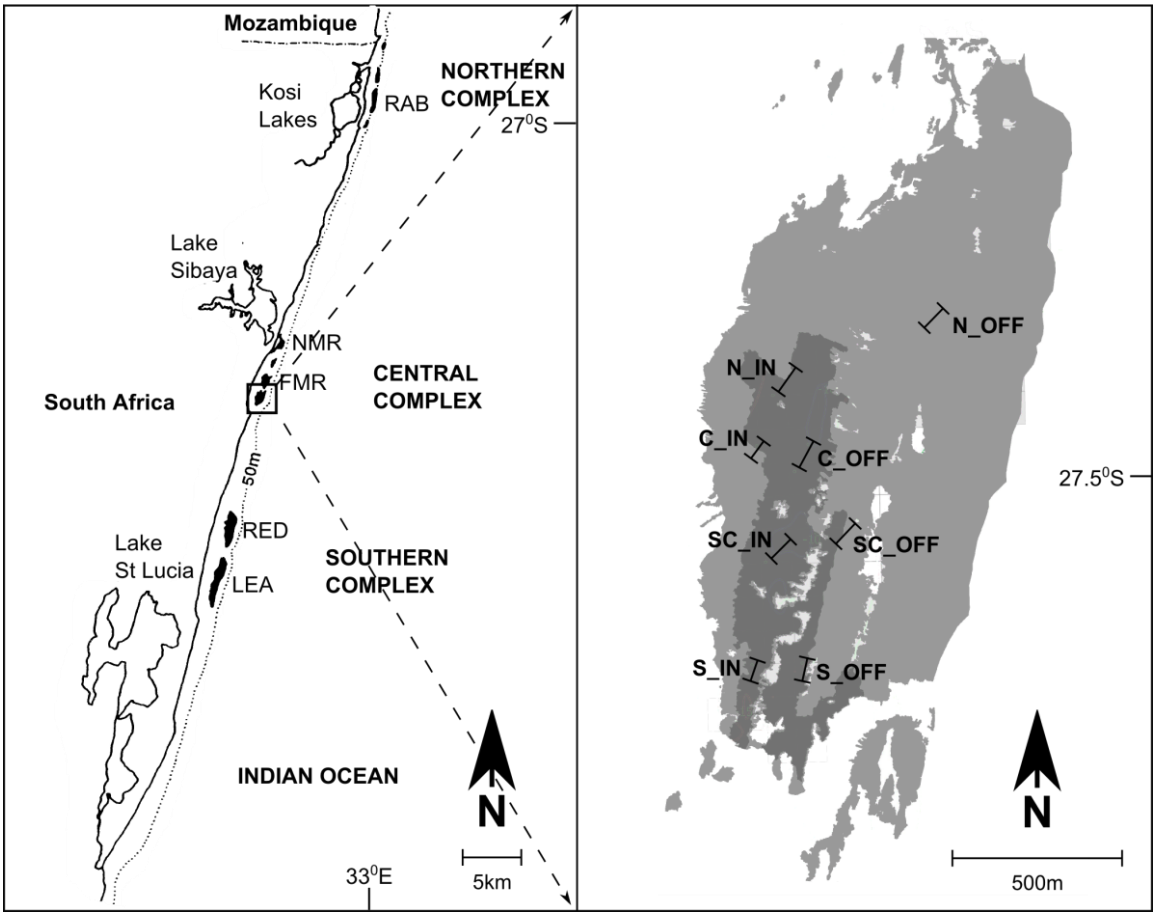

Figure 2 Location of the eight sites (transect lines) that were selected in TMR for analysis of small-scale genetic connectivity. The location of the major coral-inhabited reefs in South Africa and their grouping are also shown. N = Northern, C = Central, SC = South central, S = Southern; IN = Inshore, OFF = Offshore.

Since information on population demography can be used to substantiate patterns of gene flow (Lowe et al. 2004; Gerber et al. 2005), particularly those obtained from spatial genetic structure analyses (Summer et al. 2001; Ledoux et al. 2010), data on population density and the colony size of both species were collected at each sampling site on TMR. This task was performed by a second diver who counted and measured every single colony found within the belt transects. Total number of transects and species demographics per site are shown in Table 3.

Table 3 Mean density (D = Colonies/m^2) and colony size (MCD = cm, expressed as the geometric mean of two diameters, *d1* and *d2*) of *Acropora austera* and *Platygyra daedalea* at TMR. Estimates were calculated from 10 x 4 m transects (T) deployed continuously at each site. IN = Inshore side of reef, OFF = Offshore side of reef.

Site	*Acropora austera*					*Platygyra daedalea*				
	T	D	95% CI	MCD	Range	T	D	95% CI	MCD	Range
N_IN	10	0.04	0.00-0.07	27.07	6.32 - 80.96	10	0.34	0.21-0.46	10.93	2.00 - 32.76
N_OFF	10	0.10	0.00-0.22	17.60	1.73 - 37.52	6	0.86	0.76-0.95	15.76	2.83 - 40.89
C_IN	10	0.07	0.01-0.13	12.13	3.46 - 28.77	10	0.26	0.04-0.48	14.30	2.45 - 40.47
C_OFF	13	0.03	0.00-0.06	19.89	4.90 - 47.43	10	0.54	0.36-0.72	16.32	3.00 - 63.26
SC_IN	10	0.12	0.00-0.27	22.67	4.90 - 89.98	8	0.58	0.32-0.84	16.38	4.90 - 31.94
SC_OFF	11	0.22	0.09-0.34	18.72	3.87 - 148.09	6	0.48	0.29-0.68	20.67	3.87 - 48.99
S_IN	10	0.01	0.00-0.02	20.15	10.49 - 34.41	8	0.78	0.47-1.09	13.59	3.16 - 38.37
S_OFF	11	0.03	0.00-0.08	24.96	9.17 - 146.97	7	0.34	0.19-0.49	20.57	4.90 - 69.94
Mean (SD)	-	0.08	0.05-0.11	19.42	(18.20)	-	0.52	0.42-0.58	15.17	(9.64)

2.3 Reproductive status and size-class assignment

A better assessment of temporal genetic variation is achieved when well-defined cohorts are sampled over several generations (van Oppen et al. 2008; Ledoux et al. 2010). In this study, within-reef patterns of temporal genetic connectivity of *A. austera* and *P. daedalea*, were assessed by separating the colonies collected at TMR into size-classes (i.e. age-classes or potential cohorts). Following Mollet et al. (2000), the size and age of first maturity in both species was estimated as described in Appendix A. Briefly, a subset of sampled colonies was used to model (i.e. Generalized Linear Model) the binomial (0 = immature; 1 = mature) reproductive status (presence/absence of gametes) as a function of mean colony diameter, expressed as the geometric mean of two diameters (i.e. *d1* and *d2*; see Appendix A for model details). Parametric bootstrapping was used to estimate Mean Colony Diameters (MCD) at which the probability of

maturity is 50% (D50) and 90% (D90), (Barot et al. 2004; Mollet et al. 2000). Knife-edge maturity was assumed at D50, yielding a size of first maturity in *A. austera* on TMR at around 15 cm MCD, and 7 cm MCD in *P. daedalea*. Following Ledoux et al. (2010), genotyped colonies from TMR with a MCD below D50 for each species were assigned to size-class 0 (i.e. immature: putative recruits); those above D50 were considered adults.

Published growth rates allowed the estimation of the age of first maturity at defined sizes. Linear growth of *A. austera* from TMR has been estimated as 2.45 cm yr^{-1} (Grimmer 2011) while for *Platygyra* spp living at 24°C sea temperatures a radial growth rate of 0.54 cm yr-1 has been reported (Weber & White 1974). Thus, the age of first maturity in *A. austera* and *P. daedalea* corresponded to colonies ca six years old. Generation times in corals are estimated to be ca ten years (Hall & Hughes 1996; Connell et al. 1997); consequently, the D90 was used to divide adult colonies into two additional size-classes with different reproductive potential. Genotyped colonies from TMR with a MCD below D90 were assigned to size-class 1, corresponding to recently mature colonies (i.e. one adult generation); those above D90 were assigned to size-class 2, corresponding to old mature colonies (i.e. >10 years, several adult generations). Individuals from size-class 1 were assumed to be old enough to be considered as potential parents for stage-class 0; the same was considered for size-class 2 compared to size-classes 0 and 1 (Ledoux et al. 2010).

2.4 Genotyping of nuclear microsatellite and intron loci

All colony fragments were preserved in either 20% dimethyl sulphoxide salt buffer (0.25 M EDTA; 20% (v/v) DMSO, saturated with NaCl) or 96% alcohol (EtOH) in the field and subsequently stored at room temperature. Genomic DNA was extracted from coral tissue using the ZR Genomic DNA Tissue extraction kit (Zymo Research) or by purified standard phenol/chloroform/isoamyl alcohol protocols. Six microsatellite primer pairs (*Amil2_07*, *Amil2_10, Amil2_23*, *EST14*, *MS181*, *MS182*) that were originally developed for *A. palmata* (Baums et al. 2005) and *A. millepora* (van Oppen et al. 2006; Wang et al. 2008) successfully amplified polymorphic loci in *A. austera* (Appendix B) and were selected for further genotyping of samples. Details of genotyping procedure are described in Appendix B. Briefly, around 50 ng of template was amplified in a four polymerase chain reactions (PCRs), two multiplex and two standard PCRs, were performed per individual and in duplicate using fluorescently-labelled primers to assay loci containing a mixture of dimer and trimer repeats. Similarly, five microsatellite loci (*Pd29-2, Pd31, Pd48, Pd61* and *Pd62*) specifically developed for *P. daedalea* were amplified according to the PCR protocol described by Miller and Howard (2003). All PCR products were separated on an ABI 3500 DNA Analyser and sized using the GeneScan LIZ 600 size standard (Applied Biosystems). Genotypes were compiled using STRand v.2.4.59 (Toonen &

Hughes 2001). Scored peaks had a minimum intensity of 5% of the most intense peak, were in phase with the locus repeat motif, and, in the case of rare alleles, were present in both replicates from the same individual. The frequency of null alleles for each locus was estimated with MICRO-CHECKER.

In addition to microsatellite loci, nuclear intron markers were genotyped. A hyper variable intron region of the carbonic anhydrase gene (*CAH 3/550*) of *A. austera* was amplified following the PCR protocol described by Macdonald et al. (2010). Two primer pairs that amplify the second intron of the single-copy Calmodulin (*Calm 2*) gene in corals (Vollmer & Palumbi 2002) and a single intron in the nuclear gene for the alpha subunit of the ATP synthase complex (*ATPSα*) (Frade et al. 2010) successfully amplified polymorphic loci in *A. austera* and *P. daedalea* and were used for further genotyping of samples. Details of genotyping procedures for these two intron loci are described in Appendix C. For all three intron loci, PCR products were cycle-sequenced directly in the forward direction using Big Dye Terminator (Applied Biosystems) chemistry on an ABI 3500 DNA Analyser. Sequences were trimmed, aligned and edited in Geneious Pro v.5.62, created by Biomatters (available from www.geneious.com), and checked by eye. The trimmed, aligned sequence length for *CAH 3-550* was 164 bp, 156 bp for *ATPSα,* and 193 bp for *Calm 2*.

In some individuals, PCR amplification products from several individuals contained mixed sequences of different length or were heterozygous for a deletion event. Mixed sequences were unravelled with the help of CHAMPURU v1.0 (Flot et al. 2007). In individuals with indels, the "find heterozygotes" plug-in for Geneious was used to assign ambiguity codes for positions downstream of the deletion event and then the allelic sequences were reconstructed using Indelligent v1.2 (Dmitriev & Rakitov 2008). Allelic phases of nuclear introns were inferred from diploid sequence data using PHASE 2.0 (Stephens et al. 2001; Stephens & Scheet 2005), coding gaps as a fifth character state. PHASE uses a Bayesian approach to reconstruct haplotypes from population genotypic data and allows for recombination and the decay of linkage disequilibrium with distance. The PHASE analysis (1000 iterations with a 1000 burn-in period) was repeated ten times to ensure consistency across runs. Although the neutrality tests conducted on Calm 2 suggested this intron might be under selection in both species (see Appendix C), this marker was kept because results with and without this marker were consistent across analysis.

Multilocus genotypes (MLGs) were constructed by combining nuclear data from the intron and microsatellite loci. This was accomplished by recoding all alleles in GenAlEx v6.5 (Peakall & Smouse 2006) after they were inferred for every sample and every marker by genotyping. Failure rates of nuclear loci (i.e. the percentage of samples that could not be scored for one or more loci, either due to unsuccessful amplification or unreliable scoring) were high in both species (see

Chapter 3 and 4). Therefore, the patterns of amplification failure across loci were compared between locations using a test of independence (i.e. chi-squared test) to assess any differential failure rates across the range that could affect the results.

As patterns of genetic differentiation may be influenced by the lack of neutrality in the screened loci, LOSITAN (http://popgen.eu/soft/lositan/) was used to detect loci under selection. This software implements the coalescent-based simulation method of Beaumont and Nichols (1996) to identify genes under selection, based on the distributions of heterozygosity and F_{ST}; any loci that present higher or lower than expected values of these two parameters are outliers and considered to be under selection (Beaumont and Nichols 1996). Loci under selection were tested by simulating 100,000 loci using the infinite allele model, and adopting the "neutral mean F_{ST}" and "forced mean F_{ST}" as recommended by Antao et al. (2008).

2.5 Contribution of asexual reproduction and identification of putative clones

The relative contribution of asexual reproduction was estimated by the ratio of N_G:N_I, where N_G is the number of unique genotypes and N_I is the number of individual coral colonies. The ratio of these two factors provides a minimal estimate of sexual reproduction in a population. However, caution was taken when interpreting N_G:N_I, as this ratio is dependent on the number of loci sampled and their respective variability (Mackenzie et al. 2004).

The average probability of identity (PI_{SIBS}) was calculated in. The PI_{SIBS} is an estimate of the probability that two independent samples will have the same identical MLG by chance (i.e. are not clone mates). This index was chosen over the standard *PI* because it takes into account the genetic similarity among siblings and therefore is a more conservative estimate of the probability of identity when the population sampled includes relatives (Waits et al. 2001). Although effort was put to avoid sampling of clones produced by fragmentation and/or relatives, putative clones were identified using the function Matches in GenAlEx and were removed from the dataset to prevent biasing allele frequencies. The "*Ignore missing*" option was selected to find putative matches despite missing data.

2.6 Data analysis

A diagram depicting the series of steps to analyse the data is shown in Figure 3.

Ecological genetic connectivity

Genetic diversity

- Estimation of measures of genetic diversity[c]
  - Rarefied allelic richness and heterozigosity (ADZE v1.1.)
  - Expected Heterozygosity (H_e) and Inbreeding Coefficient (F_{IS}) (GenAlEx v6.5)
  - Hardy-Weinberg Equilibrium; Linkage Disequilibrium (Genepop v4.1)

Population structure and genetic subdivision

- Bayesian clustering analysis
  - Non-spatial and spatial[b] clustering of groups and individuals (BAPS v6)
  - Individual-based admixture analysis with location *priors* (Structure v.2.3.4)
- Estimation of fixation and differentiation indices
  - G-statistics[c]: F_{ST}, G''_{ST} and D_{EST} (GenAlEx v6.5)
  - Hierarchical analysis of molecular variance[c] (AMOVA) (GenAlEx v6.5)
  - Effect of null alleles (FreENA)
- Isolation-by-distance by Mantel tests (GenAlEx v6.5)
- Differences in genetic diversity between subpopulations
  - Friedman's Test non-parametric repeated-measures ANOVA (R v3.0.0)
- Differences in population demographics[b]
  - ANOVA for population density and size-structure between subpopulations (R v3.0.0)
- Loci under selection
  - Coalescent-based simulation method (LOSITAN)

Estimation of migration and gene flow

- Assignment tests[a]
  - Detection of first generation migrants (GENECLASS2)
  - Exclude population as origin of individuals (GENECLASS2)
  - Assign potential recruits to population of origin (GENECLASS2)
- Spatial genetic structure and neighbourhood size estimates[b]
  - Spatial autocorrelation analysis (GenAlEx v6.5)
  - Neighbourhood size (Nb) and effective gene dispersal range (σ) estimates (SPAGeDi v1.4)

Factors influencing the small-scale genetic structure[b]

- Contribution of independent variables to genetic variation
  - Hierarchical partitioning (HP) (Rv3.0.0 and GESTE v2.0)
  - Correlation between genetic variables and demographic/environmental variables
  - Bootstrapped Pearson product moment correlation coefficient (r_P) (Resampling v1.3.)

Figure 3 Step by step process to analyse the large-scale and small-scale genetic connectivity of *A. austera* and *P. daedalea* on south-east African reefs. a. Test conducted exclusively for analysis at the large-scale; b. tests conducted exclusively for analysis at the small-scale; and, c. test conducted between size-classes for the analysis of temporal genetic connectivity. The software packages used for each test are also given.

2.6.1 Analysis of large-scale (between-reef) genetic connectivity

To test the research hypothesis that *A. austera* and *P. daedalea* along the south-east African comprise single large populations showing significant levels of contemporary gene flow between reef subpopulations, the genetic information from all reefs was combined in a dataset, hereafter referred as the "regional dataset". Colonies collected from TMR that were defined as size-class 0 (immature colonies; see Results) were removed from the regional dataset, leaving only size-classes 1 and 2 (i.e. adults). In so doing, the effects that sampling mixed cohorts in the TMR sample might have on patterns of genetic variation (e.g. heterozygote deficits) were reduced. Note that the microsatellite *MS182* and nuclear intron *Calm 2* loci were removed from the analyses of genetic differentiation in *A. austera* because they had more than 25% missing data at some reefs.

Genetic diversity

Estimates of allelic richness (A_r), private allelic richness (A_p), expected heterozygosity (H_e) and inbreeding coefficient (F_{IS}) were calculated for each reef to describe their genetic diversity. The rarefaction method implemented in ADZE v1.1 (Szpiech et al. 2008) to estimate A_r and A_p accounting for differences in sample sizes was used; the minimum number of samples compared corresponded to the minimum number of samples at any one reef multiplied by two (i.e. diploid). All other indices of genetic diversity were calculated using GenAlEx v6.5. The random union of gametes was tested for each locus and reef using an exact test for heterozygote deficit; genotypic linkage disequilibrium between pairs of loci was tested using a Fisher's test. All tests were performed in Genepop v4.1 (Raymond & Rousset 1995; Rousset 2008) with default parameters. Significance levels were corrected using a false discovery rate (FDR, $\alpha = 0.05$) correction for multiple tests when necessary.

Population structure and genetic subdivision

The Bayesian non-spatial clustering of group of individuals method implemented in BAPS v6 (Corander et al. 2008) was used to detect heterogeneity in the sample. Any subdivision in the sample was considered as evidence of genetic discontinuity with the resulting populations or clusters representing genetically distinct populations that meet traditional assumptions in population genetics theory (i.e. Hardy-Weinberg and linkage-equilibrium within populations). Bayesian assessment in BAPS differs from other Bayesian clustering methods (e.g. Structure) in that it can treat groups of sampled individuals as units rather than considering individuals separately; it determines which groups of individuals have different allele frequencies rather than different MLGs; and it clusters non-differentiated groups of individuals and re-calculates the allele

frequencies after merging these groups (Pearse & Crandall 2004). The best partition for each species dataset, corresponding to the most likely number of clusters (*K*), was estimated running initially population mixture analyses of 10 replicates for each value of *K*. The function non-spatial clustering of group of individuals was used and each colony was grouped into its sampled location. Admixture analyses were then run using 100 iterations, 50–150 reference individuals (depending on fixed-*K* value), and 20 iterations for each reference individual.

It has been suggested that the use of different clustering methods constitutes good practice when analysing genetic data as similar conclusions will be indicative of the presence of a strong genetic signal (Guillot et al. 2009). Accordingly, the individual-based Bayesian clustering method implemented in Structure v2.3.4 (Pritchard et al. 2000) was also used to detect genetic subdivisions and estimate *K*. An admixture model with correlated allele frequencies, locations as informative priors, and burn-in period of 1 000 000 followed by 5 000 000 iterations, provided low-variance estimates of *K*. *K* was estimated using the ΔK method of Evanno et al. (2005) as implemented in Structure Harvester (Earl & VonHoldt 2012). In both analyses, the upper bound values of *K* were the number of samples reefs for each species plus one and the number of runs was 10.

Analysis of Molecular Variance (AMOVA) and G-statistics implemented in GenAlEx were used to assess the magnitude of genetic structure and differentiation among locations and clusters. The AMOVA was used to estimate the hierarchical distribution of genetic variation which included within reefs, among reefs within clusters and among clusters. Significance was addressed using 9999 permutations of the original dataset. Pairwise F_{ST} and D_{EST} were calculated to address genetic structure and differentiation between locations and between clusters; D_{EST} was used as it is considered a true estimator of genetic differentiation (Jost 2008). Finally, a Mantel test in GenAlEx was used to test the hypothesis that distance alone is having an effect on the observed levels of subdivision i.e. that is gene flow is reduced by increasing distance between reefs. The F_{ST} and D_{EST} pairwise matrices were correlated with a geographical distance matrix estimated as the log-linear distance (Log (x+1)) between pairs of sampling reefs and between pairs of clusters. The Friedman's Test, a non-parametric repeated-measures ANOVA (analysis of variance), was used to test statistical differences on genetic diversity between clusters. Here, rarefied allelic richness (A_r) and H_e in each cluster were treated as repeated measures data because each point corresponded to the values of the metric on the same genetic marker.

Before analyses, we used the software FreeNA (Chapuis & Estoup 2007) to assess the effect that the presence of null alleles in genotyped microsatellites (Appendix B) had in estimates of genetic structure. This software implements the ENA correction method which corrects for the positive bias induced by the presence of null alleles in estimation of F_{ST} (Chapuis & Estoup 2007).

The robustness of the results in the light of potential null alleles was estimated by calculating global and pairwise F_{ST} from 1000 bootstrap replicates, both using and without using the ENA correction and compared with the Friedman's Test non-parametric repeated-measures.

Estimation of migration and gene flow

Assignment tests were conducted to estimate recent migration among the clusters identified above. Firstly, first generation migrants were detected in each clusters using the software GENECLASS2 (Piry et al. 2004) and implementing the likelihood computation "*L = L_home* ", which is appropriate when not all source populations have been sampled (Paetkau et al. 2004). The frequency-based method of Paetkau et al. (1995) was used as the criteria for computation and the probability that an individual was a resident of a cluster was estimated by comparing its genotype to the distribution of likelihoods of 10 000 simulated genotypes, generated using a MC algorithm (Paetkau et al. 2004). Secondly, individuals that were considered first generation migrants (i.e. probabilities of originating from the cluster where they were sampled were below $P \leq 0.05$) were removed from the datasets. Finally, these reduced dataset were used as reference populations in the estimation of migration and gene flow by assignment tests.

The assignment tests consisted of using the population exclusion method implemented in GENECLASS2 to assign each individual to its most likely source cluster of origin (i.e. reference populations). For each colony, the likelihood that it originated within the cluster containing its sampling reef was calculated using the partial Bayesian criterion of Rannala and Mountain (1997) and compared to the distribution of likelihoods of 10 000 simulated genotypes, generated using a MC algorithm (Paetkau et al. 2004). When an individual was excluded from its source population based on its exclusion probability ($P < 0.01$), the individual was considered to belong to another population when $P \geq 0.1$ and it was assigned as an immigrant to the highest-probability population. However, the individual was left unassigned when the probability was too low ($P < 0.1$). Finally, pairwise F_{ST} values were also used to calculate the effective number of migrants (N_em) under an island model ($N_em = (1/F_{ST} - 1)/4$; Wright 1969); although the validity of this method has been recently criticized (Broquet & Petit 2009), it was considered relevant for comparison with previous studies.

A second approach was followed to evaluate migration and gene dispersal between clusters using the putative recruits that were sampled on TMR. Migration or dispersal is believed to be mediated by oceanographic currents along the south-east African coast, predominantly in a north to south direction, with northern reefs assumed to be sources of coral larvae (see Introduction). Therefore, putative recruits (size-class 0) on TMR, which were removed from the regional dataset, were designated as 'unknowns' and GENECLASS2 was used to assign these genotypes to the reef

as well as the cluster containing their most probable reef of origin. Test parameters were the same as for the first assignment.

2.6.2 Analysis of small-scale (within-reef) genetic connectivity

To investigate patterns of genetic variation at the finest scale which could assist the understanding of the observed regional patterns, data from the eight sites sampled on TMR only were combined for each species in datasets, hereafter referred as the "reef dataset". The reef datasets, including colonies of all sizes and after removal of putative clones, comprised 147 *A. austera* and 127 *P. daedalea* samples. Note that the microsatellite *MS181* and nuclear intron *ATPSα* loci were removed from the analyses of genetic differentiation because they had more than 25% missing data at some sites. In addition to the analysis conducted for the regional dataset, the following tests modifications and additional test were performed for the reef dataset.

Genetic diversity

In addition to the analysis of spatial genetic diversity (i.e. sampling locations), a cohort analysis was conducted to assess temporal genetic variation on TMR (Flowers et al. 2002, Hedgecock et al. 2007). Variations within and between size-classes in allelic richness, private alleles, expected heterozygosity, and HWE were assessed as described for the regional dataset.

Genetic differentiation and subdivision

Genetic subdivision within TMR was analysed by performing Bayesian clustering analyses in BAPS v6 and Structure 2.3.4. The most likely number of genetically distinct populations of both species on TMR was initially estimated as described for the regional dataset but with an upper bound value of $K = 9$. Once K was estimated, the spatial clustering of individuals and groups (i.e. sites) available in BAPS v6 was used to ascertain the spatial configuration of the potential population structures suggested by the initial Bayesian clustering analyses. In this second set of analyses, K was fixed to the values estimated in BAPS and Structure while the coordinate values given corresponded to UTM-transformed GPS coordinates of the individual and sites (taken as the centroid of all individual coordinates in a particular site).

Mantel tests were conducted in GenAlEx and F_{ST} and D_{EST} pairwise matrices were correlated with a geographical distance matrix estimated as the log-linear distance (Log (x+1)) between sampling sites. Hierarchical AMOVA tests were conducted for all potential population structures namely, among reef regions (inshore-offshore) and among estimated clusters in BAPS and Structure. Temporal genetic structure was addressed by G-statistics and AMOVA using three

hierarchical levels: among size-classes, among colonies within size-classes and within colonies. For all tests, significance was addressed using 9999 permutations of the original dataset. The ANOVA was used to test for differences in squared-root transformed population density and size structure between clusters in each species after group clustering.

Estimates of spatial genetic structure and neighbourhood size estimates

Significant spatial genetic structure (SGS) was assessed to determine the presence of non-random spatial distribution in genetic variation, for which limited gene flow would be one explanation (Lowe et al. 2004).

Firstly, spatial autocorrelation analyses were conducted in GenAlEx and gene dispersal distances for each species within each sampling site and the entire TMR were assessed directly from the resulting correlograms. The consistency of SGS across the reef was assessed by tests of heterogeneity which checks for homogeneity between the correlograms of all sites (Peakall & Smouse 2006). The following six distance categories were chosen for the spatial autocorrelation analyses as they resulted in an even number of pairs of colonies in each category: 0 to 5 m, 5 to 10 m, 10 to 20 m, 20 to 30 m, 30 to 50 m and 50 to 100 m. The first intercept where the autocorrelation coefficient *r* falls from positive to negative is denominated the genetic patch size and considered as the extent of a panmictic unit, it thus becomes an estimate of small-scale gene flow (Peakall & Smouse 2006). In an attempt to assess temporal changes in SGS, spatial autocorrelation analyses were conducted for the three size-classes. Colonies within an individual site were divided in size classes and the autocorrelation coefficient *r* for each size-class at a single site and combined across size-classes were calculated.

Lastly, the neighbourhood size (N_b) and the effective gene dispersal range (σ) were estimated with SPAGeDi v1.4 (Hardy & Vekemans 2002). Here, the genetic relatedness between pair of individuals was regressed on their pairwise spatial distance using the same distance categories as in the spatial-autocorrelation analyses; the kinship coefficient (F_{ij}) defined by Loiselle et al. (1995; in Vekemans & Hardy 2004) was used to measure genetic relatedness as it does not assume HW equilibrium in the sample and has shown low bias and variance (Vekemans & Hardy 2004). The slope of the regression (blog) is then used to estimate N_b and σ using the formulae $N_b \sim -(1-FN)/B_{\log}$ and $N_b = 4\pi D_e \sigma 2$, where FN is the inbreeding coefficient and D_e is the effective (breeding) population density; when D_e is known, σ can be obtained from N_b following an iterative procedure (Vekemans & Hardy 2004). Considering this, the population densities calculated for each species at TMR (Table 3) were used to assess D_e, which has been considered to be ca 11% of the density of a population (Richards et al. 2008). Linear regression was limited to a range of σ up

to 20σ as the default set; standard errors and significance were estimated by jack-knifing over loci and random permutations with 9999 repetitions in all tests, respectively.

Factors influencing the small-scale genetic structure of *A. austera* and *P. daedalea*

From the analyses of small-scale genetic connectivity, differences in the population demographics of the identified genetic clusters were apparent. Therefore, hierarchical partitioning (*HP*) was used to explore the demographic or environmental factors that influenced the genetic variation and spatial genetic structure of the two coral species on TMR. This method assesses the independent (*I%*) and joint (*J%*) contribution to the explanation of variance in a dependent variable for every variable in a set of predictors (Goudet 2005). The package "*hier.part"* in R was used for the analyses, log-likelihood as a measure of goodness-of-fit and a randomization test (1000 repetitions) for the significance of the contribution of each variable to explained variance. Dependent variables corresponded to the local F_{ST}, a site-specific metric based on allelic differentiation estimated in GESTE v2.0 (Foll & Gaggiotti 2006), and local relatedness (*r*), the autocorrelation coefficient at the first distance class at each sampling site. The population demographic estimates (i.e. density and mean colony size), the location on the reef (i.e. inshore [0] or offshore [1] as binomial), the median depth in metres, and the latitude coordinates of each site were used as predictor or independent variables in the *HP* analysis. The latter was included to test for influences with a latitudinal gradient. Density and mean colony size were squared-root transformed and all independent variables were standardized to a mean of zero and standard deviation of one. Because five independent variables were tested, a variable was considered to have high explanatory power when its contribution to explained variance (*I%*) was higher than $1/5$ or 20% of the total variance.

Independent variables that were found in the *HP* analysis to have a considerable or significant explanatory power were correlated to the respective dependent variables; in so doing, the nature of the influence was explored. Accordingly, the Pearson product moment correlation coefficient (*rP*) between a pair of variables and the 95% CI of the mean were estimated from a 10000 bootstrap replicates.

CHAPTER 3

GENETIC CONNECTIVITY OF *ACROPORA AUSTERA*

3.1 Results

3.1.1 Nuclear loci analysis

Multilocus genotypes (MLGs) were obtained from 288 colonies of *A. austera* from the nine study reefs employing nine nuclear markers (six microsatellites and three introns) (Table 4). The overall failure rate for the nuclear loci averaged 5.9% (± 7.1 SD), and ranged from 0% for Amil2-10 and Amil2-23 to 19.6% (± 10.1 SD) for MS182. A chi-squared test suggested that the failure rates of the nuclear loci did not differ between locations ($\chi^2_{(8)} = 9.09$, $P > 0.05$). Correspondingly, tests for selection indicated that the screened loci are evolving as neutral markers (Appendix H).

3.1.2 Contribution of asexual reproduction and identification of putative clones

Of the total sample, 97% of the individuals with complete nine-locus genotype data (159 colonies) were identified as unique individuals or had a unique MLG. The N_G:N_I values were high (>0.88), suggesting a low contribution of asexual reproduction and a prevalence of sexual reproduction in the coral populations (Table 4). Indeed, only seven MLGs were shared between 16 colonies in the total genotyped. The number of colonies sharing the same MLG was less than three at a single reef, which suggests genotypic evenness. Considering this and the high probability of identity (PI_{SIBS}= 0.014, i.e. 1 in 71 colonies will share the same MLG by chance) for the entire dataset, colonies sharing an MLG were treated as belonging to the same individual and included only once in further analyses.

3.1.3 Between-reef genetic variation and connectivity

The following results are based on the analysis of a regional dataset of 218 samples of *A. austera*, after the removal of colonies of size-class 0 and putative clones (See Chapter 2).

Table 4 Collection localities, MPAs, reefs or sites with an unique identifier (Code) and the number of samples (N) of *Acropora austera* colonies with nine-locus genotypes (N_I), unique nine-locus genotypes (N_G), and the relative contribution of asexual reproduction (N_G:N_I).

Country	MPA/Reef/site	Code	N	N_I	N_G	N_G:N_I
Reunion Island		REU	6	6	6	1
Mozambique	Bazaruto Archipelago National Park	BAZ	20	9	9	1
	Ilhas da Inhaca e dos Portugueses Faunal Reserve	INH	13	5	5	1
South Africa	iSimangaliso Wetland Park					
	Rabbit Rock Reef	RAB	18	13	13	1
	Nine-mile Reef	NMR	17	9	9	1
	Five-mile Reef	FMR	16	9	9	1
	Two-mile Reef	TMR	154	83	78	0.94
	Northern inshore	N_IN	17	4	4	1
	Central inshore	C_IN	25	12	11	0.92
	South central inshore	SC_IN	28	11	11	1
	South inshore	S_IN	4	4	4	1
	Northern offshore	N_OFF	16	6	6	1
	Central offshore	C_OFF	9	7	7	1
	South central offshore	SC_OFF	49	34	30	0.88
	South offshore	S_OFF	6	5	5	1
	Red Sands Reef	RED	22	13	13	1
	Leadmans Shoal	LEA	22	12	12	1
	Total/Mean		288	159	154	0.99

Genetic diversity

Rarefied allelic richness (A_r) per reef was 3.55 (± 0.53 SE); however, loci were not polymorphic at all reefs (Appendix D). Microsatellite loci *Amil2_07* and *Amil2_10* were monomorphic at REU, RAB, FMR, RED and LEA. *Amil2_23* was monomorphic at REU. Further, none of the measures of genetic diversity manifested a latitudinal gradient. Indeed, the rarefaction approach showed that REU, the northernmost study reef, and FMR in the iSWP, had the lowest allelic richness (A_r) of all reefs (Figure 4). Contrastingly, NMR, one of the northernmost reefs in South Africa, and BAZ had allelic richness values that were about two-fold higher than any other reef (Figure 3). Similarly, these two reefs had the highest mean number of private alleles (Figure 4), suggesting that they are distinct from the other reefs (Kalinowski 2004).

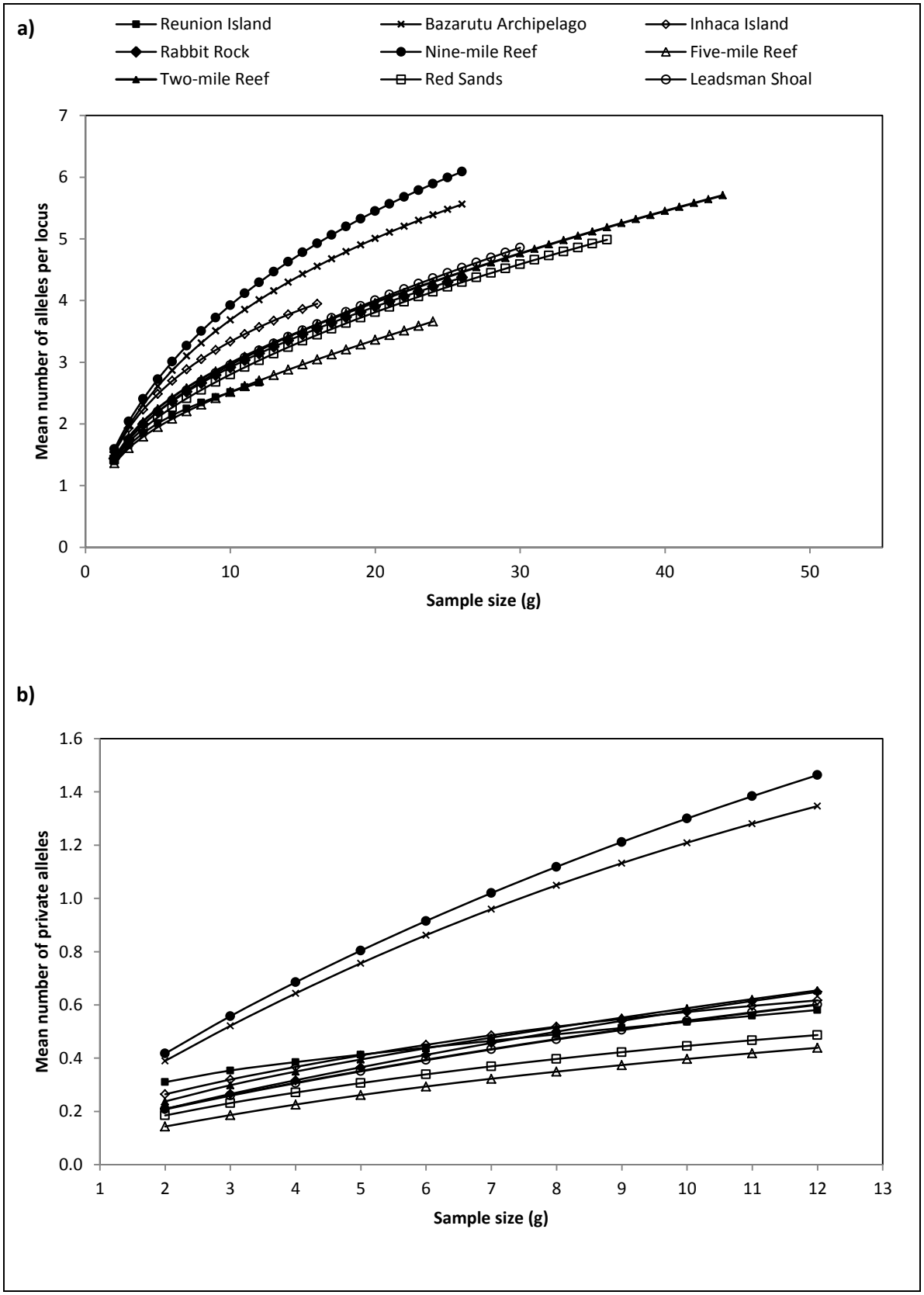

Figure 4 Genetic diversity of *Acropora austera* (a. Allelic richness; b. Private allelic richness) as a function of standardized sample size for the nine study reefs. Only loci with less than 25% missing data on reefs were plotted.

The expected average multilocus heterozygosity (H_e) was 0.466 (± 0.033 SE), ranged from 0.052 to 0.795, and was larger than the observed heterozygosity (H_o) on all reefs. The multilocus inbreeding coefficient (F_{IS}) for all reefs was 0.314 (± 0.041) and ranged from 0.07 to 0.50. Deviations from Hardy-Weinberg equilibrium (HWE) in the form of heterozygosity deficits were observed in 41 of 65 possible tests for all reefs and loci. Of these, only 25 deficits were significant after FDR correction. However, the overall test suggested a general deviation from HWE in the same form (Score U Test, P = 0.000). Only two pairs of loci (*EST14 – MS181* and *MS182 – Calm 2*) remained in linkage disequilibrium after FDR correction.

Population structure and genetic subdivision

The Friedman's tests computed between all loci and per locus F_{ST} estimates with and without ENA correction (Appendix E) suggested that the ENA-corrected F_{ST} values (i.e. corrected for presence of null alleles) were much higher than those calculated with the uncorrected data (Appendix E). Therefore, it was considered that the analysis of the uncorrected data will correspond to an underestimation of the real genetic variation and differentiation between reefs, suggesting a conservative approach.

Acropora austera exhibited significant population structure (F_{ST} = 0.095, P = 0.001) across the south-east African region in terms of loci and locality (Table 5); the signal of population genetic structure was stronger in nuclear intron (F_{ST} = 0.109, P = 0.003) than microsatellite (F_{ST} = 0.084, P = 0.022) loci. Similarly, measures of genetic differentiation (G-statistics) were all significant (Table 5), supporting the evidence of genetic discontinuity throughout the sampled area. Indeed, all pairwise F_{ST} (from 0.006 to 0.302) and 20 D_{EST} (-0.004 to 0.212) values of the 36 possible were significant after FDR (Table 5). However, the genetic differentiation appeared to be influenced by the inclusion of samples from REU and FMR. As the pairwise F_{ST} and D_{EST} between these two reefs and all other reefs were significant, measures of genetic differentiation (G-statistics) were re-calculated excluding samples from REU and FMR. Overall G-statistics were then not significant (P >0.05) (Table 5). Nonetheless, pairwise comparisons remained significant after the removal of the REU and FMR samples from the analyses (Table 5). Altogether, the study reefs could not be considered part of a single large random-mating genetic population.

Table 5 Large-scale genetic structure in and population differentiation estimated for the south-east African reef populations of *Acropora austera*. a) Per locus estimates of genetic structure; G''_{ST} is Hedrick's standardized G_{ST}, further corrected for bias when number of populations is small (Peakall and Smouse 2006) and D_{EST} is the genetic divergence index proposed by Jost (2008). Upper (CIU) and lower (CIL) 95% confidence intervals and standard errors (SE) from bootstrapping and jack-knifing loci are given. b) Pairwise genotypic differentiation between the reefs; F_{ST} values below diagonal and D_{EST} above diagonal. *<0.05 and **<0.01 are significant pairwise comparisons. Significant (P <0.05 after FDR) pairwise comparisons after FDR are in bold. Pairwise comparisons that remained or became significant after exclusion of samples from REU and FMR are underlined (P <0.05; significance after FDR). See Table 4 for description of reef codes.

a)

Locus	All locations			Excluding REU and FMR		
	F_{ST}	G''_{ST}	D_{EST}	F_{ST}	G''_{ST}	D_{EST}
Microsatellite	0.084*	0.088**	0.032*	0.045	0.032	0.012
Amil2_07	0.050	0.019	0.001	0.044	0.023	0.002
Amil2_10	0.084	0.044*	0.003	0.077	0.053	0.005
Amil2_23	0.095	0.065*	0.014	0.077	0.062	0.016
EST14	0.113**	0.280**	0.205**	0.028	0.014	0.010
MS181	0.055	0.091*	0.068	0.045	0.078	0.057
Nuclear Intron	0.109**	0.255**	0.195**	0.049	0.067	0.051
ATPs	0.099*	0.326**	0.281*	0.054	0.126	0.107
CAH 3-550	0.122**	0.231**	0.154**	0.042	0.033	0.022
All loci (SE)	0.095* (0.013)	0.118* (0.037)	0.056* (0.031)	0.047 (0.006)	0.036 (0.009)	0.017 (0.008)
CIL - CIU	0.064 - 0.105	0.064 - 0.214	0.012 - 0.112	0.037 - 0.058	0.020 - 0.056	0.007 - 0.037

b)

Reef	REU	BAZ	INH	RAB	NMR	FMR	TMR	RED	LEA
REU		**0.119****	**0.116****	**0.148****	**0.192****	**0.212****	**0.158****	**0.145****	**0.108****
BAZ	**0.129****		-0.004	**0.043****	0.022	**0.057****	**0.033****	0.021*	0.021
INH	**0.137****	0.012		0.019	0.006	**0.072****	0.021*	0.002	-0.002
RAB	**0.192****	**0.052****	**0.032***		0.023	**0.046****	0.003	0.016	-0.001
NMR	**0.166****	**0.030***	0.020	**0.030****		**0.070****	**0.035****	0.028*	**0.034***
FMR	**0.302****	**0.076****	**0.102****	**0.075****	**0.081****		**0.028****	**0.048****	**0.081****
TMR	**0.195****	**0.046****	**0.036****	0.006	**0.046****	**0.042****		**0.015***	0.015*
RED	**0.198****	**0.034****	0.018	**0.029***	**0.039****	**0.081****	**0.025****		0.019
LEA	**0.139****	**0.032****	0.013	0.007	**0.042****	**0.116****	**0.025****	**0.034****	

The Bayesian clustering analyses suggested that there were four genetically distinct populations in the sample. BAPS estimates revealed (with 99.5% probability) that $K = 4$ and placed the REU and BAZ samples in two independent clusters, INH and NMR in a third cluster (INH-NMR) and the rest of the South African (SA) reefs together in the fourth cluster (Figure 5). Four

genetically distinct populations in the sample were also identified with Structure (Appendix F); however, the clusters did not clearly correspond to either individual or groups of reefs (Figure 5). The G-statistics confirmed that the BAPS clusters were genetically different (F_{ST} = 0.097, 95% CI = 0.059, 0.132; G''_{ST}= 0.159, 95% CI = 0.059, 0.325; D_{EST} = 0.081, 95% CI = 0.017, 0.231; all P <0.05). Pairwise cluster comparisons varied from 0.017 (INH-NMR vs. SA) to 0.132 (REU vs SA) for F_{ST} and 0.080 (BAZ vs INH-NMR) to 0.148 (REU vs SA) for D_{EST}; all but BAZ vs INH-NMR were significantly different from zero (P <0.05). The AMOVA analysis showed that 2% of the genetic variation was found among the *A. austera* clusters, 4% among reefs within clusters while 94% was found within reefs. Correspondingly, there were statistically significant differences in rarefied allelic richness ($\chi^2_{(3)}$ = 18.467, P = 0.001) and expected heterozygosity ($\chi^2_{(3)}$ = 13.652, P = 0.003) among clusters (Figure 4). Post-hoc analysis with Wilcoxon paired-rank tests showed that cluster 3 (INH-NMR) was significantly more diverse (i.e. larger rarefied allelic richness and expected heterozygosity) than cluster 1 (REU).

Mantel tests revealed that distance alone did not have an effect on the observed levels of subdivision in the region. The Mantel test was only significant for F_{ST} (F_{ST}: r^2 = 0.564, P = 0.043; D_{EST}: r^2 = 0.539, P =0.060; n = 9) when all reefs were included, whereas the tests yielded weaker and insignificant correlations in both measures of genetic differentiation (F_{ST}: r^2 = 0.067, P = 0.179; D_{EST}: r^2 = 0.006, P =0.429; n = 7) when the outlier reefs, viz. REU and FMR, were excluded. Contrastingly, the Mantel test between clusters revealed that the BAPS clustering pattern followed a model of isolation-by-distance (IBD); the correlations between genetic and geographic distances were significant (F_{ST}: r^2 = 0.772, P = 0.050; D_{EST}: r^2 = 0.731, P = 0.036; n = 4) in all clusters. Therefore, to further assess whether REU and FMR influenced the clustering of the reefs, two additional Bayesian clustering analyses were conducted in BAPS, one excluding the REU and FMR samples and another including South African reefs only. These analyses yielded no changes in the conformation of clusters, suggesting that the groups were well-structured. For instance, the clustering analysis of only South African reefs identified a two-cluster partition that corresponded to NMR and the rest of South Africa as the most probable division (64.5% probability); the remaining 35.5% probability was for a three-cluster partition where TMR formed a third cluster. G-statistics indicated that the two-cluster partition corresponded to genetic populations that are significantly distinct (F_{ST} = 0.026, 95% CI = 0.016, 0.050; G''_{ST} = 0.057, 95% CI = 0.020, 0.118; D_{EST} = 0.030, 95% CI = 0.010, 0.054; all P <0.01). Finally, Mantel tests indicated that gene flow between South African reefs only did not follow an IBD pattern (F_{ST}: r^2 = 0.027, P = 0.355; D_{EST}: r^2 = 0.086, P = 0.270; n = 6)

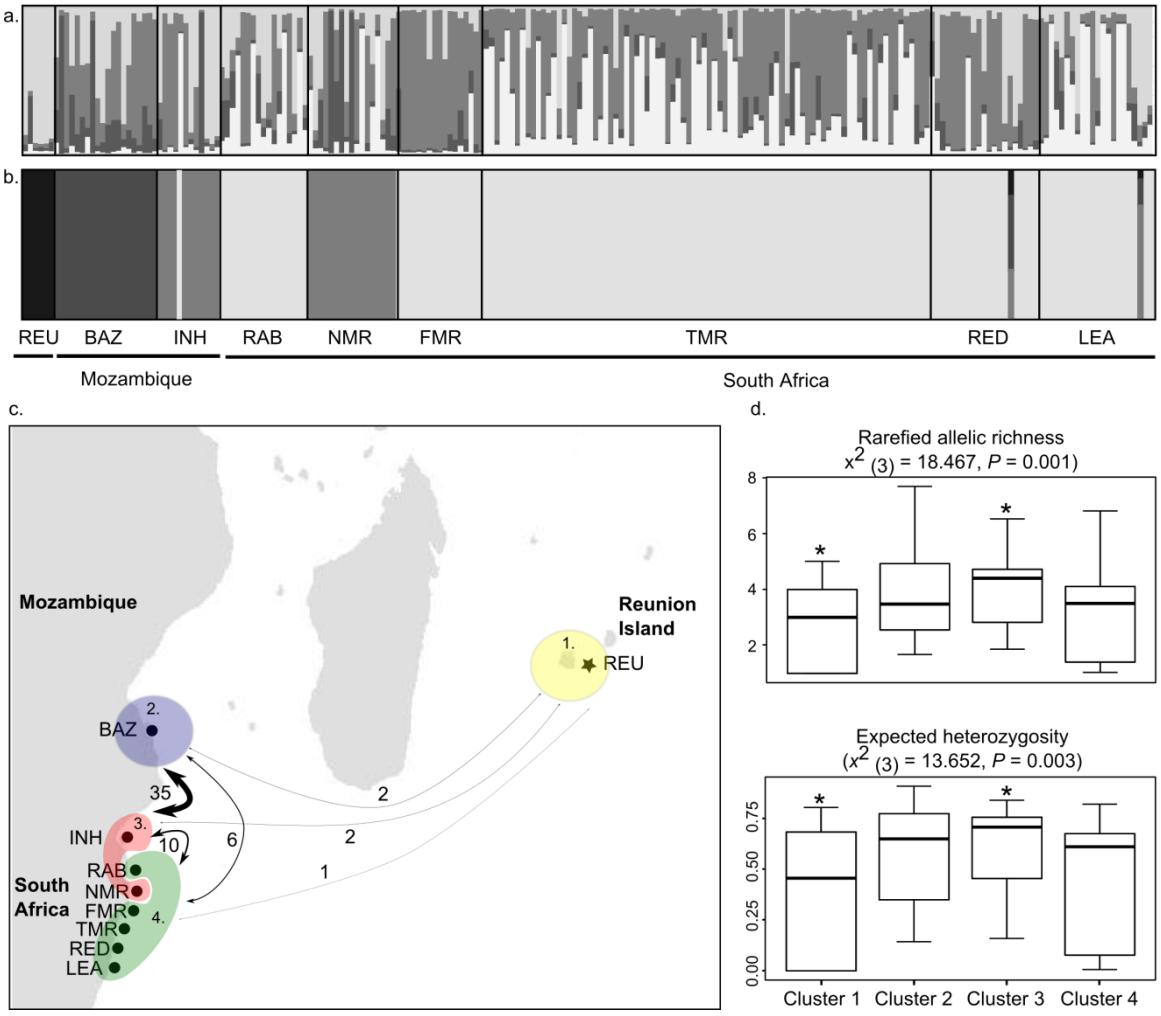

Figure 5 Large-scale genetic structure of *Acropora austera* along the south-east African coast estimated by Bayesian clustering analyses. Structure (a) identified four clusters in the study area with no major correspondence to geographic areas. BAPS (b) also identified four clusters and the graph corresponds to the most probable partition. Shown in (c) are long-term gene flow (arrows) and estimated number of migrants per generation (N_em, numbers) between clusters (numbered and coloured areas) identified in BAPS. Box plots (d) depict the distribution of genetic diversity in each cluster; thick lines correspond to the median and whiskers represent the interquartile range. * = Significant pairwise differences after FDR. See Table 4 for description of reef codes.

Estimation of migration and gene flow

Estimates of number of migrants per generation (N_em) between genetic clusters suggest that long-term migration is occurring between populations of *A. austera* located in southern Mozambican and northern South African reefs (Figure 5). The highest numbers of migrants per generation were found between clusters formed by these reefs; in contrast, the lowest N_em were found between the furthermost sampled locations (i.e. REU) and any other sampled reef (Figure 5).

However, the results of the assignment tests suggest that migration between the populations is not relevant at ecological time scales and that there is a high degree of self-

recruitment on each reef. On one hand, only eight colonies of *A. austera* (three from NMR and one from REU, BAZ, INH, TMR and LEA) were identified by GENECLASS2 as first generation migrants (i.e. were excluded from the cluster representing their sampling reefs) and all were left unassigned having a lower than 10% probability of belonging to any of the four clusters used as reference populations. On the other hand, the assignment tests of putative recruits from TMR resulted in more than half of them (61.6%) being assigned to the cluster comprised of South African reefs rather than any other cluster (Table 6). When the assignment test was conducted with each single reef as a reference population, 60% were assigned to TMR, 21.5% to NMR, and 8.1% to LEA.

Table 6 Results of assignment tests conducted in GENECLASS2 with the *Acropora austera* putative recruits from TMR. The distributions of recruits assigned to clusters a) and individuals reefs b) are shown. Recruits were determined by size as described in Chapter 2. See Table 4 for description of reef codes.

a)

Clusters	REU	BAZ	INH/NMR	SA	Unassigned
Number of recruits (61)	-	4	15	37	5
Percentage (%)	-	6.5	24.6	61.6	8.1

b)

Reefs	REU	BAZ	INH	RAB	NMR	FMR	TMR	REA	LEA
Number of recruits (61)	-	4	2	1	13	-	36	-	5
Percentage (%)	-	6.5	3.3	1.6	21.3	-	59.2	-	8.1

3.1.4 Within-reef genetic variation and connectivity

Demographics and size-class assignment

The density of *A. austera* on TMR varied significantly between sites (ANOVA $F = 3.62$, $df = 7, 76$, $P = 0.002$). Colonies were on average more abundant at the SC_OFF and C_IN sites whereas they were scarcer at the two southern sites viz. S_IN and S_OFF. Although size-frequency distributions of *A. austera* varied across TMR, small- to medium-sized (<38.6 cm mean colony diameter or MCD) colonies were predominant. Of the 147 colonies from TMR, 61 (42%) were considered immature (SC0) based on an estimated size at first maturity of 15 cm (Appendix A). The remaining were considered mature colonies; within these, 62 were assigned to size-class 1

(SC1, MCD >15 cm, <39 cm) and the remaining 24 colonies were assigned to size-class 2 (SC2, MCD >39 cm).

Genetic diversity

On TMR, the individuals that shared an MLG originated from the same sampling site and were collected within 12 m from each other, this being determined by plotting them on a map of TMR using the GPS and x- and y-coordinates of their location on the transects (See Figure 6 for an example). This suggests that colonies sharing the same MLG resulted from fragmentation. Considering this, colonies sharing an MLG were treated as belonging to the same individual and included only once in further analyses.

Variations in within-reef spatial genetic diversity were marginal on TMR. The rarefaction approach showed that allelic richness varied between 1.99 (± 0.13 SE) for S_IN site and 2.57 (± 0.42) in the S_OFF site, while private allelic richness ranged from 0.35 (± 0.19) for the S_IN site to 0.47 (± 0.20) for the N_OFF site (Appendix 4). The mean H_e per site ranged between 0.29 (± 0.13) and 0.47 (± 0.11). Deviations from HWE in the form of heterozygosity deficits were only observed in 10 of the possible 72 tests; however, the overall test suggested a general deviation from HWE.

Temporal genetic diversity was homogeneous across size-classes (Appendix 4). Using the rarefaction approach, the overall mean allelic richness was 5.04 (± 0.08), while the overall mean private allelic richness was 1.77 ± 0.09. As in the spatial analysis, the size-classes showed deviations from HWE in the form of heterozygosity deficits (Appendix D). The mean H_e per size-class was 0.46 (± 0.01). Furthermore, mean allelic richness ($\chi^2_{(2,9)}$ = 0.400, P = 0.819), mean private allelic richness ($\chi^2_{(2,9)}$ = 1.086, P = 0.581), mean H_e ($\chi^2_{(2,\ 9)}$ = 2.250, P = 0.325) and inbreeding coefficients (F_{IS}; $\chi^2_{(2,\ 9)}$ = 0.743, P = 0.690) were homogeneous across size-classes. A coupled analysis of spatial and temporal genetic diversity showed that this homogeneity in the genetic diversity of putative cohorts was constant at individual sites (data not shown).

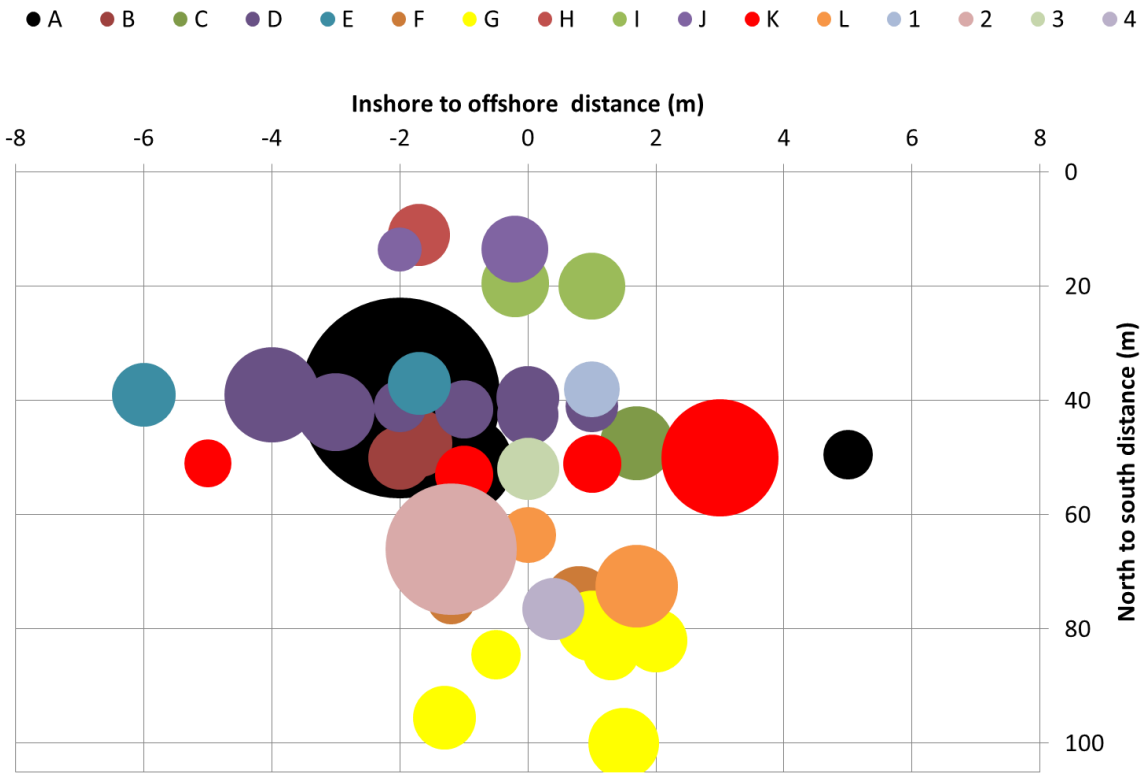

Figure 6 Spatial distribution of colonies of *Acropora austera* (49 samples/16 MLG) that shared an MLG at the SC_OFF site plotted on a map of TMR using the GPS and x- and y- coordinates of their location on their transect. This provides an example of the degree to which colonies that shared a unique multi-locus genotype (MLG) resulted from fragmentation and were therefore potential clones. Each colour/letter represents a unique MLG; circles are colonies and circle size represents colony size.

Population structure and genetic subdivision

Since the number of samples collected at the southern sites (i.e. S_OFF and S_IN) was low (<6), data from these two sites were pooled when calculating measures of genetic differentiation. There was no evidence to suggest that the presence of null alleles affected the observed patterns of genetic differentiation between the sites. This was evident after Friedman's t-tests were computed between per locus F_{ST} estimates with and without ENA correction (i.e. corrected for presence of null alleles) (Appendix E).

Acropora austera exhibited moderate and significant population structure on TMR (Table 7). On the one hand, estimates of genetic variation and differentiation among sites were all high and significant; correspondingly, pairwise comparisons between sites were mostly significant after FDR correction (Table 7). The Mantel test between geographic distance and genetic distance of

pair of sites revealed no correlation between the two (Mantel test: F_{ST}: r^2 = 0.001, P = 0.372, n = 8), suggesting distance alone is not responsible for any genetic structuring on TMR.

Table 7 Small-scale genetic structure and population differentiation estimates of populations of *Acropora austera* on TMR. a) Per locus estimates of genetic structure; G''_{ST} is Hedrick's standardized G_{ST}, further corrected for bias when number of populations is small (Peakall and Smouse 2006) and D_{EST} is the genetic divergence index proposed by Jost (2008). Upper (CIU) and lower (CIL) 95% confidence intervals and standard errors (SE) from bootstrapping and jack-knifing over loci are given. b) Pairwise genotypic differentiation between the sites; F_{ST} values below diagonal and D_{EST} above diagonal. *<0.05 and **<0.01 are significant pairwise comparisons after FDR. See Table 4 for description of site codes.

Locus	F_{ST}	G''_{ST}	D_{EST}
Microsatellite	0.220*	0.248*	0.071*
Amil2_07	0.149	0.122	0.006
Amil2_10	0.018	-0.025	0.000
Amil2_23	0.031	-0.034	0.000
EST14	0.097*	0.190*	0.131*
MS182	0.371*	0.580*	0.362*
Nuclear Intron	0.224*	0.411*	0.276*
Calm 2	0.366*	0.577*	0.387*
CAH 3-550	0.093	0.141	0.099
All loci (SE)	0.233* (0.058)	0.347* (0.103)	0.151* (0.088)
CIL - CIU	0.117 - 0.323	0.157 - 0.517	0.039 - 0.348
After removal of MS182 and Calm2			
All loci (SE)	0.050* (0.012)	0.026* (0.023)	0.010* (0.009)
CIL - CIU	0.028 - 0.062	-0.006 - 0.069	-0.001 - 0.042

b)

Site	N_OFF	C_OFF	SC_OFF	S_OFF+S_IN	N_IN	C_IN	SC_IN
N_OFF		0.043*	0.040**	0.022	0.194**	0.146*	0.233**
C_OFF	0.070**		0.003	-0.002	0.291**	0.214**	0.328**
SC_OFF	0.054**	0.028		0.011	0.275**	0.213*	0.309**
S_OFF+S_IN	0.048	0.032	0.035		0.273**	0.202*	0.305**
N_IN	0.156**	0.268**	0.249**	0.234**		0.026*	0.011
C_IN	0.115**	0.197**	0.187**	0.172**	0.036*		0.039**
SC_IN	0.185**	0.302**	0.281**	0.262**	0.022	0.044**	

On the other hand, the Bayesian non-spatial clustering analysis in BAPS revealed (with 99.6% probability) four genetically distinct clusters of *A. austera* on TMR (i.e. K = 4), while Structure detected two clusters (Figure 7, Appendix F). The best genetic partition identified by BAPS corresponded to N_OFF-S_OFF (Cluster 1), N_IN-SC_IN (Cluster 2) and S_IN-C_OFF-SC_OFF (Cluster 3); the fourth cluster corresponded to C_IN (Cluster 4) which had the largest stand of *A. austera* found on TMR. Similarly, the first cluster in Structure comprised the three inshore sites (i.e. N_IN, C_IN and SC_IN) while the second cluster consisted of all offshore and the S_IN sites (Figure 7). The spatial clustering of individuals and groups in each potential population genetic structure yielded similar patterns, supporting the initial clustering results of Structure and BAPS. Mixing of the different genetic populations occurs predominantly at the N_OFF, C_IN and SC_OFF sites; contrastingly, the N_IN, SC_IN, and S_IN sites are each dominated by a single genetic population (Figure 7). Taken together, there appears to be considerable genetic structure in *A. austera* populations on this reef with a clear inshore-offshore pattern.

The G-statistics and AMOVA analyses showed that the genetic structuring was significant. The G-statistics for both population structures were significant (P < 0.01), although those for Structure's two-cluster partition (F_{ST} = 0.097, 95% CI = 0.013, 0.222; G''_{ST} = 0.294, 95% CI = 0.030, 0.572; D_{EST} = 0.154, 95% CI = 0.010, 0.394) were predominantly higher than for the four-cluster partitioning suggested by BAPS (F_{ST} = 0.112, 95% CI = 0.038, 0.218; G''_{ST} = 0.218, 95% CI = 0.035, 0.407; D_{EST} = 0.106, 95% CI = 0.014, 0.246). The AMOVA analysis showed that the highest percentage of genetic variation in the population genetic structures on TMR occurred between the two-cluster partition identified in Structure (23.2%), followed by the four-cluster partition of BAPS (20.3%) and the inshore-offshore reef structure generated by sampling design (18.4%) (Table 8). The Friedman tests suggested that differences in rarefied allelic richness ($\chi^2_{(3)}$ = 1.308, P = 0.727) and expected heterozygosity ($\chi^2_{(3)}$ = 2.231, P = 0.526) between clusters occurred in neither the two-cluster (Data not shown) nor the four-cluster (Figure 7) partitions. Significant differences in mean colony size were only found in the four clusters identified by BAPS ($F_{(3, 289)}$ = 4.039, P = 0.007). Although the largest, single stand of *A. austera* on TMR fell in Cluster 4 (C_IN), the colonies were on average significantly smaller in this cluster than in Cluster 2 (N_IN_SC_IN) and cluster 3 (C_OFF, SC_OFF, S_IN) (Figure 8).

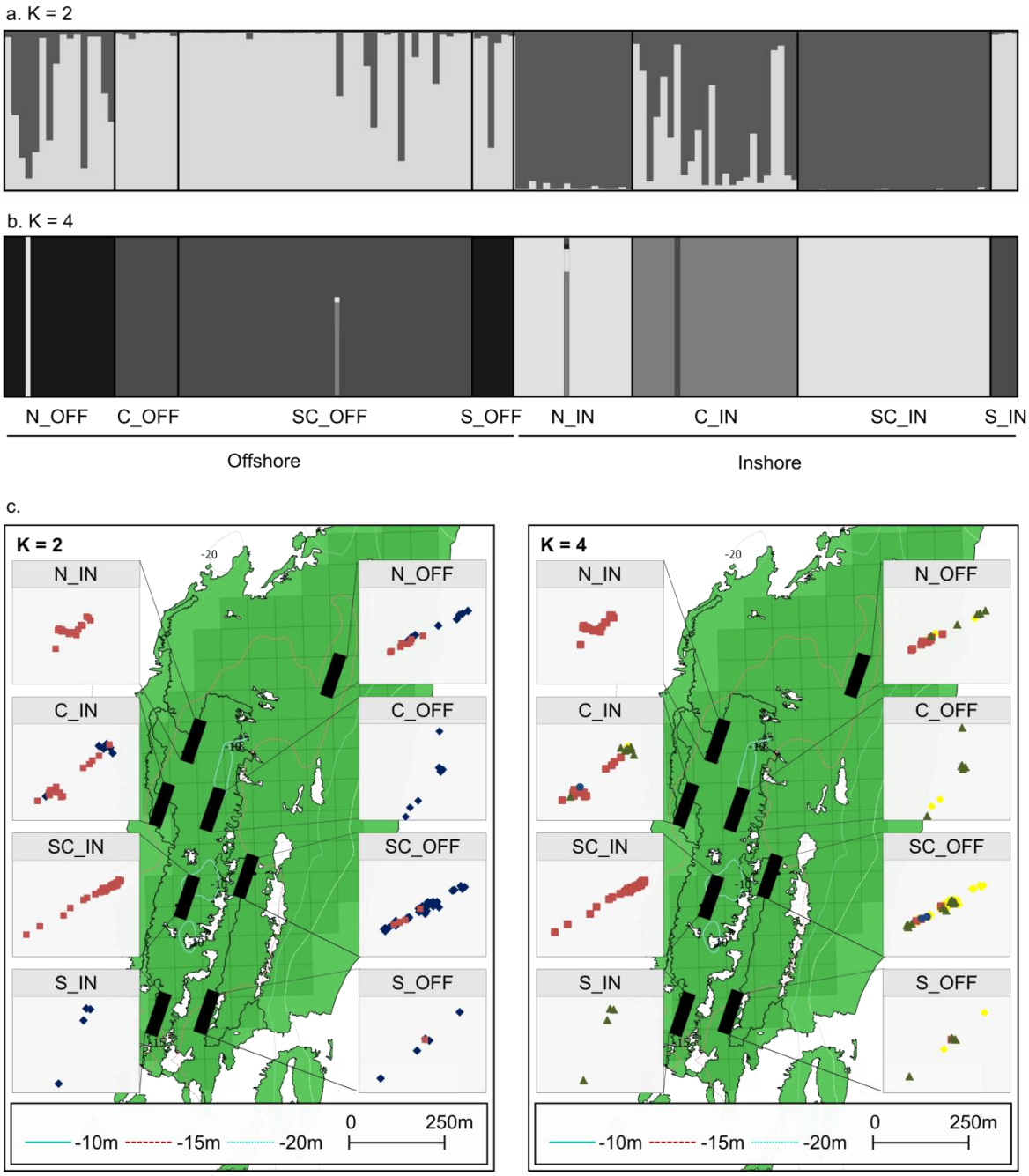

Figure 7 Small-scale genetic population structuring in *Acropora austera* on TMR estimated by Bayesian clustering analysis. a) Structure identified two clusters amongst the sampling sites while b) BAPS identified four. The BAPS spatial clustering of individuals assuming these two potential structures is depicted in c). Each point represents an individual colony and each colour represents a cluster. See Table 4 for description of site codes.

With regards to temporal genetic discontinuities, there appears to be a weak variation in allele frequencies among putative cohorts (i.e. size-classes). While none of the measures of genetic differentiation (F_{ST} = 0.015, 95% CI = 0.007, 0.022; G''_{ST} = 0.007, 95% CI = -0.010,

0.024; D_{EST} = 0.003, 95% CI = -0.005, 0.013) among size-classes were significant (all P <0.39), the AMOVA analysis and the pairwise comparison between size-class 0 (SC0) and size-class 1 (SC1) were significant (P <0.05) after FDR (Table 8).

Table 8 Estimates of genetic structure of *Acropora austera* on TMR. These are based on different potential population structures (F_{RT}) namely: a) inshore/offshore groups, b) groups designated by Structure 2.3.4, c) groups designated by BAPS 6, and d) size-classes (SC0 = immature; SC1 = young adults; SC2 = old adults). Also shown is the pairwise genotypic differentiation between the three size-classes. e) F_{ST} values below the diagonal and *P* values above the diagonal. *df*: degrees of freedom, %: percentage of variance, *P*: *P* value. *<0.05 significant after FDR.

a)

Source (F_{RT} = 0.184)	***df***	**%**	***P***
Among reef regions	1	18.4	0.001*
Among sites within reef regions	6	7.9	0.001*
Within colonies	286	73.7	0.001*

b)

Source (F_{RT} = 0.279)	***df***	**%**	***P***
Among Structure clusters (i.e. 2)	1	23.2	0.001*
Among sites within clusters	6	4.7	0.001*
Within sites	286	72.1	0.001*

c)

Source (F_{RT} = 0.228)	***df***	**%**	***P***
Among BAPS clusters (i.e. 4)	3	20.3	0.001*
Among sites within clusters	4	2.5	0.001*
Within sites	286	77.2	0.001*

d)

Source (F_{ST} = 0.012)	***df***	**%**	***P***
Among size-classes	2	1.2	0.016*
Among colonies within size-classes	144	19.3	0.001*
Within colonies	147	79.5	0.001*

e)

Size-class	**SC0**	**SC1**	**SC2**
SC0		0.012*	0.506
SC1	0.017		0.635
SC2	0.009	0.007	

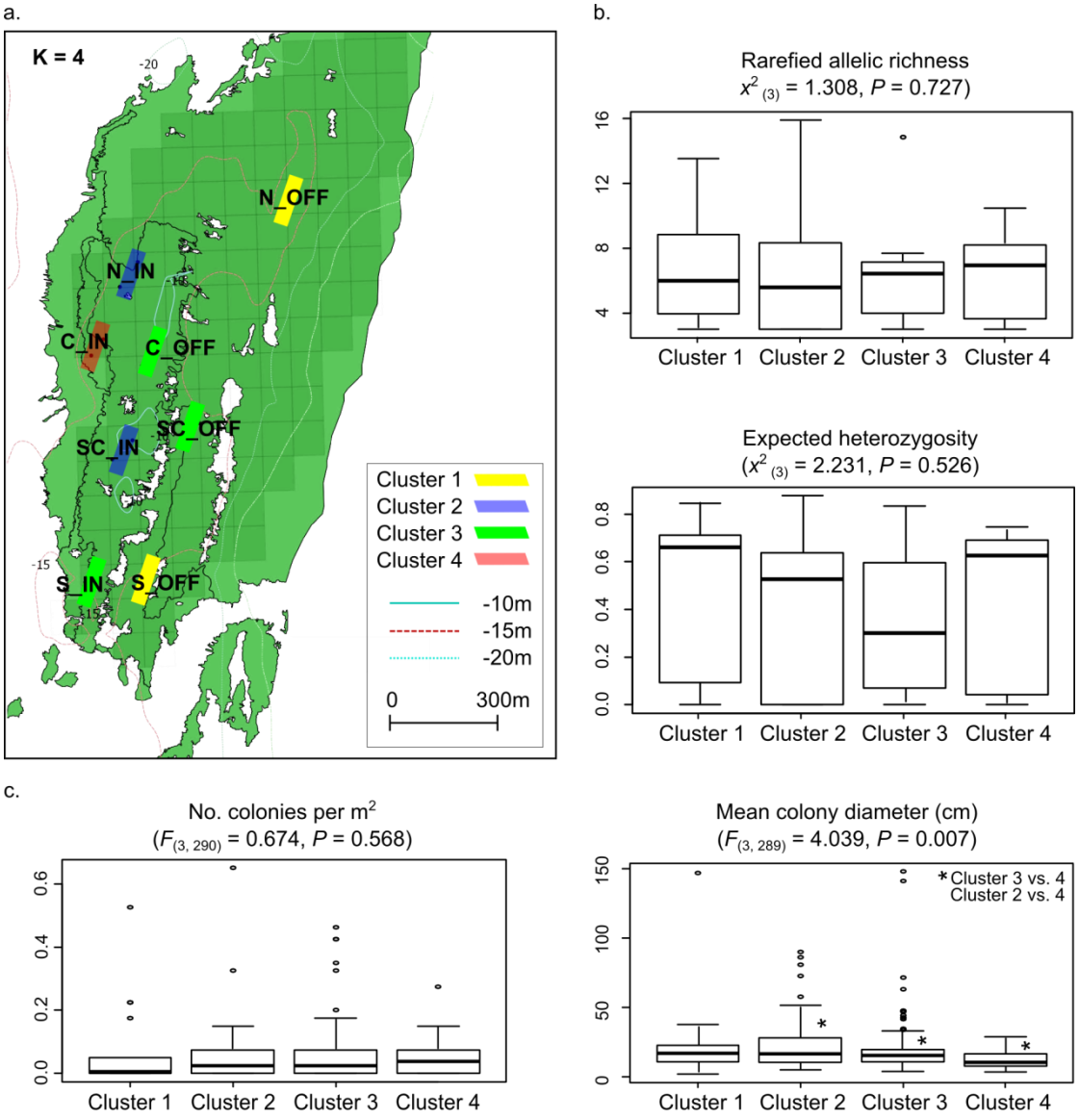

Figure 8 Population structure (a) of the four (*K* = 4) genetic populations of *Acropora austera* on TMR suggested by Bayesian spatial clustering of groups implemented in BAPS. Labels correspond to the sampling sites and colours to clusters (See Chapter 2 for a description of the sites). Box plots depict the distribution of genetic diversity (b) and population demographics (c) between clusters for *K* = 4; thick lines correspond to the median and whiskers represent the interquartile range.. * = Significant pairwise differences after FDR. See Table 4 for description of site codes.

Fine-scale spatial genetic structure and gene dispersal range

Indeed, the autocorrelation coefficient (r) estimated in GenAlEx and the kinship coefficient (Fij) calculated in SPAGeDi for the entire TMR were highly significant (*P* <0.001) (Table 9), which suggests that there is a non-random distribution of genotypes on TMR. These results help explain the considerable population structure and genetic subdivision found on TMR. Note that three sites

(C_OFF, S_IN and S_OFF) were excluded from the analysis of fine-scale spatial genetic structure (SGS) as they were represented by fewer than ten colonies each.

The spatial autocorrelation analyses detected significant spatial genetic structure (SGS) in *A. austera* within the 100 m transects on TMR (Figure 9). The autocorrelation coefficient *r* was significantly positive for the first distance class (*r* = 0.135 at 0–5 m), after which *r* decreased and then oscillated between its 95% confidence intervals; the r correlation coefficient only became significantly negative at the 30 - 50 m distance class. The plot of the *r* correlation coefficient of multiple distance classes of increasing size added evidence of non-random distribution of genotypes at TMR; *r* remained highest in the first distance class (0 to 5 m) and was still significant up to 40 m. The associations between individual-by-individual pairwise relatedness and spatial distances in GenAlEx were significant at individual sites (Table 9; Appendix 6), while the heterogeneity test was not significant (Omega = 33.7, *P* = 0.635), which suggests that the associations within distance classes were consistent across sites.

Wright's neighbourhood size (N_b) for *A. austera* at TMR was estimated to be ca 30 individuals, while the effective gene dispersal distance (σ) was found to be less than 15 m (Table 9). Although not all regression and iterative analyses converged, the few gene dispersal distances estimated by SPAGeDi were similar to those obtained from the spatial autocorrelation analyses in GenAlEx. The intercepts of the correlograms yielded a gene dispersal range of 5.6 to 35.8 m (Table 9). These estimates suggest that *A. austera* on TMR has a narrow dispersal distance, which appears to be less than 40 m and possibly explains the significant SGS observed in this species.

Table 9 Fine-scale spatial genetic structure and gene flow of *Acropora austera* on TMR and within sampling sites. Note that three sites were excluded from the analyses as they were represented by fewer than ten colonies each. F_{ij}: the average kinship coefficient between individuals in the first distance class; B_{log}: slope of regression of kinship coefficient on distance; N_b: Wright's neighbourhood size; CI: 95% confidence intervals; σ: estimate of effective gene dispersal distance inferred from N_b; first intercept: genetic patch size estimated from the first intercept that autocorrelation coefficient *r* falls from positive to negative. NC: not converged; *: significant at 0.05; **: significant at 0.01. See Table 4 for description of site codes.

Site	F_{ij}	B_{log}	N_b	95% CI	σ (m)	95% CI	First intercept (m)
N_IN	-0.051 (0.036)	0.011	NC	-	NC	-	17.0**
C_IN	0.060 (0.038)	-0.004	23.4	(16.7, 30.1)	13.4	(11.5, 15.4)	8.8**
SC_IN	0.049 (0.043)	-0.013*	NC	-	NC	-	8.6**
N_OFF	0.058 (0.036)	-0.063	10.0	(8.8, 11.1)	8.9	(8.3, 9.3)	35.8**
SC_OFF	0.150 (0.031)	-0.060**	NC	-	NC	-	9.4**
All sites	0.183 (0.063)	-0.015**	9.5	(7.1, 12.2)	8.6	(7.4, 9.8)	9.5**

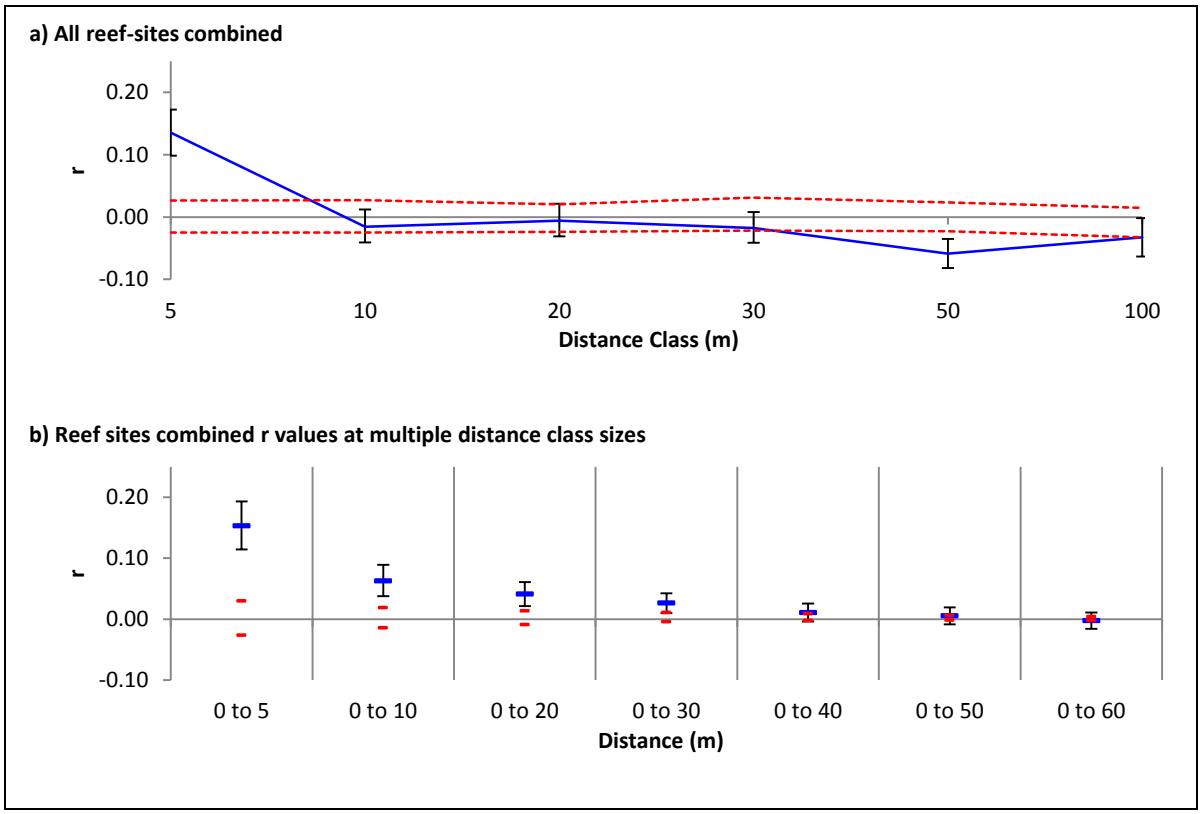

Figure 9 Spatial autocorrelation analysis of *Acropora austera* on TMR using GenAlEx. Dashed lines represent the 95% confidence intervals of a null hypothesis of no autocorrelation in 9999 permutations, and error bars delineate standard errors from jack-knifed estimates. (a) Combined correlogram plots for all sites depicting the individual-by-individual pairwise relatedness as a function of distance. (b) Multiple distance class plots, showing the influence of different distance classes on combined genetic correlation (*r*) across sites.

Factors influencing the small-scale genetic structure

Hierarchical partitioning (*HP*) showed that the location of a site, either inshore or offshore, on TMR made a large (50%) and significant contribution to the variance in local F_{ST} (Table 10). This is in agreement with the analyses of genetic variation and subdivision which revealed a significant inshore-offshore pattern in population structure. According to the other variables evaluated, including the mean colony size (MCD), mean population density (D) and latitude (LAT), only MCD was informative in the matter, accounting for 24% of the variation in local F_{ST}. Bootstrap correlations indicated that the local F_{ST} was negatively correlated with location (binomial key: inshore = 0, offshore = 1), suggesting that the genetic structure decreases towards the offshore side of the reef. Although the correlation was good (*rP* = -0.446), the 95% confidence interval of the correlation was not different from zero (Table 10). On the other hand, mean population density

made a significant contribution of >55% to the variation in local relatedness (*r*), while the other variables made contributions of <18%. The bootstrap correlations showed that there was a strong (*rP* = 0.733) and significant (95% CI = 0.035, 0.988) positive correlation between the density of *A. austera* and genetic relatedness (Table 10), indicating that colonies found at sites with a high abundance of *A. austera* were more genetically related.

Table 10 Table of hierarchical partitioning of genetic variation in *Acropora austera* from TMR, providing the percentage variance explained by individual variables (*I%*), variance explained by individual variables (*I*), variance explained together with other variables (*J*), total variance (Total) and *Z* score from the randomization procedure. Variables in bold are significant based on randomization (*Z* ≥ 1.65) and the 95% confidence limit. The bootstrapped Pearson correlation coefficient (*rP*) and its 95% confidence interval (CI) are also provided.

Variable	*I%*	*I*	*J*	Total	*Z*	*rP*	95% CI
Local F_{ST}							
Mean colony size	24.043	1.726	-1.541	0.185	0.160	-0.213	(-0.327,0.825)
Density	18.887	1.356	-0.572	0.784	0.190		
Location	**49.818**	**3.577**	**-2.692**	**0.886**	**2.300**	**-0.446**	(-0.968,0.416)
Latitude	0.957	0.069	-0.025	0.043	-1.000		
Depth	6.295	0.452	-0.446	0.006	-0.790		
Local *r*							
Mean colony size	10.330	0.776	1.069	1.845	-0.060		
Density	**54.652**	**4.105**	**-0.467**	**3.638**	**2.240**	**0.733**	(0.035,0.988)
Location	5.943	0.446	0.581	1.028	-0.540		
Latitude	17.149	1.288	-1.035	0.253	-0.330		
Depth	11.926	0.896	0.184	1.080	-0.330		

3.2 Discussion

In terms of geographic coverage and the number of genetic markers used, this study represents the most comprehensive assessment of genetic connectivity of a marine invertebrate on the south-east coast of Africa. The nine nuclear loci genotyped in this study have shown that *A. austera* from Reunion Island and along the south-east coast of Africa, appear to be comprised of several discrete populations of sexually produced individuals; which have varying genetic diversity that does not appear to follow a latitudinal gradient; significant levels of genetic structuring at large (ca 100 km) and fine (< 100 m) spatial scales; and limited contemporary gene flow between and within populations by non-random dispersal of coral larvae. The fact that similar patterns of genetic variation and subdivision were obtained using different statistical approaches and models (e.g.

mixture in BAPS and admixture in Structure) strengthens these findings. Concomitantly significant genetic variation and differentiation emerged, this despite low sample numbers at some locations and the high failure rates (i.e. microsatellites) and low polymorphism in the loci. Note that the *A. austera*-specific nuclear intron *CAH 3-550* and the microsatellite locus *EST14* which was developed from well-conserved coding sequences produced the highest estimates of genetic variation and differentiation (Table 5). Thus, an even stronger signal of limited contemporary genetic connectivity between south-east African populations of *A. austera* could be expected if a larger number of samples and additional markers were genotyped, particularly if new markers were developed for the polymorphism characteristic of the south-east African coral populations.

3.2.1 Large-scale (between-reef) genetic connectivity

Local populations of *A. austera* along the south-east African coast exhibited a recruitment mode and heterozygosity similar to other coral species (e.g. Underwood et al. 2007, Ridgway et al. 2008, Maier et al. 2009; *inter alia*) but a different pattern of genetic diversity. The populations receive a relative contribution of recruits from sexual reproduction at the reef level of 99% which is towards the high end of the range (65 – 100%) observed for other branching coral species sampled at a similar scale (i.e. colonies separated by ≥ 2 m), both in the region (*Pocillopora verrucosa*, ≥1 m apart Ridgway et al. 2001) and worldwide (*A. nasuta*, ≥5 m, Mackenzie et al. 2004; *A. tenuis*, ≥2 m, Underwood et al. 2009). Similarly, a general departure from HWE in the form of heterozygote deficits that were detected in this study is in agreement with previous studies on corals and this appears to be related to the occurrence of localized inbreeding and limited dispersal (discussed below; Mackenzie et al. 2004; Underwood et al. 2009; Underwood et al. 2007). On the other hand, genetic diversity in *A. austera*, measured as allelic richness and expected heterozygosity, did not decrease with increasing latitude as has been observed in previous studies conducted in the region (Ridgway et al. 2008; Macdonald et al. 2010) and at other locations (Underwood et al. 2009). In contrast, rarefied allelic richness was significantly higher at NMR, at the centre of the sampling area, while significantly lower at REU, the most tropical location. Although this departure from the latitudinal pattern can be attributed to stochastic variability resulting from the inclusion of different reefs, they might also reflect genetic variability related to recent changes in population demographics on individual reefs (Miller et al. 2009; Lowe et al. 2004). For instance, *A. austera* was once a dominant species on the reef-crest of FMR (Celliers & Schleyer 2007) but, during this study, the species was noticeably scarce on this reef; the reduction in the abundance of this species possibly explains the lower estimates of genetic diversity obtained for this reef (Lowe et al. 2004; Shearer et al. 2009), the second lowest encountered in the study.

Four genetically distinct populations (i.e. clusters) of *A. austera* were identified in the sampled area by Bayesian clustering analysis (Figure 5). These genetic populations exhibited moderate and significant levels of genetic structure (F_{ST} = 0.095) which appeared to be affected by distance, following a stepping-stone pattern which results from limited gene flow between distant locations due to the absence of continuous reef habitat (Vollmer & Palumbi 2006). Notwithstanding the above, the cluster arrangement detected here may also be related to the nature of reef development that is characteristic of the latitude at which the reefs are found. For instance, Reunion Island (Cluster 1) is located within the tropics which are characterized by high mean annual and seasonal temperatures and the high aragonite saturation state needed for coral growth (Kleypas et al. 1999). These optimal conditions decrease with increasing latitude, making southern Mozambican reefs and South African reefs more marginal in nature than their tropical counterparts.

Although the high estimates of migrants per generation (N_em) between southern Mozambican and South African reefs (Figure 5) are suggestive of considerable gene flow between these populations and explain their weak differentiation, results from the analyses of genetic diversity, genetic subdivision, assignment tests and the oceanic patterns in the region suggest that the level of connectivity is not that great at ecological time-scales. NMR had the highest mean number of private alleles (Figure 4), indicating that this reef is either isolated from the other reefs or that it has a source of gene flow that is different from Mozambican reefs. The south east coast of Madagascar is currently under consideration in this regard. Pairwise F_{ST} and D_{EST} comparisons between FMR and all other reefs were also significant. This suggests that gene flow from Mozambican and South African reefs to this reef has not been sufficient to reduce fixation of alleles (van Oppen & Gates 2006; Lowe et al. 2004) after a recent reduction in its population of *A. austera* (see Chapter 2 and Celliers & Schleyer 2007), despite healthy populations occurring to the north (Celliers & Schleyer 2001). Furthermore, no immigrants were assigned to FMR and few to the other reefs by the assignment tests. Similarly, the largest proportions of putative recruits sampled on TMR were assigned to the South African cluster and, more specifically, to TMR itself when independent reefs were analysed, providing evidence that most recruits are highly philopatric (Underwood et al. 2007; Saenz-Agudelo et al. 2009; Berumen et al. 2012). Therefore, these findings suggest that *A. austera* larvae disperse less than 100 km, if the geographic distance between the two closest genetic clusters (i.e. INH/NMR and SA) is considered, and do not periodically disperse over much greater distances (>100 km). The findings also add weight to the argument that the contemporary gene flow in broadcast spawning acroporid species (*A. palmata*, Baums et al. 2005; *A. digitifera* and *A. aspera*, Whitaker 2004; *A. tenuis*, Underwood et al. 2009) is restricted at meso (10s of kms) and large (100s of kms) scales; more importantly, the findings

support the genetic discontinuity suggested by Macdonald et al. (2010) and Ridgway et al. (2008) between South African and Mozambican coral populations at ecological time scales.

As in previous studies on genetic connectivity of coral communities in the region, this study did not sample the reefs south of Inhaca Island down to Kosi Bay at the Mozambique-South African border. This un-sampled stretch of coast extends ca 90 km and contains predominantly small patch reefs dominated by soft corals (POPMMP 2009). However, the reef known as Techobanine is 13km long and up to 56% of its cover at one time consisted of branching *Acropora* spp. (Robertson et al. 1996) which have undergone considerable degradation (MH Schleyer, unpub. data). Therefore, these reefs might well be acting as un-sampled source populations to the South African coral populations, possibly explaining the few migrants that were left unassigned (Piry et al. 2004) and the clustering of samples from INH and NMR.

A better explanation comes from the oceanographic current patterns found between 25°S and 27°S (between INH and NMR), an area where the waters of the Mozambique Channel Eddies (MCE) and eddies from the East Madagascar Current (EMC) are believed to meet and give rise to the Agulhas Current (Lutjeharms 2006; Lutjeharms et al. 2012; Ridderinkhof et al. 2013). As in this study, Macdonald et al. (2010) found that genetic diversity (expressed as haplotype diversity and H_e) in *A. austera* was highest at INH and RAB. In addition, genetic diversity in populations of the coral *Pocillopora verrucosa* (Ridgway et al. 2008) and the mangrove fiddler crab *Uca annulipes* (Silva et al. 2010) at Kosi Bay, the northernmost South African reef (not sampled in this study), was also shown to be higher than at other localities in the region. Therefore, it is possible that northern South African reefs may be acting as "landing-sites or sinks" for larvae that are transported from other locations besides Mozambique by the MCE and EMC systems (Bryden et al. 2005; Roberts 2006; Morris 2009) before they radiate to the rest of the south-east African reefs. This concept is supported by the Structure and BAPS plots (Figure 5) as they show co-occurrence rather than admixture of the identified genetic clusters. This would explain the higher levels of genetic diversity at these migratory "landing-sites" due to admixture (Macdonald et al. 2010), accounting for the large number of unassigned migrants on NMR encountered in this study, and the recurring disjunction in the ecological genetic connectivity of southern Mozambican and South African reefs (Ridgway et al. 2008, Macdonald et al. 2010; this study). Since the contribution of the MCE (5 x 106 m^3 s^{-1}) to the Agulhas Current is probably smaller than that of the EMC (20 x 106 m^3 s^{-1}) (Lutjeharms 2006), contemporary gene flow between South African reefs and those in southern Madagascar is presently being investigated to ascertain whether reefs from Madagascar are an important source of coral larvae for South African reefs.

3.2.2 Small-scale (within-reef) genetic connectivity

Results from this study suggest that the small-scale genetic connectivity of *A. austera* on TMR is influenced by environmental and demographic factors. For one thing, the two clusters identified by Bayesian clustering analysis in Structure corresponded to an inshore and offshore subdivision of the reef, which would account for up to 23% of the observed genetic variation on TMR (Table 8). An inshore – offshore subdivision on the reef would be expected due the local currents and a gradual change in environmental conditions (e.g. depth and surge-energy) as one moves away from the longitudinal axis of the reef (Chapter 2). Indeed, hierarchical partitioning (HP) analysis showed that location on the reef alone explained half (49.8%) of the variation in genetic structure on this reef. Interactions between the north-easterly and south-westerly winds that prevail in this region, reef topography, tidal flux and wave energy produce localised hydrodynamic conditions (Werner et al. 2007) that could result in the dispersal and settlement of larvae in a longitudinal pattern, with reduced cross-reef mixing of propagules. This would result in the genetic "isolation" of the inshore from the offshore regions of the reef.

Commonalities observed in the clustering analysis in BAPS, the ANOVA tests between clusters, and the HP analysis suggest that, in addition to physical factors, some biological and population-level factors also appear to be influencing the genetic structure of *A. austera* on TMR. For instance, the C_IN site had the largest mono-specific patch (7 x 12 m) of *A. austera* and BAPS identified the C_IN as a separate, genetically distinct cluster; the ANOVA tests showed that, once the large patch was removed, colonies at this site were on average significantly smaller, probably younger. Positive relationships between adult abundance or coral cover with coral recruitment have been found before in *Acropora* spp. (Suzuki et al. 2008; Suzuki et al. 2012) and pocilloporids (Nakamura & Sakai 2009); these relationships have been attributed to the concept that denser populations produce more larvae than those less dense, and to the fact that the presence of conspecifics, particularly adults, appear to constitute a cue which indicates to larvae that habitat conditions will favour settlement, survival and growth (Suzuki et al. 2008; Kingsford et al. 2002; Sponaugle et al. 2002; Nakamura & Sakai 2009). However, an inverse density-dependent settlement has been documented in some corals (see Vermeij & Sandin 2008). In this study, the density of *A. austera* varied between sites and genetic clusters were better represented at those sites where the species was more abundant relative to sites poorer in this species (Figure 7). In this regard, density alone explained 55% of the variation in the genetic relatedness between individual colonies, with the two variables showing a significant positive correlation. Lastly, the average coefficient of relatedness (0.183) suggested that the range of kinship relationships between closely spaced individuals on the reef could be interpreted as grandparent–grand-offspring, half sib, first cousin and great grandparent–great grand-offspring relationships (Blouin 2003). Considering this,

it is likely that, on TMR, larvae of *A. austera* prefer areas of the reef where conspecifics are abundant and also where relatives are found, which will explain the strong genetic subdivision found on this reef. A similar trend observed in *Acropora* corals on a Japanese reef (Suzuki et al. 2012). This also implies that there is non-random or limited dispersal of coral larvae on TMR (Bohonak 1999; Underwood et al. 2007; Lowe et al. 2004).

Indeed, the genetic discontinuity observed in *A. austera* at the finer scale (<100 m) and the significant spatial genetic structure (SGS) encountered in this study further suggest that this species exhibits non-random dispersal of its larvae on TMR. The spatial autocorrelation analyses and estimates of gene dispersal distances suggested that a significant number of *A. austera* coral larvae that settled within 40 m of each other are more related than could be expected by chance. Significant spatial genetic structure among colonies separated by <10 m has been previously encountered in a broadcast-spawning acroporid (*A. digitifera*); however, this was attributed to the inclusion of clone mates as the SGS disappeared once these were removed from the analysis (Stoddart 1988). In this study, putative fragments were excluded during sampling and the genetic analyses excluded putative clones; therefore, the fact that significant SGS was found may provide the first evidence of non-random dispersal at the finest spatial scale in an acroporid species that is not the result of fragmentation or asexual reproduction. This non-random dispersal also explains the predominance of heterozygote deficits (Underwood et al. 2007; Ridgway et al. 2008; Mackenzie et al. 2004) on TMR and other reefs, particularly because the presence of null alleles did not appear to influence the results and the SGS was constant across size-classes (see Results and Appendix D). Altogether, the results add to the growing evidence of non-random dispersal of larvae on coral reefs (Vollmer & Palumbi 2006, Levin 2006, Underwood et al. 2007, Constantini et al. 2007, Miller & Ayre 2008; *inter alia*).

Estimates of dispersal distances obtained by SGS analyses are commonly interpreted as natal (parent-offspring) dispersal; that is, the distance larvae will settle from their natal reef or parental colonies. However, in contrast to sessile brooding invertebrates (e.g. hard corals: *Seriatopora hystrix* Maier et al. 2005, Underwood et al. 2007; sponges: *Crambe crambe* Calderón et al. 2007; soft corals: *Corallium rubrum* Constantini et al. 2007, Ledoux et al. 2010) where limited dispersal of sexually-derived individuals at a scale of tens of meters has been documented, as well as philopatric dispersal of their larvae, evidence of limited dispersal at a similar spatial scale in broadcast-spawners like *A. austera* is scant. A more parsimonious explanation would be that propagules coming from the same spawning event upstream would be inherently related and could be transported together in currents and settle in group downstream; however, this explanation is not fully supported by the findings of this study (e.g. genetic discontinuity at large and small-spatial scales and higher assignment of individuals to their reef of origin).

Two alternative explanations emerge from a similar study on the broadcast-spawner *P. daedalea*. Miller and Ayre (2008a) found that the dispersal range in *P. daedalea* on the Great Barrier Reef (GBR) was ca 20 m which they related to larval behaviour or development (e.g. rapid settlement), or intense site-specific selection in this species. However, the authors found both explanations highly unlikely due to their knowledge of larval development in this species and the environmental conditions on the GBR. A minimum of 2.5 days pass between spawning and settlement (see Miller & Mundy 2003, Graham et al. 2008, Nozawa & Harrison 2008; *inter alia*) and the dynamic environment found on tropical reefs implies that larvae of broadcast-spawners must be transported long distances before they are ready to settle.

Notwithstanding the above, the explanations provided by Miller and Ayre (2008) may be valid on South African reefs due to the predominant environmental conditions found locally and the marginal nature of the reefs. The core of the Agulhas Current runs 200 km offshore (Lutjeharms 2006), suggesting that this is not the most important factor driving the direction of surface currents which ultimately will affect the dispersal of positively buoyant egg-sperm bundles as those produced by *A. austera* (Black et al. 1991). Wind patterns are a more likely factor to which the direction of surface currents is linked (Morris 2009). The climate and oceanography during the peak spawning period of broadcast-spawners at Sodwana Bay, January and February (Glassom et al. 2006), is characterised by strong southerly and south-easterly winds (8.8 – 11.1 m s^{-1}), a higher frequency of calms (periods of little water movement), current speeds between 0.11 and 0.20 m s^{-1} regardless of their direction, and wind-induced northward current reversals (Morris 2009). Given these conditions during the time of spawning, two potential scenarios or a combination of both would be plausible and would suggest that limited dispersal of coral larvae on TMR occurs. In the first, *A. austera* could time its spawning to coincide with periods of little water movement, something that has been observed at other localities (Suzuki et al. 2011; Babcock & Heyward 1986), and ensures the retention of coral larvae close to their natal colonies. In the second scenario, larvae of *A. austera* could be transported alongshore in one direction, e.g. by northerly or southerly winds, and then brought back in the other direction, e.g. by a wind reversal or subsurface currents after a minor buoyancy control, to the natal reef, resulting in an almost negligible net dispersal. Laboratory experiments suggest that *Acropora* larvae are ready to settle within two to five days of spawning (Szmant & Meadows 2006; Nozawa & Okubo 2011; Suzuki et al. 2011). Added to this, the ability of coral larvae to adjust their swimming pattern, velocity and depth (Pizarro et al. 2008; Gleason et al. 2009); to select particular settlement surfaces (Price 2010; Mason et al. 2011); the evidence that settling close to relatives may enhance fitness in corals (Amar et al. 2008); and, the influence of population density on relatedness found in this study, suggest that *A. austera* do not disperse widely.

Finally, chaotic (or fluctuating) genetic patchiness or sweepstakes recruitment occurs when processes like reproduction, settlement, recruitment and mortality vary considerably in space and time, resulting in genetic "*variation among sites that lacks clear geographic trends or shows temporal instability*" (Selkoe et al. 2010). Considering this, and the fact that the observed non-random dispersal of genotypes on TMR was always significant between sites and size-classes, may suggest that none of these processes varied substantially during the temporal scale of this study (two to three generations) and did not influence the predominant pattern of genetic diversity and population structure of *A. austera* on this reef (Botsford et al. 2009). Nonetheless, the AMOVA analysis between size-classes suggested the existence of a weak temporal genetic discontinuity in the sample. Coral bleaching and crown-of-thorns outbreaks (Schleyer & Celliers 2003b) are recent impacts documented at TMR that might have caused a reduction in the effective population size of *A. austera*; these impacts could have resulted in recent recruits being outsourced from neighbouring reefs and not from TMR itself (Coulon et al. 2006; Underwood et al. 2007; Wang et al. 2011), and could possibly explain the changes in allele frequencies observed between putative recruits (SC0) and young adults (SC1) (Becker et al. 2007; Pinsky et al. 2010). Cohort analysis has its limitations (e.g. old but fragmented colonies could have been classified as recruits or young adults). Thus, it is recommended that future studies include samples from different years and recruits collected from settlement tiles to ascertain the extent of temporal genetic variation of *A. austera* on TMR and the influence of environmental impacts to which the reefs are exposed.

CHAPTER 4

GENETIC CONNECTIVITY OF *PLATYGYRA DAEDALEA*

4.1 Results

4.1.1 Nuclear loci analysis

Multilocus genotypes (MLG) of 334 *P. daedalea* colonies from the twelve study reefs were obtained from seven nuclear markers (five microsatellites and two introns) (Table 11). The overall failure rate for nuclear loci averaged 4.3% (± 8.0 SD), and ranged from 0.61% (± 2.01 SD) for Pd62 to 10.6% (± 11.0) for Pd48. A chi-squared test suggested that the failure rates of nuclear loci differed between locations ($\chi^2_{(10)}$ = 30.1, P <0.05).Tests for selection also indicated that intron locus Calm 2 was likely to be under positive selection (Appendix H). As presented below, removal of this locus in the analysis for population subdivision (Table 12) reduced the G-statistic estimates (e.g. All loci: F_{ST} = 0.094, G''_{ST} = 0.098, D_{EST} = 0.041; excluding Calm 2: F_{ST} = 0.075, G''_{ST} = 0.061, D_{EST} = 0.024) but did not change the significance of the results (All P-values < 0.05; Table 12). Therefore, the following analyses include this locus.

4.1.2 Contribution of asexual reproduction and identification of putative clones

Of the total, 79% of the individuals with complete seven-locus genotype data (281 colonies) were identified as unique individuals or had a unique MLG. The $N_G:N_I$ values were high (mean 0.83), suggesting a low contribution of asexual reproduction and a prevalence of sexual reproduction in the populations (Table 11). The low polymorphism levels in the nuclear markers (see below) resulted in a high probability of identity (PI_{SIBS} = 0.04, i.e. 1 in 25 colonies will share the same MLG by chance) in the dataset. Indeed, 19 MLGs were shared among 78 colonies, while the overall number of colonies sharing the same MLG averaged 4.1 (± 3.07) and ranged between 2 and 17. Nonetheless, colonies sharing an MLG were treated as belonging to the same individual and included only once to avoid bias in subsequent analyses.

4.1.3 Between-reef genetic variation and connectivity

The following results are based on the analysis of a regional dataset of 259 samples of *P. daedalea* after the removal of colonies of size-class 0 and putative clones (See Chapter 2).

Table 11 Collection localities, MPAs, reefs or sites with an unique identifier (Code) and the number of samples (N) of *Platygyra daedalea* colonies with seven-locus genotypes (N_I), unique seven-locus genotypes (N_G), and the relative contribution of asexual reproduction (N_G:N_I)

Country	MPA/Reef/site	Code	N	N_I	N_G	N_G:N_I
Chagos Archipelago		CHA	17	7	6	0.86
Kenya	Mombasa Marine Park	KEN	4	2	2	1.00
Tanzania	Mtwara	TAN	13	4	4	1.00
Mozambique	Bazaruto Archipelago National Park	BAZ	18	14	14	1.00
	Pemba Bay	PEM	16	13	13	1.00
	Ilhas da Inhaca e dos Portugueses Faunal Reserve	INH	21	19	16	0.84
South Africa	iSimangaliso Wetland Park					
	Rabbit Rock Reef	RAB	19	17	17	1.00
	Nine-mile Reef	NMR	20	17	16	0.94
	Five-mile Reef	FMR	15	15	15	1.00
	Two-mile Reef	TMR	152	148	123	0.83
	Northern inshore	N_IN	24	24	22	0.92
	Central inshore	C_IN	19	19	17	0.89
	South central inshore	SC_IN	21	20	17	0.85
	South inshore	S_IN	18	16	15	0.94
	Northern offshore	N_OFF	17	17	16	0.94
	Central offshore	C_OFF	20	18	18	1.00
	South central offshore	SC_OFF	15	15	15	1.00
	South offshore	S_OFF	18	18	17	0.94
	Red Sands Reef	RED	20	16	16	1.00
	Leadmans Shoal Reef	LEA	18	9	9	1.00
	Total/Mean		334	281	251	0.96

Genetic diversity

Three (i.e. *Pd29-2, Pd48* and *Pd62*) of the five microsatellite loci genotyped in this study were monomorphic on some reefs. For instance, only one allele of locus *Pd29-2* was present in colonies from KEN, BAZ, INH, FMR and RED while only one allele of locus Pd61 was found in CHA, KEN and TAN. Overall, the average number of alleles per locus per reef in samples of a minimum size of eight individuals, excluding KEN as the sample comprised only three colonies, was homogeneous on all the reefs (mean 2.51 ± 0.27). However, it was evident from the rarefaction analysis that RAB and NMR had above average mean allelic richness, while CHA, the northern-most study reef, had the lowest allelic richness (A_r) of all the reefs (Appendix D; Figure 10). Similarly, NMR and RAB had the highest mean number of private alleles (Figure10), suggesting that these reefs are distinct from the others (Kalinowski 2004).

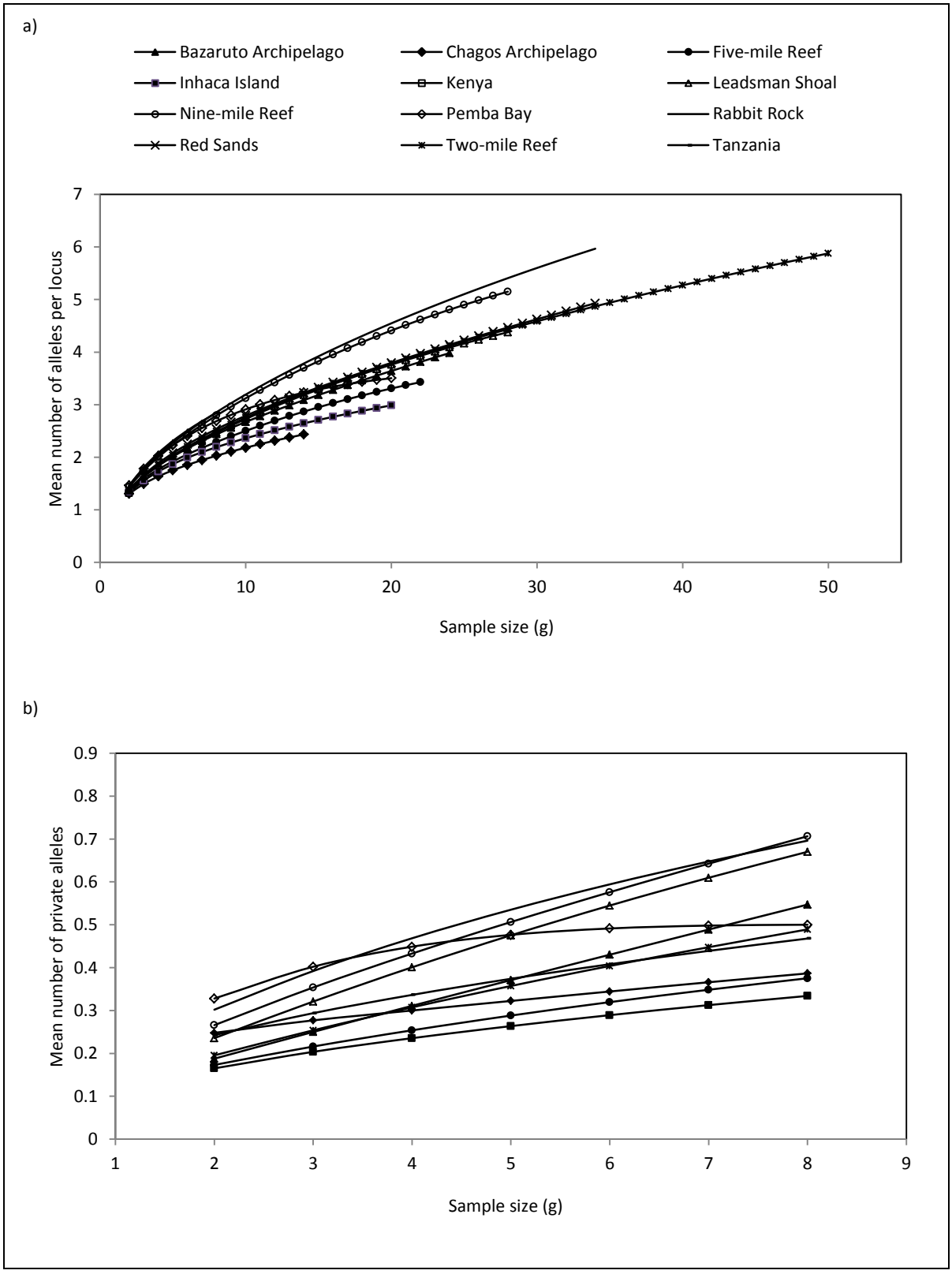

Figure 10 Genetic diversity of *Platygyra daedalea* (a. Allelic richness; b. Private allelic richness) as a function of standardized sample size for the twelve study reefs.

The average multilocus expected heterozygosity (H_e) was 0.369 (± 0.030 SE) and ranged between 0.262 - 0.449 (Appendix D). The overall multilocus inbreeding coefficient was 0.202 (± 0.040) and ranged from -0.104 to 0.390. Deviations from Hardy-Weinberg equilibrium (HWE) in the form of heterozygosity deficits were observed in 25 of the 60 tests conducted across all reefs and loci. Of these, only 19 deficits were significant after FDR correction. However, the overall test suggested a general deviation from HWE in the same form (Score U Test, P = 0.000). Linkage disequilibrium remained in only one pair of loci (*Pd29-2* and *Pd31*) after FDR correction.

Population structure and genetic subdivision

There was no evidence to suggest that the presence of null alleles affected the observed patterns of genetic differentiation between the reefs. This was evident after Friedman's tests were computed between per locus F_{ST} estimates with and without ENA correction (Appendix E). Correspondingly, F_{ST} estimates calculated with the ENA correction (i.e. corrected for presence of null alleles) were much higher than those calculated with the uncorrected data (Appendix E). Similarly, the analysis of microsatellite loci by Micro-checker did not detect null alleles, scoring errors or large allele dropout in the final dataset.

Measures of genetic differentiation in *Platygyra daedalea* (G-statistics) for all loci and reefs in the study area (pooling KEN and TAN together due to their small sample sizes) were high and significant (e.g. F_{ST} = 0.094, P < 0.01; Table 12), suggesting that there is moderate genetic discontinuity throughout the study area. Separate analyses of genetic markers revealed that genetic structuring in the population was stronger in intron than in microsatellite loci (Table 12). Of the 55 possible pairwise F_{ST} and D_{EST} comparisons between reefs, 26 were significant but only 18 remained significant (P < 0.05) after FDR (Table 12).

Table 12 Large-scale genetic structure in and population differentiation estimated for the south-east African reef populations of *Platygyra daedalea*. a) Per locus estimates of genetic structure; G''_{ST} is Hedrick's standardized G_{ST}, further corrected for bias when number of populations is small (Peakall and Smouse 2006) and D_{EST} is the genetic divergence index proposed by Jost (2008). Upper (CIU) and lower (CIL) 95% confidence intervals and standard errors (SE) from bootstrapping and jack-knifing loci are given. b) Pairwise genotypic differentiation between the reefs; F_{ST} values below diagonal and D_{EST} above diagonal. *<0.05 and **<0.01 are significant pairwise comparisons. Significant (P <0.05 after FDR) pairwise comparisons after FDR are in bold. Pairwise comparisons that remained or became significant after exclusion of samples from CHA are underlined (P <0.05; significance after FDR). See Table 11 for description of reef codes.

a)

Locus	Including CHA			Excluding CHA		
	F_{ST}	G''_{ST}	D_{EST}	F_{ST}	G''_{ST}	D_{EST}
Microsatellite	0.081**	0.068**	0.021**	0.079**	0.067**	0.020**
Pd29-2	0.068*	0.031*	0.003*	0.074*	0.039*	0.004*
Pd31	0.075**	0.124**	0.083**	0.072**	0.122**	0.082**
Pd48	0.092**	0.065**	0.014**	0.093**	0.070**	0.016**
Pd61	0.093**	0.116**	0.056**	0.089**	0.106**	0.050**
Pd62	0.028	-0.014	-0.001	0.026	-0.015	-0.001
Nuclear Intron	0.114**	0.217**	0.156**	0.050	0.001	0.004
ATPs	0.073*	0.106*	0.084*	0.048	-0.013	-0.011
Calm2	0.165**	0.300**	0.193**	0.052	0.017	0.010
All loci SE	0.094** (0.020)	0.093** (0.037)	0.041** (0.023)	0.065** (0.008)	0.043** (0.020)	0.018** (0.010)
CIL - CIU	0.072 - 0.133	0.043 - 0.172	0.011 - 0.106	0.052 - 0.081	0.008 - 0.080	0.004 - 0.040
After removal of Calm 2						
All loci SE	0.075** (0.003)	0.061** (0.014)	0.024** (0.012)	-	-	-
CIL - CIU	0.068 - 0.083	0.040 - 0.104	0.008 - 0.062	-	-	-

b)

Reef	CHA	TAN	PEM	BAZ	INH	RAB	NMR	FMR	TMR	RED	LEA
CHA		**0.140****	**0.168****	**0.157****	**0.147****	**0.190****	**0.123****	**0.105****	**0.146****	**0.144****	**0.130****
TAN	**0.194****		0.017	0.017	0.033	0.014	0.001	0.014	0.009	-0.014	0.012
PEM	**0.212****	**0.043***		0.027	**0.045***	**0.069****	0.031*	0.032*	**0.052****	**0.043***	**0.050***
BAZ	**0.232****	**0.043***	**0.050***		-0.011	0.010	-0.010	0.017	-0.001	0.006	0.019
INH	**0.242****	**0.073****	**0.080***	0.000		0.018	-0.014	0.032*	0.000	0.016	0.022*
RAB	**0.222****	**0.031***	**0.083****	0.020	**0.035***		0.001	**0.074****	0.012*	0.010	**0.030***
NMR	**0.169****	0.023	**0.051****	0.000	0.000	0.010		0.017	-0.006	-0.003	0.002
FMR	**0.177****	0.040*	**0.058****	**0.037***	**0.070***	**0.099****	**0.035***		**0.030****	0.023*	0.027*
TMR	**0.206****	**0.030***	**0.083****	0.003	0.007	**0.023****	0.000	**0.053****		-0.002	0.010*
RED	**0.208****	0.000	**0.067***	0.017	**0.037****	0.019*	0.006	**0.045***	0.001		0.005
LEA	**0.193****	**0.035***	**0.076***	**0.037****	**0.047****	**0.044****	0.014	**0.051****	**0.021****	0.014	

The genetic differentiation appeared to be influenced by the inclusion of samples from CHA, the farthest location (See Chapter 2). Firstly, pairwise F_{ST} and D_{EST} suggested most genetic differences were between CHA and all the other reefs (Table 12). Secondly, a Mantel test revealed a significant relationship between genetic and geographic distances when samples from CHA were incorporated (F_{ST}: r^2 = 0.68, P = 0.039; D_{EST}: r^2 = 0.60, P = 0.071; n = 55) but no significant when this location was excluded (Mantel test; F_{ST}: r^2 = 0.056, P = 0.155; D_{EST}: r^2 = 0.04, P = 0.232; n = 45). However, the permuted overall P-values of G-statistics were still significant when CHA was excluded from the test. Separate analyses of genetic markers revealed that G-statistics for the intron loci lost statistical significance upon the removal of CHA samples (Table 12). Similarly, some pairwise comparisons remained while other became significant after the removal of the CHA samples from the analyses (Table 12). Altogether, there appears to be genetic subdivision across subpopulations of *P. daedalea* in the region that caution them being considered as part of a single large random-mating population.

Indeed, Bayesian individual-based clustering analysis by Structure suggested that the clusters in the sample were most probably four in number (Appendix F) and it clearly identified CHA as a separate genetic population; however, the other three clusters corresponded to neither individual nor groups of reefs. Correspondingly, the non-spatial clustering analyses conducted in BAPS suggested the existence of five (i.e. 97.3 % probability of K = 5) genetically distinct populations in the sample and these corresponded to groups of neighbouring reefs. As in Structure analyses, BAPS identified CHA as a separate group (cluster 1). Contrastingly, KEN, TAN and PEM in northern Mozambique formed a second grouping (cluster 2), while the other two Mozambican reefs, BAZ and INH, were grouped as cluster 3; the fourth cluster comprised South African reefs excluding LEA, the southernmost reef, which formed the final cluster (Cluster 5). The G-statistics (F_{ST}: = 0.107, 95% CI = 0.051, 0.182; G''_{ST} = 0.159, 95% CI = 0.048, 0.326; D_{EST} = 0.068, 95% CI = 0.011, 0.186; all P <0.01) and pairwise cluster comparisons (all P values <0.05) showed that these BAPS clusters corresponded to distinct genetic populations with moderate genetic differentiation.

Two additional analyses of the dataset, one excluding the CHA samples (K = 4 with 98.2 % probability) and another on the South African reefs alone (K = 2 with 99.9 % probability), yielded no changes in the conformation of clusters, suggesting that the groups were well-structured. A Mantel test revealed that the BAPS clustering pattern conformed to a model of isolation-by-distance (IBD); the correlation between both genetic (F_{ST} and D_{EST}) and geographic distances between clusters was significant (Mantel test; F_{ST}: r^2 = 0.484, P = 0.048; D_{EST}: r^2 = 0.485, P =0.038; n = 10). There were no statistically significant differences in rarefied allelic richness ($\chi^2_{(4)}$ = 6.014, P = 0.198) and expected heterozygosity ($\chi^2_{(4)}$ = 7.396, P = 0.116) between

clusters (Figure 11); nonetheless, these two parameters of genetic diversity were on average higher in clusters 2 and 4. Taken together, these analyses suggest a stepping-stone model of population structure in *P. daedalea* along the south-east African coast, with two separate South African populations that are genetically distinct from those found on northern reefs.

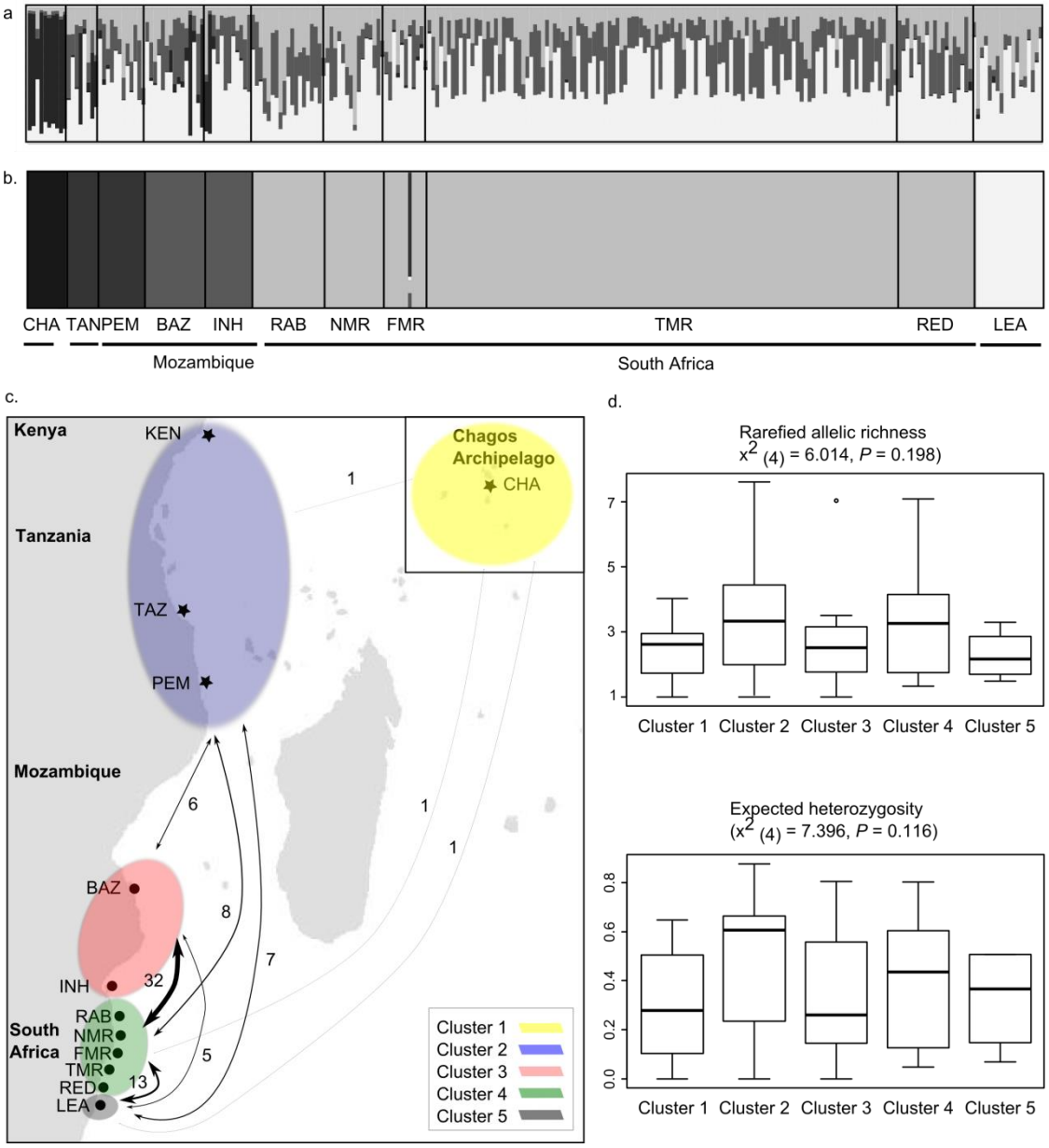

Figure 11 Large-scale genetic structure of *Platygyra daedalea* along the south-east African coast estimated by Bayesian clustering analysis. Structure (a) identified four clusters in the study area with no major correspondence to geographic areas. BAPS (b) also identified four clusters and the graph corresponds to the most probable partition. Long-term gene flow (arrows) and the estimated number of migrants per generation (N_em, numbers) between clusters (numbered and coloured areas) identified in BAPS are shown in (c). Box plots (d) depict the distribution of genetic diversity in each cluster; thick lines correspond to the median and whiskers represent the interquartile range. * = Significant pairwise differences after FDR. See Table 11 for description of reef codes.

Estimation of migration and gene flow

Estimates of the number of migrants per generation (N_em) suggest that there is long-term migration between populations of *P. daedalea* in the region, with up to 32 migrants per generation between southern Mozambican and South African reefs (Figure 11). In contrast, the lowest N_em values were found between CHA and the other reefs; the estimates of N_em between the former and the cluster formed by BAZ and INH were less than one, suggesting very weak, long-term gene flow between them (Figure 11).

The assignment tests revealed that the migration between Mozambican and South African reefs is not relevant at ecological time scales. Neither the migrants identified on South African reefs, nor the putative recruits sampled on TMR, were assigned to southern Mozambican reefs (Table 13). The five migrants that were detected in the South African reefs (i.e. two on RAB, one on NMR, one on TMR and one on LEA) were unassigned and there was <10% probability that they originated from any of the sampled genetic populations (i.e. clusters). Correspondingly, none of the putative recruits collected on TMR were excluded from the cluster containing TMR (Cluster 4: SA) or from TMR itself when the assignment test was conducted with each single reef as a reference population. Although these results might be due to the low polymorphism of the markers used, the average probability of belonging to Cluster 4 was higher (64% ± 20.1 SE) than to any other cluster whereas the probability of belonging to Cluster 3, the two southern Mozambican reefs, was low averaging only 12% (± 7.9 SE). All together, these results suggest that there is an important degree of self-recruitment in South African populations of *P. daedalea,* that migration from Mozambican reefs into South African is limited at ecological time scales, and that migrants come from an un-sampled source populations.

4.1.4 Within-reef genetic variation and connectivity

Demographics and size-class assignment

The density of *P. daedalea* on TMR varied significantly between sites (ANOVA $F = 4.47$, $df = 7, 36$, $P = 0.001$). The mean population density was 0.52 (± 0.21) colonies per m^2 and colonies were significantly more abundant at the N_OFF and S_IN sites but scarcer at the N_IN and C_IN sites. Similarly, the mean colony size varied significantly between sites (ANOVA $F = 4.28$, $df = 7, 330$, $P = 0.000$) and averaged 40.89 cm (± 3.27); colonies at the SC-OFF site were, on average, significantly larger than at the N_IN site. Of the 152 colonies that were genotyped, and based on estimates of size at first maturity (Appendix A), 77% were considered old colonies and assigned to size-class 2 (SC2, MCD >11 cm), 18% were considered young but mature colonies (SC1, 7 cm

>MCD <11 cm), and only 5% were considered immature colonies or putative recruits (SC0, MCD <7 cm).

Table 13 Immigrant colonies through larval dispersal, as estimated by using GENECLASS2 assignment test with 10 000 simulated individuals of *Platygyra daedalea*. The values in this table show the numbers of estimated immigrants from other sites. When an individual with a probability of exclusion (E) of 1% (*P* ≤0.01) belonged to another population (i.e. cluster) at 10% or more (*P* ≥0.1), the individual was assigned to the highest-probability site as an immigrant; when lower, the individual was left unassigned (U). Only reefs were immigrants were detected are shown. See Table 11 for description of reef codes.

Sampling reef	E	Assigned cluster					U
		CHA	KEN/TAN/PEM	BAZ/INH	SA	LEA	
CHA (N=10)	1	-	1	-	-	-	-
PEM (N=12)	1	-	-	-	-	-	1
BAZ (N=15)	4	-	2	-	-	-	2
RAB (N=18)	2	-	-	-	-	-	2
NMR (N=15)	1	-	-	-	-	-	1
TMR (N=118)	1	-	-	-	-	-	1
LEA (N=17)	1	-	-	-	-	-	1
Total (N=259)	11	-	3	-	-	-	8
Putative recruits							
No. of recruits (N=7)	-	-	-	-	7	-	-

Genetic diversity

No more than three colonies shared the same MLG at any sampling site on TMR and such colonies were always more than 14 m apart. Nonetheless, colonies sharing an MLG were treated as belonging to the same individual and included only once to avoid bias in subsequent analyses.

The mean number of alleles per locus per site, in a minimum sample size of 13 individuals, was 4.27 (± 0.45 SE); however, loci were not polymorphic at all sites (Appendix D). Microsatellite locus *Pd29-2* was monomorphic at all sites except S_OFF and N_IN; *Pd62* was monomorphic at SC_OFF, N_IN and SC_IN; *Pd48* was monomorphic at SC_OFF. The mean genetic diversity (H_e) per site over loci averaged 0.36 (± 0.04), which suggests that TMR is homogeneous in terms of the genetic diversity of *P. daedalea* while the wider range (0.36 for S_IN to 1.20 for N_IN) in the mean number of private alleles suggests some degree of genetic isolation at some sites (Appendix D). Deviations from HWE were observed in 11 of the 56 tests; however, the overall test suggested a general deviation from HWE (Appendix D).

A decrease in size-class mean allelic richness (A_r: 2.96 ± 0.55 to 2.29 ± 0.56), mean number of private alleles (A_p: 0.93 ± 0.45 to 0.32 ± 0.20) and mean genetic diversity (H_e: 0.38 ± 0.11 to 0.26 ± 0.11) was observed from SC2 (i.e. largest colonies, old adults) to SC0 (i.e. smallest colonies, putative recruits) (Appendix D); the inverse was observed in the per size-class inbreeding coefficient F_{IS}, which increased from SC2 (F_{IS}: 0.20 ± 0.07) to SC0 (F_{IS}: 0.31 ± 0.23). All size-classes except SC0 were in Hardy-Weinberg disequilibrium in the form of heterozygosity deficits (Appendix D). Nevertheless, these variations in genetic diversity measures between size-classes were not significant; Friedman's test revealed that the mean allelic richness ($\chi^2_{(2,7)}$ = 4.222, P = 0.121), mean private allelic richness ($\chi^2_{(2,7)}$ = 3.185, P = 0.203), mean H_e ($\chi^2_{(2,\ 7)}$ = 2.296, P = 0.317) and inbreeding coefficients (F_{IS}; $\chi^2_{(2,\ 7)}$ = 0.667, P = 0.716) were statistically homogeneous across size-classes. A coupled analysis of size-classes per site revealed similar patterns of genetic diversity (data not shown).

Population structure and genetic subdivision

There was no evidence to suggest that the presence of null alleles affected the observed patterns of genetic differentiation between the sites. This was evident after Friedman's *t*-tests were computed between per locus F_{ST} estimates with and without ENA correction (i.e. corrected for presence of null alleles) (Appendix E).

Whether patterns in the population structure of *Platygyra daedalea* were present at TMR was inconclusive. On the one hand, the G-statistics across sites were small and all permuted *P*-values were insignificant. Nevertheless, none of the confidence intervals of G-statistics overlapped zero, indicating that the estimates of genetic structuring were different from zero (Table 14). Indeed, significant pairwise comparisons between sites suggested that there were differences between sites, particularly between S_IN and the other sites; however, statistical significance was lost after FDR correction (Table 14). Correspondingly, the Mantel test between geographic and genetic distances of pairs of sites revealed that these were significantly correlated (Mantel test: r^2 = 0.225, P = 0.012; n = 8), suggesting the existence of isolation-by-distance at the reefal scale. Altogether, the results suggest that *P. daedalea* on TMR does not necessarily comprise a single homogeneous genetic population as there is a marginal degree of fixation of alleles between sites.

Table 14 Small-scale genetic structure and population differentiation estimated for populations of *Platygyra daedalea* on TMR. a) Per locus estimates of genetic structure; G''_{ST} is Hedrick's standardized G_{ST}, further corrected for bias when number of populations is small (Peakall and Smouse 2006) and D_{EST} is the genetic divergence index proposed by Jost (2008). Upper (CIU) and lower (CIL) 95% confidence intervals and standard errors (SE) from bootstrapping and jack-knifing over loci are given. *<0.05 and **<0.01 are significant pairwise comparisons. Significance (*P* <0.05) after FDR is bold. See Table 11 for description of site codes.

Locus	F_{ST}	G''_{ST}	D_{EST}
Microsatellite	0.036	0.007	0.002
Pd29-2	0.040	0.007	0.000
Pd31	0.032	0.019	0.012
Pd48	0.028	-0.006	-0.001
Pd61	0.039	0.005	0.002
Pd62	0.066	0.031	0.002
Nuclear Intron	0.047	0.022	0.014
ATPs	0.047	0.028	0.022
Calm	0.047	0.021	0.011
All loci SE	0.041 (0.004)	0.010 (0.003)	0.004 (0.002)
CIL - CIU	0.034 - 0.047	0.004 - 0.016	0.001 - 0.009

b)

Site	N_OFF	C_OFF	SC_OFF	S_OFF	N_IN	C_IN	SC_IN	S_IN
N_OFF		-0.007	0.007	0.009	0.001	0.013*	0.011	0.015
C_OFF	0.000		0.002	0.004	-0.015	-0.007	-0.008	0.008
SC_OFF	0.020	0.012		0.001	0.004	0.009	0.010	-0.006
S_OFF	0.025*	0.016	0.008		0.010	0.001	0.003	0.010
N_IN	0.009	0.000	0.014	0.024*		0.001	-0.007	0.013
C_IN	0.034*	0.000	0.025	0.011	0.011		-0.005	0.020
SC_IN	0.029	0.000	0.025	0.015	0.000	0.003		0.013
S_IN	0.032*	0.021	0.000	0.023*	0.026*	0.039**	0.028*	

On the other hand, the Bayesian non-spatial clustering of groups of individuals in BAPS failed to detect genetic structure in the *P. daedalea* samples (K = 1 with 99.5% probability) from TMR. Contrastingly, the individual-based non-spatial clustering analysis in Structure identified two clusters (K = 2; Appendix F). However, these did not clearly correspond to the geographic location of the sites and very few individuals were strongly assigned to one population or another (mean probability of membership to Cluster 1: 0.649 ± 0.006 SE, range: 0.459-0.775; Cluster 2: 0.351 ± 0.006 SE, range: 0.225-0.542) (Figure 12). Similarly, the AMOVA analyses showed that

the a priori inshore-offshore division was negligible, explaining none of the genetic variation in the samples (F_{RT} = 0.001, P = 0.638; Table 15).

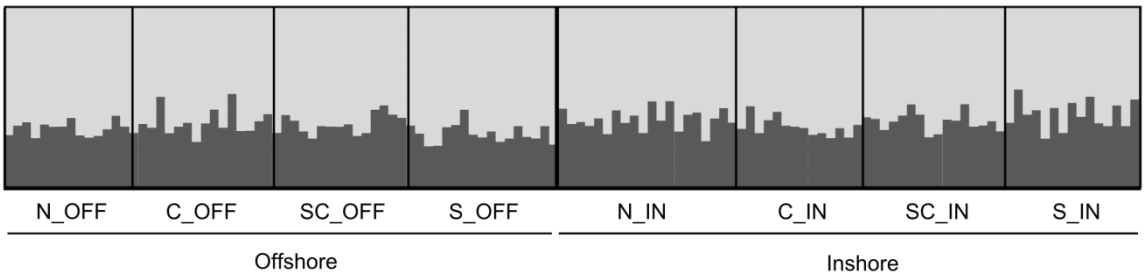

Figure 12 Small-scale genetic structure of *Platygyra daedalea* on TMR estimated by Bayesian clustering analysis. The two clusters identified using Structure. Labels correspond to the sampling sites and colours to clusters (See Chapter 2 for description of the sites).

Table 15 Estimates of genetic structure in *Platygyra daedalea* on TMR based on the potential population structure a) inshore/offshore groups. Also shown are the AMOVA results (b) among size-classes (SC0 = immature; SC1 = young adults; SC2 = old adults) and the pairwise genotypic differentiation between the three size-classes (c): F_{ST} values below the diagonal and *P* values above the diagonal. *df*: degrees of freedom, %: percentage of variance, *P*: *P* value. *<0.05 significance after FDR.

a)

Source (F_{RT} = 0.001)	***df***	**%**	***P***
Among reef regions	1	0.0	0.553
Among sites within reef regions	6	1.5	0.002*
Within sites	246	98.5	0.006*

b)

Source (F_{ST} = 0.007)	***df***	**%**	***P***
Among size-classes	2	0.7	0.058
Among colonies within size-classes	117	99.3	

c)

Size-class	**SC0**	**SC1**	**SC2**
SC0		0.023*	0.166
SC1	0.053		0.299
SC2	0.012	0.000	

The lack of genetic population structure observed between sites was also reflected in the analysis between putative cohorts (i.e. size-classes). Measures of genetic differentiation between size-classes were not significant, although higher than between sites (F_{ST} = 0.039, 95% CI = 0.014, 0.074; G''_{ST} = 0.026, 95% CI = -0.033, 0.106; D_{EST} = 0.009, 95% CI = -0.009, 0.050; all P >0.05). Similarly, the AMOVA analysis revealed small and not significant genetic variation between size-classes, with only 0.7% of the genetic variation assigned to between size-classes and 99.3% between colonies within the size-classes (Table 15). Only the pairwise comparison between size-classes SC0 and SC1 was significant after FDR correction.

Fine-scale spatial genetic structure and gene dispersal range

The spatial autocorrelation analyses failed to detect significant spatial genetic structuring (SGS) in *P. daedalea* populations on TMR. Both the overall kinship coefficient (F_{ij}) calculated using SPAGeDi and the autocorrelation coefficient (r) estimated using GenAlEx were insignificant (P >0.05) for the entire reef (Table 16), which also suggested that the distribution of genotypes on TMR is random and possibly explains the weak population structure and genetic subdivision found for this species on TMR.

The spatial autocorrelation analyses similarly failed to detect SGS in *P. daedalea* within the 100 m transects on TMR (Figure 13). The autocorrelation coefficient r oscillated within its 95% confidence interval throughout the distance classes; a similar pattern was observed in the plot of r coefficients with respect to distance classes of increasing size (Figure 13). The random distribution of genotypes was consistent across sites, this being indicated by the lack of significance in the heterogeneity test (Omega = 19.4, P = 0.092).

The associations between pairwise relationships and spatial distances were not significant at any of the sites and only the regression and iterative analyses for the S_OFF site converged (Table 16). The average kinship coefficient (F_{ij}) between individuals in the first distance class ranged from -0.071 at N_OFF to 0.040 at SC_OFF and averaged 0.183 across sites (Table 16). A Wright's neighbourhood size (N_b) of ca 45 individuals and an effective gene dispersal distance (σ) of ca 10 m can be estimated for *P. daedalea* at TMR. The intercepts of the correlograms drawn by GenAlEx yielded a gene dispersal distance of between 7.5 – 28.7 m (Table 16).

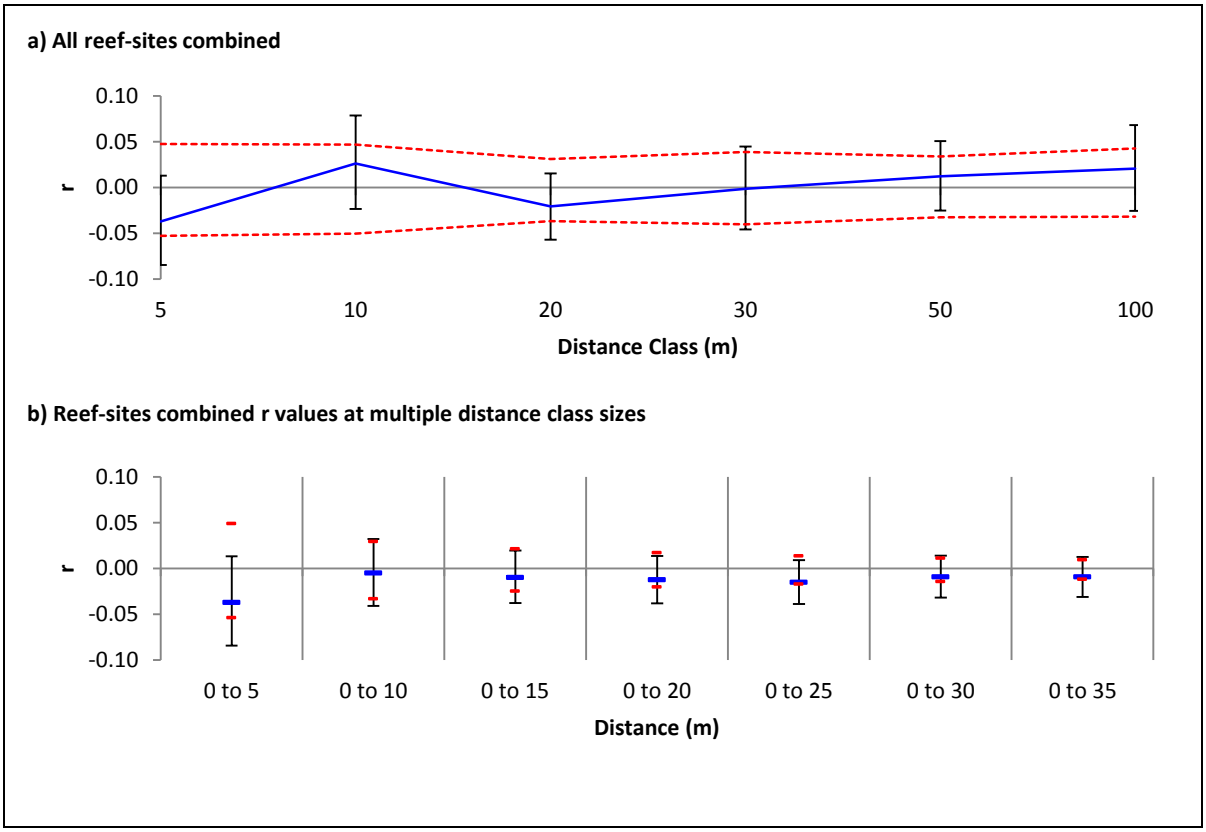

Figure 13 Spatial autocorrelation analysis of *Platygyra daedalea* at TMR using GenAlEx. Dashed lines represent the 95% confidence intervals that a null hypothesis of no autocorrelation exists in 9999 permutations, and error bars delineate standard errors from jack-knifed estimates. (a) Combined correlogram plot for all sites depicting the individual-by-individual pairwise relatedness as a function of distance. (b) Multiple distance class plot showing the influence of different distance classes on combined genetic correlation (*r*) across sites.

Table 16 Fine-scale spatial genetic structure and gene flow of *Platygyra daedalea* within TMR and within sampling sites. F_{ij}: the average kinship coefficient between individuals in the first distance class; B_{log}: slope of regression of kinship coefficient on distance; N_b: Wright's neighbourhood size; CI: 95% confidence intervals; σ: estimate of effective gene dispersal distance inferred from N_b; first intercept: genetic patch size estimated from the first intercept that autocorrelation coefficient r falls from positive to negative. NC: not converged; *: significant at 0.05. See Table 11 for description of site codes.

Site	Fij	Blog	Nb	95% CI	σ (m)	95% CI	First intercept (m)
N_IN	-0.069 (0.070)	0.015	NC	-	NC	-	14.5
C_IN	-0.058 (0.018)	-0.044	NC	-	NC	-	19.1
SC_IN	0.040 (0.018)	0.010	NC	-	NC	-	13.4
S_IN	-0.035 (0.027)	-0.014	NC	-	NC	-	16.4
N_OFF	-0.071 (0.062)	0.022	NC	-	NC	-	26.5
C_OFF	-0.023 (0.021)	0.032	NC	-	NC	-	-
SC_OFF	-0.034 (0.044)	0.004	NC	-	NC	-	28.7
S_OFF	0.034 (0.084)	-0.002	45.1	NC	9.7	NC	7.5
All sites	-0.005 (0.072)	0.005	NC	-	NC	-	15.6

Factors influencing the small-scale genetic structure

Hierarchical partitioning (*HP*) revealed that mean population density (D) contributed 34.5% to the variation in local F_{ST} and location of the site, either inshore or offshore, 23.7%, although the results were not significant (Table 17); the other variables explained less than 20% each of the variation in local F_{ST}. The bootstrap correlations indicated that local F_{ST} was positively correlated with density (*rP* = 0.443), although not statically significant. The bootstrap correlations between local F_{ST} and location of the site were very low and not significant (Table 17). Correspondingly, the *HP* analysis revealed that mean colony size (32.5%), depth (29.3%) and latitude (28.6%) contributed substantially to the variation in local relatedness (*r*), although none of the results were statistically significant. The bootstrap correlations indicated that mean colony size in *P. daedalea* and local relatedness (*r*) were positively (*rP* = 0.622) and significantly (95% CI = 0.242, 0.936) correlated (Table 17). Similarly, the bootstrap correlations between depth and local relatedness (*r*) indicated a negative (*rP* = -0.348) and significant (95% CI = -0.958, - 0.272) correlation between the two variables. Together, these results suggest that colonies of *P. daedalea* on TMR are more genetically related at shallower sites and at sites where larger colonies predominate. Note that site depth and mean colony size were not correlated (Data not shown). The bootstrap correlations between local relatedness (*r*) and latitude were negative and strong (*rP* = -0.700) yet not significant (Table 17).

Table 17 Table of hierarchical partitioning of genetic variation in *Platygyra daedalea* from TMR, providing the percentage variance explained by individual variables (I%), variance explained by individual variables (*I*), variance explained together with other variables (*J*), total variance (Total) and *Z* score from the randomization procedure. The bootstrapped Pearson correlation coefficient (*rP*) and its 95% confidence interval (CI) are also provided.

Variable	I%	I	J	Total	Z	rP	95% CI
Local F_{ST}							
Mean colony size	18.049	0.799	-0.589	0.210	1.22		
Density	34.481	1.527	-0.651	0.876	-1.01	0.443	(-0.197,0.916)
Location	23.731	1.051	-1.046	0.005	-0.53	0.036	(-0.650,0.761)
Latitude	4.401	0.195	-0.017	0.178	1.12		
Depth	19.338	0.856	-0.674	0.182	0.51		
Local r							
Mean colony size	32.463	2.811	-0.852	1.959	-0.23	0.622	(0.242,0.936)
Density	1.430	0.124	-0.093	0.031	0.83		
Location	8.151	0.706	-0.648	0.057	0.03		
Latitude	28.639	2.480	0.209	2.690	-0.80	-0.700	(-0.951,0.710)
Depth	29.318	2.539	-2.022	0.517	-0.17	-0.348	(-0.958,-0.272)

4.2 Discussion

The seven nuclear loci genotyped in this study have revealed that populations of *P. daedalea* on the east coast of Africa appear to be comprised of several discrete populations of sexually produced individuals; manifest varying genetic diversity that does not appear to follow a latitudinal gradient; are characterised by significant levels of genetic structuring at large (ca 100 km) spatial scales; and have limited contemporary gene flow between populations. However, these findings should be considered with caution as failure rates of nuclear markers varied between reefs which might be due to the existence of cryptic species, a plausible scenario that has been considered in similar studies on this species in the region (e.g. Souter & Grahn 2008; Macdonald et al. 2012) due to a lack of genetic distinction between morphotypes (Mangubhai et al. 2007). At small (10 -1000 m) spatial scales the patterns of genetic structure were unclear and its presence as a result of limited dispersal of *P. daedalea* larvae could not be confirmed. This may be an effect of the low levels of allelic diversity that characterised the microsatellite loci screened in this study. Nonetheless, the patterns of genetic variation obtained on TMR suggest that the dispersal of larvae of this species on TMR might be driven by demographic (e.g. population density, presence of adults) and habitat cues (e.g. depth).

4.2.1 Large-scale (between-reef) genetic connectivity

The mean relative contribution of sexual reproduction (96%) to populations of *P. daedalea* along the south-east African coast and the general departures from HWE in the form of heterozygote deficits that were observed in this study are in agreement with previous studies on corals (e.g. Mackenzie et al. 2004, Underwood et al. 2007, 2009, Ridgway et al. 2008, Macdonald et al. 2010). As was the case with *A. austera* (Chapter 3), the genetic diversity of *P. daedalea* (measured as allelic richness and expected heterozygosity) did not decrease with increasing latitude, which contrasts with that observed in previous studies conducted in the region (Ridgway et al. 2008, Macdonald et al. 2010) and other locations (Underwood et al. 2009). Correspondingly, its allelic richness was lowest at CHA and other tropical locations compared to the subtropical locations in this study (Figure 9). This departure from the expected latitudinal pattern may be attributable to stochastic variability or it could be explained by recent changes in population demographics on individual reefs (Chapter 3; Lowe et al. 2004; Miller et al. 2009). However, it is unlikely that *P. daedalea* undergoes significant demographic fluctuations (i.e. population density) given its high

resilience to natural threats like bleaching (Baird & Marshall 2002; Celliers & Schleyer 2002); an alternative explanation is provided later.

Five genetically distinct populations (i.e. clusters) of *P. daedalea* were identified in the study area by Bayesian clustering analysis (Figure 11). These genetic populations exhibited weak (F_{ST} = 0.065; G''_{ST} = 0.043) but statistically significant levels (F_{ST}: 95% CI = 0.052, 0.081; G''_{ST}: 95% CI = 0.008, 0.080) of genetic structure which appeared to follow a stepping-stone pattern resulting from limited gene flow between distant locations due to the absence of continuous reef habitat (Vollmer & Palumbi 2006); the genetic pattern also appeared to follow present-day oceanic currents. The major oceanographic features in the South West Indian Ocean are the east-flowing South Equatorial Current (SEC) which feeds around northern Madagascar generating the north-flowing East African Coastal Current (EACC) and the south-flowing Mozambique Channel Eddies (MEC) system (Swallow et al. 1991). At the north-eastern end of Madagascar, waters of the SEC also generate the south-flowing East Madagascar Current (EMC). The latter and the MEC are believed to give rise to the Agulhas Current which impinges on the northern South African reefs (Lutjeharms 2006; Lutjeharms et al. 2012). Considering this, it is hypothesized that propagules of *P. daedalea* are sporadically transported westward from CHA and other eastern reefs and distributed to the reefs along the east African coast by the current systems fed by the SEC, with Madagascar acting as a barrier to more direct gene flow to the Mozambican reefs. The long-term migration between CHA and the other genetic populations identified in this study; the negligible number of migrants per generation found between CHA and the BAZ-INH clusters; and the lower allelic richness in the latter cluster compared to its proximal clusters (Figure 11) support this hypothesis.

Genetic differentiation was found between RAB and LEA (Table 12), the latter having the third largest number of private alleles after RAB and NMR, and samples from LEA yielded a cluster separate and different from the rest of the South African reefs. These results mirror the findings of Macdonald et al. (2010) obtained for *A. austera*, who attributed this to chance recruitment and the same explanation may apply to this study. The differentiation of LEA from the rest of South African reefs can also be explained by a founder effect (Lowe et al. 2004). A founder effect is a type of bottleneck effect, which results from the colonization of a new habitat by a small number of individuals, resulting in different allele frequencies between a newly created population and the source or original population. As the new population is frequently small in size, in combination with the limited gene flow observed between populations in this region, the risk of extinction of a *P. daedalea* population such as that found on LEA may be higher. Leadsman Shoal is the largest (~12km in length), southernmost African reef with coral communities; it is part of the southern complex of the iSimangaliso Wetland Park and has sanctuary status. Therefore, it is

important that the level of protection of this reef is maintained and enforced, particularly given the marginal nature of this reef (Kleypas et al. 1999) and the probability of significant self-recruitment due to its larger size (Black et al. 1991).

The results also demonstrate that, at ecological time-scales, South African and southern Mozambican reefs are genetically disconnected (see Chapter 3; Ridgway et al. 2008, Macdonald et al. 2010). The higher mean allelic richness and number of private alleles at RAB and NMR (Figure 10) indicate that these reefs have a source of gene flow that is different from Mozambican reefs or that they are isolated from other reefs. Also, few migrants were identified in the assignment tests on any of the reefs, while those that were identified on South African reefs were left unassigned suggesting a source population that was not sampled. Lastly, assignment test did not exclude recruits from their sampling reef, viz. TMR, and the probability of them originating on the South African reefs (Cluster 4) was higher than on the southern Mozambican reefs (Cluster 3). Furthermore, when the genetic differentiation of LEA from the northern South African reefs (Table 12 and Figure 11), combined with the lower estimates of long-term *P. daedalea* migrants (N_em, Figure 11) between the Mozambican and South African clusters are considered, the dispersal distance in *P. daedalea* along the south-east African coast appears to be less than the 100 km suggested for *A. austera* (Chapter 3).

All these attributes are shared with *A. austera* (Chapter 3) and support the hypothesis that northern South African reefs may be acting as sinks for larvae that are transported by the MCE and EMC systems from reefs in locations other than southern Mozambique (Bryden et al. 2005; Morris 2009; Roberts 2006) before they radiate to the rest of the south-east African reefs. Again east Madagascar springs to mind. Such an alternative larval source would explain the higher levels of genetic diversity found at these migratory "landing-sites", the large number of unassigned migrants in this study, and the recurring discontinuity in the ecological genetic connectivity of southern Mozambican and South African reefs (Ridgway et al. 2008, Macdonald et al. 2010; this study). Studies incorporating samples from southern Mozambican and Madagascan reefs will be imperative to fully elucidate the level of dependence of South African reefs on other reefs, particularly reefs in Madagascar.

Notwithstanding the above, the fact that clustering of the reefs clearly only occurred with those proximal to each other may indicate parapatric speciation, which refers to species' ranges that are adjacent to each other but without significant overlap. The differential failure rates of the nuclear markers and the positive selection apparent in the Calm 2 intron provide some support for this explanation. Interestingly, non-coding regions of the cnidarian Calmodulin gene, which

regulates a calcium (Ca2+) binding protein, has proven to reliably infer phylogenetic relationships in the *Acropora* (Chen et ak. 2009) and *Stylophora* (Keshavmurthy et al. 2013) genera.

4.2.2 Small-scale (within-reef) genetic connectivity

The low variability of the nuclear markers screened in this study limited the ability to provide conclusive evidence that *P. daedalea* manifest significant population structure on TMR. Three of the five microsatellite markers were monomorphic at two or more sites. These resulted in a large number of samples sharing identical multi-locus genotypes which had to be removed to avoid bias in the analysis of genetic differentiation; the final sample size available for analysis (127) was 20% less than the number of samples collected on TMR (152). These loci, initially developed using GBR samples, have also shown lower levels of polymorphism when used in north-eastern African populations of *P. daedalea* (Souter & Grahn 2008). In this regard, the results suggest that nuclear intron loci appear to be better than microsatellites for the assessment of population structure at the finest scale (i.e. the intron loci were more polymorphic than microsatellite loci, see Appendix 4). Indeed, Miller and Howard (2003; pers. com.) reported similar difficulties in obtaining and reliably amplifying the five microsatellite markers. Consequently, the high number of repeated MLG can be explained by a combination of genetic drift and reduced levels of polymorphism in the microsatellite markers used. Although fission and fragmentation have been suggested as the cause of the replication of numerous genotypes (i.e. putative clones) in close proximity in other studies (Miller & Ayre 2008a; Souter & Grahn 2008), there is no evidence to support this theory (Mangubhai & Harrison 2008) and our findings suggest genotypes were derived from sexual reproduction (e.g N_G:N_I values were high and colonies sharing an MLG on TMR were always more than 14 m apart).

Notwithstanding the lack of statistical significance in most of the results for within-reef genetic connectivity, the data provide some information that is worth considering. First, the confidence intervals estimated for the different measures of genetic differentiation were different from zero which provides some evidence of statistical significance. Second, Bayesian clustering analysis suggested that there were a maximum of two genetic populations of *P. daedalea* on TMR, although the configuration did not group the sites in a clear pattern. Third, hierarchical partitioning (*HP*) analysis showed that site density explained 34.5% of the variation in local F_{ST}, whereas site location (i.e. inshore or offshore) explained 23.7% of this variation. Lastly, mean colony size, mean site depth and latitude all made substantial contributions (>20%) to the variation in genetic relatedness on TMR. Most of the findings discussed above are consistent with those for *A. austera* and other reef species, adding support to the idea that small-scale genetic connectivity of corals is

influenced by environmental and demographic factors (Chapter 3). However, more research is needed to ascertain the extent of these relationships.

Although the coefficients of genetic relatedness were small and suggest that genotypes were not related by descent (Blouin 2003), they still provide a measure of similarity between individuals. Considering this, the correlation between genetic relatedness and site depth might be attributable to specific substrate settlement preferences shared by coral larvae. Indeed, Baird et al. (2003) found in ex situ experiments that, when given the choice, *P. daedalea* settle preferentially on tiles conditioned on shallow reefs rather than on those conditioned on deeper reefs of the Great Barrier Reef. This behaviour may explain the negative correlation observed between genetic relatedness and site depth in this study. It would also aid in understanding the significant pairwise genotypic differentiation observed between TMR and FMR despite being less than 3 km apart. While most colonies of *P. daedalea* from TMR were collected at depths <14 m, samples from FMR Reef were collected at depths >18 m as this reef ranges in depth from 18 - 24 m.

The battery of markers used in this study failed to detect spatial genetic structure in *P. daedalea* colonies on TMR and this appeared mainly attributable to the reduced levels of polymorphism in the microsatellite markers used (Hardy & Vekemans 2002). However, the predominance of heterozygote deficits on TMR unrelated to asexual reproduction or the presence of null alleles (see Results and Appendix D), supports the idea that larval dispersal in *P. daedalea* is limited (Underwood et al. 2007; Ridgway et al. 2008; Mackenzie et al. 2004). Similarly, the isolation by distance detected at the reefal scale (see Results) indicates that its dispersal is low enough on this reef to allow minor gene-frequency differences to develop between distant sites (Palumbi 2003b). Indeed, the genetic patch size (7.5 m by GenAlEx) and effective gene dispersal (9.7 m by SPAGeDi) estimated for the S_IN site provides some evidence that dispersal is very limited on TMR or at least at this site. Furthermore, the dispersal distance (max 30 m, see Results) derived from this study is similar to the ca 20 m estimated for this species on the GBR (Miller & Ayre 2008a). Considering this, it is possible that, by screening more loci, the SGS signal indicative of non-random or limited dispersal will become more evident in *P. daedalea* larvae on TMR, particularly if new markers can be developed for the polymorphism in the south-east African coral populations.

Additional evidence of limited contemporary dispersal of *P. daedalea* on TMR is provided by the assessment of genetic variation amongst size-classes. Although differences in this were not statistically significant, genetic diversity, measured as allelic richness and expected heterozygosity, decreased from size-class 2 (largest adults) to size-class 0 (recruits), which suggests that very few new alleles are introduced regularly by immigration. The inbreeding coefficient (F_{IS}) was inverted

and suggests that the largest adults (SC2) are more outbred than recruits (SC0); this may indicate that the population was founded by a small number of individuals that, due to limited immigration, tend to inbreed. Finally, genetic variation between size-classes (possibly cohorts) was significant, although weak; young adults (SC1) appear to be genetically different from recruits (SC0) (Table 15), suggesting that allele frequencies have changed from generation to generation. These are all tell-tale signals of a rather closed population with a small effective population size, or a population that receives limited contemporary gene flow (Hedgecock et al. 2007; Lowe et al. 2004). The results are also consistent with the founder effect discussed above in terms of the genetic differentiation encountered on LEA. Thus, it is recommended that future studies include samples from different years and cohorts to ascertain the extent of temporal genetic variation of *P. daedalea* on TMR.

Finally, the findings support the hypothesis that variations in population demographics, larval behaviour and the magnitude of coral larval dispersal synergistically affect genetic connectivity. Such relationships have been proposed in various studies on the larvae of several coral species (e.g. *Montastraea annularis* and *M. faveolata*, Pizarro et al. 2008; *Porites astreoides* and *A. palmata*, Mason et al. 2011; *Paramuricea clavata*, Mokhtar-Jamaï et al. 2013) and were found in *A. austera* on TMR (Chapter 3). More importantly, this demonstrates the importance of studies at the seascape level (e.g. employing a coupled biological-genetic approach) when trying to elucidate patterns of larval dispersal and connectivity (Werner et al. 2007).

CHAPTER 5

GENERAL DISCUSSION

Population genetic studies provide insights into the relationships between and within reef populations of coral species. Ultimately, patterns in these relationships will depend on the spatial and temporal scale of dispersal of coral larvae. In this study, the magnitude of gene flow of two species of corals from south-east African reefs was addressed using multilocus genotype data (Chapters 3 and 4). The methods applied in the study provided information on recent patterns of connectivity between reefs which is of importance to managers.

5.1 Study methods

Marker polymorphism can affect the detection of genetic variation between and within populations, as was found with the microsatellite loci used in this study (Chapter 3 and 4). Low polymorphism was characteristic of the microsatellite loci screened in *A. austera* and *P. daedalea*, regardless of whether they were specifically developed for the target species or cross-amplified from sister species. This could explain the inconclusive statistical evidence for spatial genetic structure (SGS) in *P. daedalea* on TMR. Low allelic diversity was also found in the microsatellite loci of *P. daedalea* on Kenyan and Tanzanian reefs (Souter & Grahn 2008). It is recommended that hyper-variable genetic markers are developed for the polymorphism characteristic of south-east African coral populations. This may increase their resolution to detect genetic variation, particularly the assignment of individuals to their population of origin and the detection of SGS. Polymorphism was higher in nuclear Exon-primed Intron-crossing (EPIC) introns than microsatellite loci (Appendices B and C); nuclear intron loci were also easier to cross-amplify than microsatellites (Appendix C). Thus, nuclear intron loci appear to be more efficient (i.e. better resolution power) as population genetic markers, particularly in symbiotic species like corals where pure host DNA is difficult to obtain.

The results of this study support the use of allelic data from similar types of genetic markers as the significance of the measures of genetic variation estimated separately by nuclear microsatellite and intron loci were similar in both species and at all spatial scales (Chapters 3 and 4). Similar results have been obtained in studies following a multilocus approach in the study of population genetics (Mackenzie et al. 2004; Vollmer & Palumbi 2006; Sonsthagen et al. 2007). In these, the larger number of loci screened allowed the use of methods that address contemporary gene flow (Cornuet et al. 1999; Bernatchez & Duchesne 2000; Primmer et al. 2000).

The presence of null alleles in the samples (see Appendix B) could have affected the results (Selkoe & Toonen 2006), particularly in *P. daedalea* due to the significant differential failure rate across the study area. However, the measures of genetic variation and differentiation were not statistically affected by the presence of null alleles in the *A. austera* and *P. daedalea* microsatellite loci (Appendix E). Similarly, the statistical evidence for a null allele effect on the observed excess of homozygotes in *A. austera* on TMR suggested that the coefficients of genetic relatedness were not affected either. Therefore, although the relatedness values may be somewhat inflated, the overall conclusions of these analyses remain unchanged. With regards to the differential failure rates of nuclear loci in *P. daedalea*, phylogenetic research on this species is needed in the region as findings thus far (e.g. this study, Macdonald et al. 2012) are indicative of cryptic speciation.

Individual-based genotype analyses used in this study (i.e. Bayesian clustering, assignment tests and spatial autocorrelation analysis) revealed the existence of genetic structure at all spatial scales in the populations of the two coral species. They also suggested the existence of temporal genetic discontinuity in both species. Furthermore, investigation into the demographic (e.g. population density and size structure) and environmental (e.g. site location, depth and latitude) factors that may influence genetic structure in the two species resulted in a better understanding of the observed patterns. More importantly, the exercise revealed a potential species-specific response to the factors considered, as the influence of each individual variable varied between species. Although the benefits of following this seascape genetics approach (i.e. coupling genetic data with spatially-explicit environmental and demographic variables) in the analysis of genetic connectivity are evident (see Selkoe et al. 2010, Amaral et al. 2012, McInerney et al. 2012), its application in coral reef science is still scant (but see Galindo et al. 2006). Thus, similar studies are imperative if the ecological genetic connectivity of coral reefs is to be fully understood and the demographic factors that influence it are to be managed appropriately.

Finally, it is acknowledged that sample sizes in this study were less than optimal for the analysis of genetic differentiation of some populations (>30 per population) using F-statistics. Pooling locations to increase sample size was not supported by the findings of the Bayesian clustering analyses. Genetic subdivision was evident at all spatial scales using this method and pooling sites might have caused a Wahlund effect (i.e., mixing of differentiated gene pools). Furthermore, genetic Bayesian analysis does not rely on large sample sizes per location to discern genetic subdivision in an entire sample as it initially treats all locations as a single population (Pritchard et al. 2000; Corander et al. 2008).

5.2 Connectivity patterns

The results of this study do not support the hypothesis that south-east African reefs harbour single large populations of *A. austera* and *P. daedalea*, with significant levels of contemporary gene flow at all spatial scales i.e. between reef and within-reef populations. The data suggested that south-east African reef populations of the two coral species comprise several genetically distinct populations. Four genetically distinct populations (i.e. clusters) of *A. austera* and five of *P. daedalea* were identified in the study area by Bayesian clustering analysis. The spatial arrangements of the regional patterns were similar in both species (Figures 5 and 11) and they are in agreement with those in other benthic species. For instance, Silva et al. (2010) analysed morphometric and mtDNA variation in the mangrove crab *Perisesarma guttatum* and concluded that individuals from Inhaca Island and Maputo Bay were morphologically and genetically different from those in northern Mozambique, Tanzania and Kenya. Similarly, Lessios et al. (2003) found that the variation in the mtDNA of *Tripneustes* spp. sea urchins from South Africa and Madagascar was negligible compared to that between South Africa/Madagascar and Reunion Island. Lastly, analysis of mtDNA in the mud crab *Scylla serrata* from localities within the Indian Ocean revealed that South African populations of this invertebrate are different from those in Mauritius, Madagascar, Zanzibar and Kenya (Fratini & Vannini 2002). All these studies clearly suggest that genetic panmixia in these benthic marine invertebrates of southern Africa cannot be assumed, despite studies being preliminary designed to assess evolutionary genetic connectivity in the region.

Considering the similarities in the patterns of genetic differentiation measured in the present and aforementioned studies, it is tempting to suggest that limited gene flow between populations of benthic marine invertebrates is characteristic in the south-western Indian Ocean. Genetic differentiation in three benthic echinoderms (*Linckia laevigata*, Williams et al. 1998; *Acanthaster planci*, Benzie 1999; *Holothuria nobilis*, Uthicke & Benzie 2003) in the Indian Ocean has also been found to be higher than among Pacific Ocean populations, indicative of a more restricted gene flow in the Indian Ocean. As suggested by Benzie (1999) and Silva et al. (2010), the south-western Indian Ocean has fewer reefs and islands than other regions (e.g. Pacific Ocean), but has oceanographic features (e.g. mesoscale gyres) that together prevent much exchange between south-east African populations and the rest of the Indian Ocean. The present results support this and also suggest that the forces that promote genetic differentiation in these two coral species act at ecological time-scales because contemporary gene flow between southern Mozambican and South African reefs is limited (Tables 6 and 13).

Acropora austera and *P. daedalea* each have pelagic larval durations (PLDs) in excess of 100 days which, given a minimum current speed of 0.11 m s^{-1} in the region during the spawning season (Morris 2009), would suggest a dispersal range of over 1000 km. Considering this, the data indicate that the full potential of dispersal is not realized in these species. Reviews on larval dispersal and genetic structure by Palumbi (2004) concluded that marine invertebrates exhibit dispersal ranges between 10 and 100 km; the present data support this conclusion. The configuration of the genetic populations of both species (Figures 5 and 11) and the fact that genetic discontinuity was found between the northernmost South African reef sampled (Rabbit Rock) and Inhaca Island, which are separated by ca 100 km, also suggest that larvae of these two species do not normally disperse more than 100 km. Such limited dispersal would explain the frequent genetic isolation of high-latitude reefs (HLR) from more tropical reefs (e.g. LaJeunesse et al. 2004, Miller & Ayre 2008, Noreen et al. 2009, Macdonald et al. 2010, Nakajima et al. 2012). Genetic isolation of HLR occurs despite the reefs being exposed to unidirectional oceanic currents which would transport larvae from upstream reefs to those downstream. This makes HLR vulnerable to natural and anthropogenic threats as they are predominantly self-recruiting.

At the reefal scale (within-reef), the data also suggest genetic discontinuity in both species, possibly explaining the genetic discontinuity observed in the region. Two (*P. daedalea*) to four (*A. austera*) clusters were identified on TMR in these coral species and may be related to the neighbourhood sizes of each species. These clusters were all genetically distinct, although this phenomenon was weaker in *P. daedalea*. Similarly, there was evidence to suggest the existence of SGS indicative of non-random or limited dispersal in *A. austera*. The evidence for SGS in *P. daedalea* on TMR was statistically inconclusive, but literature on the species and most of the results suggest this might be the case (Chapter 4). More importantly, there was also significant relatedness between closely-spaced individuals of *A. austera*, whereas the hierarchical partitioning analysis suggested that the non-random dispersal may be the result of specific larval settlement preferences (e.g. the presence of adults) and habitat conditions (e.g. depth and location) (see Chapters 3 and 4), not only water movement. Thus, the findings suggest that, at least for *A. austera*, the dispersal of larvae might be limited to as little as a couple of tens of meters on TMR. Altogether, this challenges the assumption that the larvae of these two corals are passive swimmers.

Recent studies on behaviour and settlement of coral larvae have revealed that coral larvae are not necessarily passive particles; they have the ability to explore suitable substrata for settlement by alternating their swimming patterns (Pizarro et al. 2008; Mason et al. 2011). Coral recruitment studies on TMR have also shown that coral larvae have settlement preferences. Hart (2011) deployed three different types of settlement tiles on TMR and found that most recruits

settled on tiles that were pre-conditioned with calcareous coralline algae. This author also found that most settlement occurred on the vertical edges of tiles, which was attributed to the protection provided by such refuges compared to the flat open top surface of the tiles. Considering this, and the genetic patterns observed in this study, it is suggested that coral larvae of *A. austera* and *P. daedalea* actively "choose" where to settle and they do so close to natal or kin colonies. Similar trends have been observed in *Acropora* species (Suzuki et al. 2008; Suzuki et al. 2012) and some brooding corals elsewhere (Ledoux et al. 2010; Mokhtar-Jamaï et al. 2013). Although there are local climatic and hydrographic conditions that appear to favour this non-random dispersal of coral larvae (Chapter 3), other explanations need to be considered.

The most parsimonious explanation would be that the increased relatedness results from "patchy recruitment" whereby propagules produced on upstream reefs during the same spawning event settle together downstream; however, this explanation was not supported by the findings of this study (Chapters 3 and 4). Alternatively, the increased relatedness between closely spaced individuals might well be due to post-settlement selection of genotypes and not necessarily direct kinship (Zvuloni et al. 2008). Post-settlement selection would favour traits that increase the likelihood of survival in specific habitats, which eventually will increase genotypic similarity between individuals. This might be true of HLR where conditions for corals are not optimal and genotypes that adapt to the marginality would be advantageous. Although the reliability of Tajima's D and Fu's D as tests of neutral selection have been questioned (Nielsen 2001; Excoffier & Heckel 2006), these tests suggested that the *Calm 2* intron used in this study might be under selection (Appendix C). Therefore, genetic studies that specifically address selection pressure on South African individuals and populations of the two coral species are encouraged.

5.3 Management implications

Networks of marine protected areas (MPAs) are required for the successful protection of coral reefs. The results presented here indicate that the genetic connectivity of reefs within and between the MPAs in which the samples were collected follow a stepping-stone pattern whereby adjacent reefs appear to be more connected than distant reefs. A network of MPAs would ensure the exchange of propagules between individual MPAs and, provided their connectivity is maintained, would confer resilience to populations through replenishment from adjacent MPAs. Fortunately for the African region, efforts towards the establishment of transboundary MPA networks are already in place (e.g. WWF Eastern African Marine Ecoregion 2004, Guerreiro et al. 2010). For example, the iSimangaliso Wetland Park (iSWP), the Mozambican Ponta do Ouro Partial Marine Reserve and the Ilhas da Inhaca e dos Portugueses Fauna Reserve constitute a continuous series of protected reefs that, as the data suggest, are connected more significantly at evolutionary than at

ecological time scales. The sources of the South African samples of both species not assigned by the assignment tests may well correspond to unsampled reefs in southern Mozambique. However, the results suggest that some reefs (e.g. Rabbit Rock and Nine-mile Reef) within this MPA network have a "landing-site" function for migrants, given their geographical location and the prevailing currents. As a result, every effort must be made to maintain this transboundary network of MPAs and protect individual reefs, especially the "landing-sites" reefs, to ensure the interdependence of the coral communities in terms of genetic exchange.

The fact that *Acropora austera* and *P. daedalea* represent different life strategies in the South African reef communities yet manifest similar genetic patterns suggests that these corals are responding similarly to the forces that are driving genetic connectivity in the region. *Acropora austera* represents a fast-growing, framework-building species that can become very abundant in certain areas of the reef. In contrast, *P. daedalea* represents slow-growing, massive species (e.g. *Favia* spp., *Favites* spp., *Hydnophora* spp.) that are widely distributed on reefs but not numerically abundant. Indeed, these two species tend to occur in distinct zones of the reef with different community structures (Celliers & Schleyer 2007; Schleyer & Celliers 2005). The two species also differ in terms of their susceptibility to environmental impacts. *Acropora austera* represents fragile species that are susceptible to physical damage, bleaching and predation by crown-of-thorns starfish (e.g. *Acropora* spp., *Montipora* spp.), whereas *P. daedalea* exemplifies long-lived species that, due to their massive form, can sustain physical impacts and are less susceptible to bleaching (e.g. *Porites* spp.). The results presented here thus suggest that gene flow in both these species is limited between reef populations over ecological time scales due to non-random dispersal of their larvae. Their patterns of large-scale genetic connectivity appear to be similar to that of *Pocillopora verrucosa*, another broadcast-spawning species in which separation was found between Mozambican and South African populations and attributed to limited contemporary gene flow (Ridgway et al. 2008). This may be a generalisation to which there will be exceptions but it would constitute a conservative standard that must be considered in MPA spatial design. MPA management would benefit if further species were studied to corroborate these results.

Ecological and biological assumptions (e.g. populations are open, species with long PLD show less genetic structure, and invertebrate larvae are passive swimmers) that were accepted during the early stages of marine reserve design need to be revisited. The present results revealed significant fixation of alleles on the reefs, moderate levels of genetic differentiation between reefs, clear genetic subdivision at the largest spatial scale and considerable levels of self-recruitment on each reef, and the presence of temporal genetic structure. All of these are tell-tale signs that the populations are less open (or more closed) or that effective population sizes are smaller than initially assumed. Altogether, the results of this study add to the growing evidence that a

worldwide change is needed in the paradigms of marine reserve design (Levin 2006; Botsford et al. 2009; Ashe et al. 2010; Palumbi 2003b; Palumbi 2004; Miller & Ayre 2008a).

Population demographics and larval behaviour need to be considered to understand and model population connectivity. The availability of demographic and environmental data (e.g. population density, size structure, site depth) on TMR helped in the elaboration of its small-scale patterns of genetic connectivity. Multi-scale modelling to measure dispersal and connectivity has recently been given attention as it can incorporate biological traits (e.g. PLD, swimming ability) and habitat information (e.g. nursery/settlement habitats, spawning populations) with oceanographic data (Paris et al. 2007), ensuring fidelity between actual larval dispersal and model predictions (Botsford et al. 2009; Paris et al. 2007; Werner et al. 2007). Botsford et al. (2009) suggested that settlement and recruitment depend on the presence of suitable habitat and may also depend on the density of potential settlers and adults. Considering this, the results of this study indicate that, when available, models should include information such as population density and size structure as these significantly influenced connectivity patterns on TMR. Variations in these parameters are probably responsible for the location of larval settlement. The reliability of connectivity models as a management tool will depend on the inclusion of these parameters. Pelagic larval duration, mortality and vertical movement are commonly included in such model simulations (e.g. Baums et al. 2006; Paris et al. 2007; Kool et al. 2011); population density and size structure have yet to be included.

Finally, the current literature suggest that the consequences of climate change on coral reef connectivity extend to reduced pelagic larval duration, earlier reef-seeking behaviour, habitat loss and fragmentation, and changes in local hydrodynamics (Munday et al. 2009). These are all consequences that make South African reefs vulnerable, given that the reefs are rather isolated and already show limited dispersal of coral larvae. Their vulnerability is already evident (Celliers & Schleyer 2007; Celliers & Schleyer 2002; Schleyer & Celliers 2003b; Schleyer & Tomalin 2000) and might be higher compared to other isolated reefs. For example, the large patch of *A. austera* that was conspicuous on TMR during sampling is showing signs of senescence (J Hart pers. comm. July 10 2013; P.H. Montoya-Maya pers. obs.) and recovery of this species on FMR appears to be slow and related to its restricted dispersal (Chapter 3). Underwood et al (2007) observed that populations of the brooder *Seriatopora hystrix* on an isolated system of reefs in northern Western Australia that was heavily affected by bleaching in 1998 took just five years to recover to pre-bleaching levels. These authors were able to demonstrate through assignment tests that a single reef which was not decimated by bleaching was responsible for the recovery of the affected reefs due to the export of larvae; this despite high levels of localized recruitment on the reefs (Underwood et al. 2007). Although patterns of genetic connectivity at small-scales can vary from

year to year due to demographic stochasticity, it is recommended that genetic studies be included in long-term coral monitoring strategies. In so doing, the temporal variation of genetic diversity can be assessed and interpreted relative to reef responses to climate change related impacts.

5.4 Conclusions and recommendations

A central problem for reef managers is the establishment of marine protected areas (MPAs) that either include genetic populations of concern, or the size and configuration of which allow the connectedness of such populations (Cowen et al. 2006; McCook et al. 2009; Jones, Geoffrey P. et al. 2009; Botsford et al. 2009). For management purposes, the genetically distinct populations identified at each of the spatial scales analysed in this study may correspond to management units (MUs), or evolutionarily significant units (ESUs) (Pearse & Crandall 2004; Palsbøll et al. 2007). At least in South Africa, the iSWP appears to incorporate the full genetic extent of the South African populations of these two species, which provides grounds for some confidence in the functioning of this MPA for their protection. Nonetheless, the evidence of limited dispersal in these species, their significant spatial genetic structure at the reefal scale, and the potential role that northern reefs play as "landing sites" for putative migrants suggest that the scope of management for their protection should not be too broad; each reef should be managed as a single unit, part of the whole rather than a representative of the whole. Since some reefs appear to act as "landing-sites" for migrants (NMR) and there is evidence of significant within-reef genetic structure (TMR), an adaptive management framework would be the best option for this MPA. Such a framework would allow management to be adapted to the heterogeneity of each reef and the different function each reef plays within the MPA (Tompkins & Adger 2004; Beeden et al. 2012); scientific input will be needed to provide information on these functions (Kruger & MacFadyen 2011).

Altogether, the results of this study are consistent with the isolation observed in other studies using less variable markers, and support the hypothesis that there is demographic discontinuity between the coral populations along the south-east African coast. Therefore, it is suggested that South African populations are, at ecological time scales, independent of gene flow from northern coral populations. Several small reefs found in southern Mozambique, from Ponta do Ouro at the border to Inhaca Island, were not sampled in this study and might be sources of coral larvae for South African reefs. Thus, it is recommended that geographical gaps in the study be filled, notably with the inclusion of sites in Madagascar and more sites along the Mozambique coast, especially in the south. In this regard, it is recommended that contemporary gene flow between South African reefs and south eastern Madagascar be investigated to ascertain whether reefs from Madagascar are also an important source of coral larvae for South African reefs.

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APPENDIX A. Estimation of size at first maturity in two species of corals from South Africa

Although size at first reproduction and subsequent reproductive events in clonal organisms are influenced by environmental factors which can result in extremely variable sizes (see Harvell & Grosberg 1988 and Hall & Hughes 1996), size-structured models seem to be more relevant than age in understanding population demography in clonal organisms such as corals (Babcock 1991; Artzy-Randrup et al. 2007). Here, the methods and results of estimating the size of first maturity for two species of corals following Mollet et al. (2000) are described. This estimation was carried out to group sampled individuals of *Acropora austera* and *Platygyra daedalea* into size-classes (i.e. potential generations), which was required to investigate within-reef patterns of temporal genetic connectivity across individuals of both species.

Methods

To define stage-classes (i.e. adult and recruit colonies) of *A. austera* and *P. daedalea* at Two-mile Reef (TMR, South Africa), colonies of these species were sampled within the 2010 coral spawning season in South Africa (i.e. January – March) (Glassom et al. 2006; Masse et al. unpub. data). Colonies of a wide size range were selected randomly for reproductive assessment at different sampling sites on TMR; the source site and the maximum colony diameter (*d1* and the diameter at right angles to this (*d2*) were measured to the nearest cm. Notwithstanding the above, only healthy (i.e. no visual evidence of disease or injury) and non-fragmented colonies were selected in an effort to minimize the influence of environmental factors in their reproductive status (see Baird & Marshall, 2002 and Okubo et al. 2006). Sampling comprised the collection of three fragments from individual colonies of each species. Colony fragments corresponded to non-marginal areas where polyps tend to have a reproductive role compared to marginal polyps (Sakai 1998).

In the laboratory, the macroscopic maturity stage was inferred by visual examination under a stereomicroscope of the fragments and using a binomial key: 1 = gametes present; 0 = gametes absent. Mature eggs usually become pigmented close to the spawning period while sperm and immature eggs are visible in broken sections of coral (Wilson & Harrison 2003). Hence, a colony was considered mature when at least one of its fragments had either eggs or sperm or both.

Multiple logistic regression was initially used to model the binomial (0 = immature; 1 = mature) reproductive status data as a function of mean colony diameter (MCD, expressed as the geometric mean of two diameters i.e. *d1* and *d2*), site of origin and density estimates (no. colonies m^{-2}). Only MCD had a significant effect on the probability of being mature (data not shown) and goodness-of-fit tests (Hosmer-Lemeshow test, Hosmer et al. 1997) indicated that the most adequate model corresponded to a Generalized Linear Model (GLM) of form $P = \beta_0 - \beta_1 d$, where P is the natural log of the odds of a colony being mature, d is MCD, and β_0 (intercept) and β_1 (slope) are the estimated model parameters. This model was then used construct the maturity ogive for each species (Figure A1).

Parametric bootstrapping was used to estimate MCDs at which the probability of maturity is 10% (D10), 25% (D25), 50% (D50), 75% (D75) and 90% (D90), (Mollet et al., 2000; Barot et al. 2004). Random data sets of the same original sample size were simulated with replacement from the fitted model. Colony diameters were calculated from this simulated data set. This sampling process was repeated for 1000 iterations and the distribution of the calculated diameters was used to determine the MCD (i.e. 5% trimmed mean) and to derive confidence intervals with

the 95% percentiles bias-corrected method (Quinn & Keough, 2002). All analyses were conducted in R environment (R Core Team, 2013)

Results and Discussion

Acropora austera

A total of 45 colonies ranging in size from 3.5 to 130.0 cm MCD were sampled. The smallest mature colony measured 9.5 cm MCD and the largest non-mature was 43.3 cm MCD. The parameter estimates and statistics for the GLM model were as in Table A.1.

Table A.1 Parameters estimates and descriptive statistics for the two GLM models ($P = \beta_0 + \beta_1 d$) fitted to estimate size at first maturity in *A. austera* and *P. daedalea*. CI = 95% confidence interval; AIC = Akaike Informative Criterion.

Acropora austera (n=45)

Parameters	Estimate	Std. Error	z value	*P*	Odds ratio	Lower CI	Upper CI
b0	-1.484	0.713	-2.083	0.034	0.227	0.056	0.916
b1	0.096	0.039	2.463	0.014	1.101	1.020	1.188
Null deviance:		60.571 on 44 degrees of freedom					
Residual deviance:		23.679 on 43 degrees of freedom					
AIC = 50.99							

Platygyra daedalea (n= 40)

Parameters	Estimate	Std. Error	z value	*P*	Odds ratio	Lower CI	Upper CI
b0	-3.752	1.973	-1.902	0.057	0.023	0.001	0.639
b1	0.540	0.224	2.412	0.016	1.716	1.212	3.021
Null deviance:		33.817 on 39 degrees of freedom					
Residual deviance:		19.061 on 38 degrees of freedom					
AIC = 23.06							

A Hosmer-Lemeshow goodness-of-fit statistic provided no evidence for lack of fit of the model (H-L = (9, N = 45) = 0.922, $P > 0.05$). The probability ratio of an *A. austera* colony being mature was estimated as 1.101. This suggests that for one cm increase in colony diameter, a sampled colony has 1.101 chance of being mature. The finding supports the idea that, under general conditions, there is a positive relationship between reproductive potential and size (Hall & Hughes, 1996); the latter either measured as number of polyps per area (Sakai 1998), mean radius (Kojis 1986) or surface area (Nozawa et al. 2006).

Bootstrap estimates and 95% confidence intervals for D10, D25, D50, D75, and D90 are shown in Table A.2. Assuming knife-edge maturity (Jennings et al. 2001), the bootstrap estimates for D50 suggest that *A. austera* from TMR matures at 15.01 cm MCD with 95% CI from 9.25 to 20.75 cm MCD. This estimated size range at first maturity is similar to the 14 cm colony diameter

reported for *A. hyacynthus* from Satsukigaura, southwestern Japan (Nozawa et al. 2006), also a high-latitude reef as TMR. However, these values are much larger than those reported for tropical Acroporid species (2 -4 cm colony diameter; Kojis 1986; Hall & Hughes 1996), which could be the reflection of marginal conditions for corals found at higher latitudes (Kleypas et al. 1999). In this study, colonies as small as 4.03 cm MDC had a 25% probability of being mature (Table A.2, D25). For *A. austera* is a branching species, it is likely that these small mature colonies have arisen from fragments of larger mature colonies (Okubo et al. 2006).

Table A.2 *Acropora austera* bootstrap estimates and 95% confidence intervals (CI) for D10, D25, D50, D75, and D90. Age estimated from a growth rate of 2.45 cm yr^{-1} (Grimmer 2011)

	D10	D25	D50,	D75	D90
Actual estimate	0	4.027	15.493	26.960	39.526
Trimmed mean (5%)	0	4.083	15.078	26.306	38.623
Lower CI	0	-9.952	9.253	19.174	26.472
Upper CI	4.877	10.693	20.751	36.707	57.472
Estimation bias*	-0.463	-0.056	0.415	0.654	0.903
Standard error	0.298	0.171	0.098	0.156	0.278
Estimated age (years)	<2	<4	3 - 8	8 - 15	11 - 23

*estimation bias = actual estimate – trimmed mean (Quinn & Keough, 2002). Negative estimates have been replaced with zero.

The age of *A. austera* colonies can be estimated from linear growth data collected by Grimmer (2011). The mean linear extension of this species at TMR was 2.45 cm yr^{-1}. This means that colonies of *A. austera* reach maturity at around six years old (3 – 8 years old from 95% CI of D50) but that colonies 2 years old and younger could be mature. It also means that colonies with more than 75% probability of being mature are more than 11 years old and that the largest colony sampled (i.e. 130 cm MCD) was more than 53 years old. The age of first maturity of most Acroporid species is typically four years; however, it can vary between three to eight years (Connell et al. 1997; Harrison & Wallace 1990). Nonetheless, this is similar to the age estimates for *A. austera* at TMR.

Platygyra daedalea

A total of 40 colonies ranging in size from 4.5 to 70.8 cm MCD were sampled. The smallest mature colony measured 7.9 cm MCD and the largest non-mature was 15.5 cm MCD. The parameter estimates and statistics for the GLM model are shown in Table A.1.

A Hosmer-Lemeshow goodness-of-fit statistic provided no evidence for lack of fit of the model, H-L = (9, N = 40) = 2.183, P = > .05. The probability ratio of a *P. daedalea* colony being mature was estimated as 1.716. This suggests that for one cm increase in colony diameter, a sampled colony has 1.716 chance of being mature. This is larger than that estimated for *A. austera* but expected for slow growing, long-lived faviid species like *P. daedalea* (Babcock 1991; Sakai 1998).

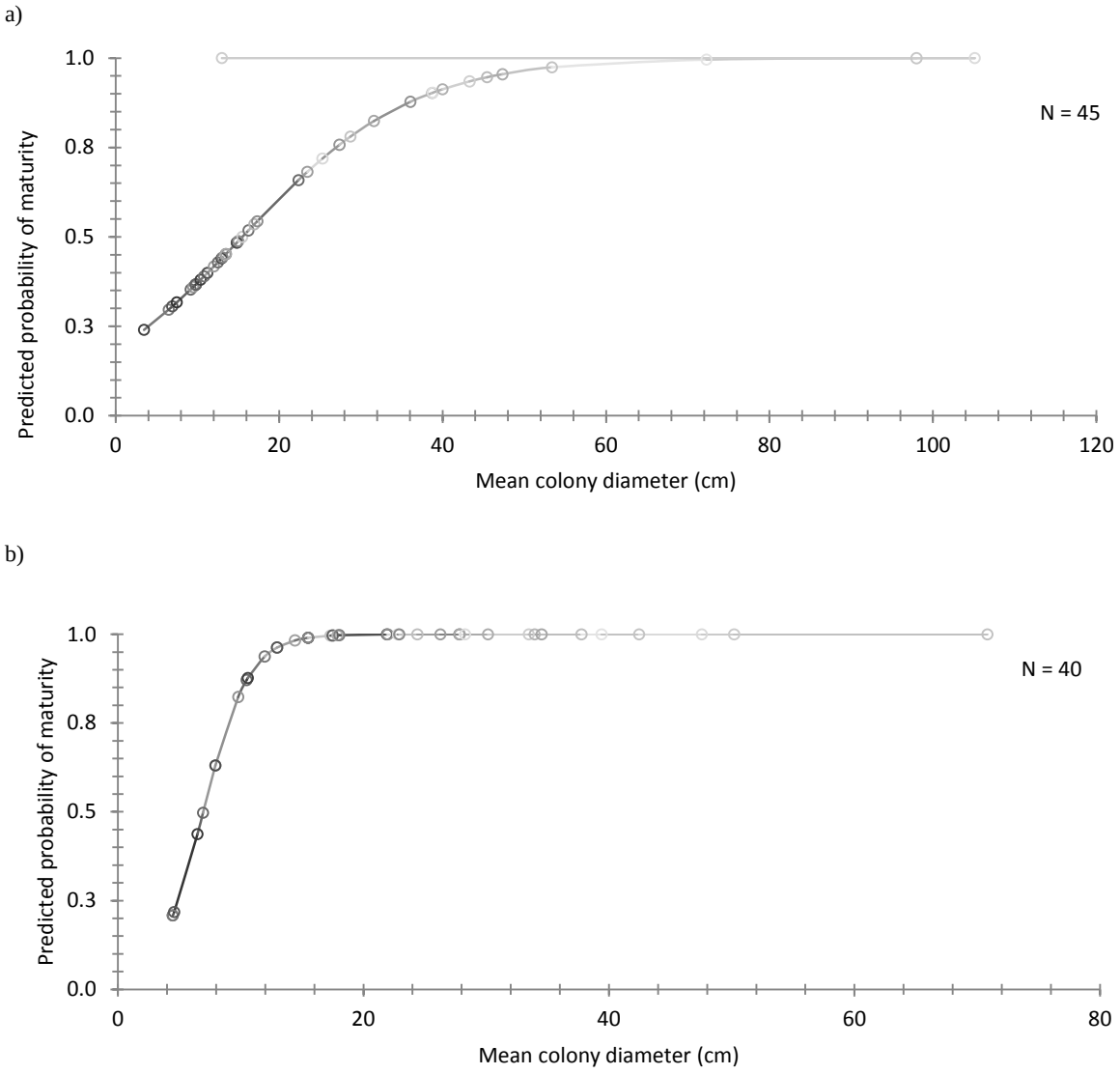

Figure A.1 Scatterplots of the predicted probabilities of maturity from GLM model (binomial, logit link) of reproductive status in relation to colony mean of sampled colonies of *Acropora austera* (a) and *Platygyra daedalea* (b) from Two-mile Reef (Sodwana Bay, South Africa).

The bootstrap estimates and 95% confidence intervals for D10, D25, D50, D75, and D90 are shown in Table A.3. Assuming knife-edge maturity, the bootstrap estimates for D50 suggest that *P. daedalea* from TMR matures at 6.86 cm MCD with 95% CI from 4.55 to 8.39 cm MCD. Although the estimated confidence interval includes values reported in other studies, the central value is larger than that of *P. sinensis* from the GBR (5.6 cm colony diameter; Babcock 1991) and of *P. contorta* from high-latitude reefs in southwestern Japan (4.6 cm; Nozawa et al. 2006). Fragmentation is uncommon in massive corals (Babcock 1991) and slow growing faviids species such as *P. daedalea* species (Soong 1991) are known for achieving maturity at very small sizes, <4 cm in diameter, in tropical reefs (Sakai 1998; Hall and Hughes 1996, Soong 1991). Therefore, a smaller size of first maturity in *P. daedalea* is possible and may explain the 10% probability of maturity found for colonies as small as 3.07 cm MCD (Table A.3).

Table A.3. *Platygyra daedalea* bootstrap estimates and 95% confidence intervals for D10, D25, D50, D75, and D90. Age estimated from a mean radial growth rate of 0.54 cm yr^{-1} (Weber & White 1974).

	D10	D25	D50	D75	D90
Actual estimate	2.684	4.913	6.947	8.981	11.210
Trimmed mean (5%)	3.067	5.077	6.885	8.716	10.759
Lower CI	0	1.356	4.547	6.724	8.007
Upper CI	6.014	7.262	8.390	10.291	13.267
Estimation bias*	-0.383	-0.164	0.061	0.265	0.451
Standard error	0.073	0.049	0.033	0.030	0.045
Estimated age (years)	<6	1 - 7	4 - 8	6 - 10	7 - 12

*estimation bias = actual estimate – trimmed mean (Quinn & Keough, 2002). Negative estimates have been replaced with zero.

Reports on growth rates for *P. daedalea* were not available. However, a radial growth rate range of 0.38 – 0.69 cm yr-1 for *P. sinensis* from the GBR was reported by Babcock (1991) while Weber & White (1974) estimated a radial growth rate of 0.54 cm yr^{-1} for *Platygyra* spp living at 24°C sea temperature, which is within the mean seasonal sea-surface temperatures range (22°C - 26°C) for South African coral communities (Schleyer et al. 2008). Using these figures, the estimated age of first maturity for *P. daedalea* at TMR would range between 5 to 9 years old and be centred around six years. These age estimates are similar to the age of first maturity estimated for *P. sinensis* (5 – 8 years; Babcock 1991) and that estimated for most haermatypic corals (3 – 8; Hall & Hughes 1996). It is estimated that colonies with more than 90% probability of being mature are more than ten years old and that the largest colony sampled (i.e. 70 cm MCD) was more than 64 years old.

Conclusions

The findings in this study support the positive relationship between reproductive potential and colony size found in previous studies. Although the small samples sizes may have resulted in wider confidence intervals, the estimated sizes at first maturity in *A. austera* (~15 cm MCD) and *P. daedalea* (~7 cm MCD) from TMR are within the range reported for their congeners. Similarly, the estimated age of first maturity for both species, ca six years, was similar to previous estimates. It is expected that the findings from this study assist the effective management of the coral communities at TMR, especially in understanding the time coral populations might take to recover after a major disturbance.

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APPENDIX B. Cross-amplification and characterization of microsatellite loci in *Acropora austera* from the south-western Indian Ocean

Paper accepted for publication in Genetics and Molecular Research

Cross-amplification and characterization of microsatellite loci in *Acropora austera* from the south-western Indian Ocean

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Running title: Cross-amplified microsatellite markers for *Acropora austera*

Abstract

Here, we report the successful cross-species amplification of previously published acroporid microsatellite markers in the coral *Acropora austera* from the south-western Indian Ocean. This fast-growing species is a major reef-building coral on South African reefs; however, it is the most damaged coral by SCUBA diving activity, and is known to be very susceptible to coral bleaching. Neither genetic information nor symbiont-free host tissue was available to develop novel microsatellite markers for this species. Cross-species amplification of previously published microsatellite markers was considered as an alternative to overcome these problems. Of the 21 microsatellite markers tested, 6 were reliably amplified, scored, and found to contain polymorphic loci (3–15 alleles). The results of this study and previous research indicate that the microsatellite sequences are well conserved across *Acropora* species. A detailed screening process identified and quantified the sources of error and bias in the application of these markers (e.g., allele scoring error, failure rates, frequency of null alleles), and may be accounted for in the study of the contemporary gene flow of *A. austera* in the south-western Indian Ocean.

Key words: Acroporidae; Genetic markers; Genotyping; Marker transferability; Gene flow; South-east Africa

Introduction

Microsatellite loci are the genetic markers of choice in studies of gene flow or genetic connectivity, because they are co-dominant, highly polymorphic, species-specific, and offer adequate genetic resolution (e.g., Baums et al., 2006; Ridgway et al., 2008); however, microsatellite markers also have drawbacks. The isolation of novel microsatellite markers is expensive, time-consuming, and requires

genetic information about the target species, which is often missing for non-model organisms (Selkoe and Toonen, 2006). For symbiotic cnidarians, such as acroporid corals, genetic information is obtained from symbiont-free host gametes (e.g., Baums et al., 2005; van Oppen et al., 2007; Nakajima et al., 2009), which is a technique that ensures molecular markers are cnidarian in origin. Mining for microsatellites in public sequence databases (e.g., Wang et al., 2008; Baums et al., 2009) and the cross-species amplification of previously published microsatellite markers (e.g., Nakajima et al., 2009) present alternative ways of overcoming the paucity of symbiont-free host tissue in cnidarians or of genetic information on non-model organisms.

Acropora austera is a reef-building, fast-growing coral, with a high population turnover, and is regarded as being opportunistic among reef corals (Macdonald et al., 2010). This branching coral is widespread across the Indian and Pacific Oceans. Although the species is found in a wide range of habitats on South African reefs, the colonies are more abundant in shallow areas (10–15 m) exposed to a certain amount of wave action (Celliers and Schleyer, 2001). It is the most affected coral species by SCUBA diving on South African reefs, and is very susceptible to coral bleaching (Schleyer MH and Montoya-Maya PH, personal observation). In addition, it is known to be preyed on by the crown-of-thorns starfish (Schleyer MH, unpublished data).

Macdonald et al. (2010) suggested that populations of *A. austera* along the south-east African coast exhibit a latitudinal gradient in genetic diversity (with it being higher in the northern reefs of the region), and that South African and Mozambican populations are connected. However, the same authors found a significant amount of fixation of allele frequencies among populations, which indicates a certain extent of demographic isolation (i.e. at ecological time scales) between *A. austera* populations in southern Mozambique and South Africa. To assess this apparent isolation at ecological time scales, the connectivity of this species is currently being assessed at various spatial scales using assignment methods and spatial autocorrelation analysis, which benefits substantially from the use of microsatellite markers (Manel et al., 2005; Selkoe and Toonen, 2006).

Microsatellite markers were missing for *A. austera*, while the *de novo* development of microsatellite markers was hindered by unsuccessful attempts to isolate symbiont-free host DNA (Montoya-Maya PH, unpublished data, 2011) and the paucity of genetic information on this species in public DNA databases. Cross-species amplification of previously published microsatellite markers was considered as an alternative to overcome these problems. Here, we successfully describe the cross-species amplification of 6 previously published acroporid microsatellite markers in *A. austera* from reefs along the south-western Indian Ocean. This study focuses on the identification of transferable microsatellite markers, marker polymorphism evaluation, and quality control screening. The results of this study will facilitate individual-based genotyping of coral colonies in studies of contemporary genetic connectivity between reefs along the South West Indian Ocean.

Methods

Selection of microsatellite markers for transferability tests

We searched for previously published *Acropora* microsatellite loci in the literature, and selected 21 based on their high polymorphism and transferability across *Acropora* species, with preference being given to markers developed for Indo-Pacific species. Five markers (*MS166, MS181, MS182, MS192, MS207*) were selected that were originally developed for the Caribbean coral *A. palmata* (Baums et al., 2005), 7 markers (*Amil2-02, Amil2-06, Amil2-07, Amil2-08, Amil2-10, Amil2-22, Amil2-23*) for *A. millepora* from the Great Barrier Reef in Australia (van Oppen et al., 2007), and 9 markers (*EST014, EST016, EST032, EST122, EST196, WGS051, WGS092, WGS101, WGS196*) for the same species from the public expressed sequence tag (EST) and whole-genome shotgun (WGS) NCBI databases (http://www.ncbi.nlm.nih.gov/genbank/; Wang et al., 2008).

Coral samples and DNA extraction

A total of 287 tissue samples of *A. austera* from different reefs along the coasts of South Africa and Mozambique were collected by the Oceanographic Research Institute in South Africa, as part of a large coral genetic connectivity study. Sampled reefs included those within the iSimangaliso Wetland

Park in South Africa, and the reefs at Inhaca Island and the Bazaruto Archipelago in Mozambique. Tissue samples were preserved in either 20% dimethyl sulphoxide salt buffer (0.25 M EDTA; 20% (v/v) DMSO, saturated with NaCl) or 96% alcohol (EtOH) in the field, and subsequently stored at room temperature. In the laboratory, DNA was extracted from coral tissue using the ZR Genomic DNA Tissue extraction kit (Zymo Research, Irvine, USA) and following the manufacturer protocol.

Marker transferability, specific amplification, and variability

The 21 selected markers were initially amplified in 8 samples of *A. austera*. Around 50 ng of template was amplified in a 15 µl polymerase chain reaction (PCR) containing 2.5 mM MgCl, 0.5 µM of each primer, 0.2 mM dNTPs, 0.7 mg/ml BSA, and 0.7 U Maxima HS Taq DNA polymerase (Fermentas). *Pfu* DNA polymerase (Fermentas) was added to the Maxima HS DNA polymerase in a 16:1 (unit to unit) ratio, to increase the fidelity and accuracy of PCR amplification (Matz M, personal communication, 2010). The following thermal cycle was used for the PCR: [95°C for 5 min], 35 X [(95°C for 30 s), (annealing temperature, -0.1°C/cycle, for 60 s) (72°C for 60 s)], [72°C for 10 min], [10°C∞]). The annealing temperature was 51°C for all markers, except EST14, which was 61°C. Gel electrophoresis analysis (1% Agarose) indicated unsuccessful amplification in 6 loci (*MS192, EST122, EST196, WGS092, WGS101, WGS196*) and nonspecific amplification (i.e., more than 3 bands) in 4 loci (*MS166, Amil2-08, EST032, WGS051*); these loci were discarded from all subsequent analyses. The amplification of corresponding microsatellite sequences was confirmed by direct sequencing, in both directions, of 1 amplification product from each remaining locus using the Applied Biosystems BigDye Terminator v1.1 chemistry (Perkin-Elmer, Boston, MA) on an ABI 3500 DNA Analyser (data not shown).

Eleven (*Amil2-02, Amil2-06, Amil2-07, Amil2-10, Amil2-22, Amil2-23, MS181, MS182, MS207, EST14, EST16*) successfully amplified microsatellite loci were tested on an additional 20 samples of *A. austera*. PCR products from the 1st and 2nd PCR screening were resolved on 8% polyacrylamide gels to determine size variation. Three loci (*Amil2-02, Amil2-22, EST16*) were invariant, and were excluded from all subsequent analyses.

Genotyping of successfully amplified and variable loci

Labeled primers were ordered for the remaining 8 loci, and used to amplify, in duplicate, the entire collection of 287 samples of *A. austera*. Four PCRs (2 multiplex and 2 standard) were carried out, as described in the previous section, except for primer concentrations, which varied, as shown in Table 1. Products were separated on an ABI 3500 DNA Analyser, and sized using the GeneScan LIZ 600 size standard (Applied Biosystems). Genotypes were compiled using STRand v2.4.59 (Toonen and Hughes, 2001). Scored peaks had a minimum intensity of 5% of the most intense peak, were in phase with the locus repeat motif, and, in the case of rare alleles, were present in 2 replicates. Two loci, *MS207* and *Amil2_06*, were discarded, because they produced ambiguous peak patterns that made allele scoring difficult.

Table 1. Characteristics of six cross-amplified microsatellite loci in 287 samples of *Acropora austera* from the south-western Indian Ocean. The primer concentration in single or multiplex PCR reaction (Plex), number of genotypes (N), number of alleles (N_A), observed (*Ho*) and expected (*He*) heterozygosity, and null allele frequency (*r*) are presented. Significant ($P < 0.05$) deviations from HWE and null allele frequencies are shown in bold.

Locus	Primer sequences (5' – 3')	Plex (µM)	Size range (bp)	N	N_A	H_O/H_E	*r*	Species (source)
Amil2_07	F: FAM-TAATGAGCAAACTCATTCATGG	I	96-126	283	3	**0.028/0.035**	0.05	*A. millepora* (van Oppen et al., 2007)
	R: CTTTTCCAAGAGAAGTCAAGAA	(0.4)						
Amil2_10	F: TET-CAGCGATTAATATTTTAGAACAGTTTT	II	100-156	287	5	**0.014/0.045**	**0.15**	
	R: CGTATAAACAAATTCCATGGTCTG	(0.2)						
Amil2_23	F: HEX-GCAAGTGTTACTGCATCAAA	I	127-133	287	4	**0.035/0.135**	**0.23**	
	R: TCATGATGCTTTACAGGTGA	(0.1)						
MS181	F: FAM-TTCTCCACATGCAAACAAACA	Single	118-205	246	15	**0.642/0.734**	**0.06**	*A. palmata* (Baums et al., 2005)
	R: GCCAGGATAGCGGATAATGA	(0.5)						
MS182	F: FAM-TCCCACAACTCACACTCTGC	II	132-228	236	12	**0.322/0.521**	**0.21**	
	R: ACGCGGAAATAGTGATGCTC	(0.2)						
EST014	F: TET-CAGCTCCTTCATCTTCATCCT	Single	124-166	282	13	**0.663/0.650**	0.01	*A. millepora* (Wang et al., 2008)
	R: AGCCGAAGAGGGGACAGAGT	(0.5)						

Quality control screening of microsatellite loci

As quality control measures, we estimated the allele scoring error and average failure rates for each locus. Allele scoring error was assessed by comparing the duplicated genotypes, and was estimated from the number of incorrect genotypes divided by the number of repeated reactions (i.e., 287). Failure rates corresponded to the percentage of samples that could not be scored for 1 or more loci, either by unsuccessful amplification or unreliable scoring. Micro-Checker v2.2.3 (van Oosterhout et al., 2004) was used to assess microsatellite genotyping errors caused by stuttering and large allele drop-out.

Locus characteristics and Hardy-Weinberg equilibrium

The estimated number of alleles, and observed and expected heterozygosities, were calculated using GenAlEx v6.4 (Peakall and Smouse, 2006). The frequency of null alleles for each locus was estimated by Micro-checker. Departures from Hardy–Weinberg Equilibrium (HWE) and evidence of linkage disequilibrium were tested in Arlequin v3.5.1 (Excoffier et al., 2005).

Results and Discussion

The results demonstrated that 6 microsatellite markers (Table 1) previously developed for *A. millepora* and *A. palmata*, from both genomic DNA libraries and EST databases, could be applied to *A. austera* from the south-western Indian Ocean. This figure provides a 28% success rate (6 out of 21 primer pairs tested) in the cross-species amplification of acroporid microsatellite markers. The figure is comparable to the value of 33% obtained in a similar study in Japan (Nakajima et al., 2009), and is much higher compared to the success rate (<11%) in developing novel microsatellite markers via genomic DNA library construction (van Oppen et al., 2007). Conversely, previous studies have found that many of the markers tested in this study, including those from the Atlantic *A. palmata* (e.g., *MS181, MS182*), successfully amplified reliable microsatellite loci in other Pacific *Acropora* species (Nakajima et al., 2009; Tang et al., 2010). In addition, previous studies have also reported nonspecific amplification in some markers (e.g., *MS166*, van Oppen et al., 2007), indicating that microsatellite sequences might be conserved; however, amplification steps require further optimization (Selkoe and Toonen, 2006). The results of this study and previous research indicate that the microsatellite sequences that exist are well-conserved across *Acropora* species, particularly AAT microsatellites (Tang et al., 2010).

The allele scoring error of successful markers ranged from 0.1% (*Amil2-07*) to 6.4% (*MS181*), and averaged 2.9% across loci. This average scoring error rate is similar to the 2.7% obtained by Underwood et al. (2009) in a study of gene flow in *A. tenuis* using cross-amplified microsatellite markers. Although Micro-checker identified allele scoring errors because of stuttering for locus Amil2-23, the re-analysis of its DNA electropherograms showed no evidence of stuttering in this locus. The average failure rate was 5.9% across loci, and ranged from 0% (*Amil2-10 and Amil2-23*) to 17.8% (*MS182*). No evidence of scoring errors because of large allele drop-out was found in the dataset. It is recommended that the influence of these error rates should be assessed in estimates of gene flow, particularly for those based on individual multilocus genotypes (Selkoe and Toonen, 2006). Fortunately, there are software packages that offer bootstrapping techniques to accomplish this requirement.

Departures from HWE were observed in all loci ($P < 0.05$), while linkage disequilibrium was observed between locus *Amil2-23* and loci *Amil2-10, MS181*, and *EST14* ($P < 0.05$). The departures from HWE observed in these cross-amplified microsatellite markers are not sufficient reason to discard the loci (Selkoe and Toonen, 2006). The heterozygosity deficits that were detected in this study are in agreement with previous studies on corals and other marine organisms (see Underwood et al., 2009). In particular, similar results were observed for nuclear intron sequence data in the same species (Macdonald et al., 2010) and microsatellite loci in *Pocillopora verrucosa* (Ridgway et al., 2008) from the same reefs. Departures from HWE might be caused

either by inbreeding, a Wahlund effect (i.e., mixing of differentiated gene pools leading to the compounding of genotypes from different reefs), or the presence of null alleles (Selkoe and Toonen, 2006). Although inbreeding and clonality might account for some of the HWE deviations, they are most likely caused by a Wahlund effect and the presence of null alleles (Table 1). The presence of null alleles is expected when cross-amplifying microsatellite loci (Selkoe and Toonen, 2006; Chapuis and Estoup, 2007), and is a bias that may be accounted for in population genetic studies (Selkoe and Toonen, 2006; van Oosterhout et al., 2004). The rejection of linkage disequilibrium for Amil2-23 might be linked to the observed departures from HWE (see Excoffier and Slatkin, 1998).

Conclusions

Six carefully screened and selected genetic markers are now available for the study of genetic connectivity of *A. austera*, despite the unavailability of symbiont-free host tissue and genetic information in public DNA databases to develop novel host-specific primers. The microsatellite loci of *Acropora* species appear to be well-conserved, even across transoceanic species. An assessment of the value of these loci as genetic markers in phylogenetic studies of this genus is recommended. It is also encouraging that the sources of error and bias in the application of these markers could be quantified in contrast to similar studies (Selkoe and Toonen, 2006), and may be accounted for in the study of population structure of *A. austera* in the south-western Indian Ocean. Indeed, the population genetic structure of *A. austera* that is inferred from these microsatellite markers is discussed in another paper focusing on the genetic connectivity of this species in this region. Studies of this nature are invaluable in formulating a management strategy to ensure that south-east African coral reefs retain their biodiversity and resilience to climate change.

Acknowledgements

We thank the South African Association for Marine Biological Research (SAAMBR) for financial support, and colleagues for *ad hoc* assistance and help during the fieldwork. PHMM thanks Christiaan Labuschagne from Inqaba Biotechnical Industries (Pty) Ltd and Debora Sweby from SASRI for their instruction in applicable techniques. We are especially grateful for critical comments and suggestions from various anonymous reviewers. A South African National Research Foundation (NRF) PhD grant (to PHMM) supported this work, with additional support from the Western Indian Ocean Marine Association (WIOMSA) through a MASMA program grant (MASMA CC201006) to PHMM and MHS.

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APPENDIX C. Cross-species amplification of nuclear introns using exon-primed intron-crossing (EPIC)-PCR primers.

Introduction

The number of available population-level microsatellite markers and nuclear introns for *Acropora austera* and *Platygyra daedalea* is limited. In Appendix B, six microsatellite markers cross-amplified in *A. austera* are described whereas Miller and Howard (2003) provided five microsatellite loci for *P. daedalea*. Correspondingly, two nuclear introns have been used in population genetic studies of these two species, namely the carbonic anhydrase (*CAH*) *3-550* nuclear intron of *A. austera* and the nuclear ribosomal ITS region of *P. daedalea* (Macdonald et al 2008). Although *CAH 3-550* was found to be very informative for *A. austera*, this was not the case for the ITS region in *P. daedalea* and its use as a population level marker is discouraged (Macdonald et al 2008).

Because the assessment of contemporary genetic events benefits substantially when large numbers of hyper variable markers (e.g. microsatellites and nuclear introns) are used (Cornuet et al. 1999; Bernatchez and Duchesne 2000; Christie 2010), efforts to obtain additional nuclear intron markers were pursued. Accordingly, metazoan nuclear intron markers that had EPIC-PCR primers were sought in the literature and transferability tests were conducted in *P. daedalea* and *A. austera*. Exon-primed intron-crossing – Polymerase Chain Reaction (EPIC-PCR) is a technique whereby primers for the PCR amplification of introns are designed in the flanking exons of the gene of interest. By following this approach, cloning can be avoided and cross-species amplification becomes easier because the exon sequences flanking the introns are well conserved across species (Palumbi & Baker 19945; Gupta and Varshney 2000; Bierne et al 2000). This Appendix presents the results of this effort. Although laborious, the exercise yielded nuclear intron loci suited to the analysis of contemporary gene flow in *A. austera* and *P. daedalea*.

Material and Methods

Selection of previously published molecular markers

Selected nuclear introns for testing included an EPIC-PCR primer pair for the second intron from the *Calmodulin* gene (*Calm 2*) in corals (Vollmer and Palumbi 2002); one pair for an intron in the *β-Tubulin* gene in faviids (Lopez & Knowlton 1997); one pair for a single intron from the signal recognition particle 54-kDa subunit (*SRP54*) of this polypeptide complex (Jarman et al. 2002; Frade et al. 2010); and, one for a single intron in the nuclear gene for the alpha subunit of the ATP Synthase complex (*ATPSα*) in the genus *Madracis* (Frade et al. 2010).

Transferability test

The four intron regions were initially amplified in eight samples of each species collected from different reefs in South Africa and Mozambique. DNA was extracted from coral tissue using the ZR Genomic DNA Tissue extraction kit (Zymo Research). Around 50 ng of template was amplified in a 25 µl polymerase chain reaction (PCR) containing 2.5 mM MgCl, 0.5 µM of each primer, 0.2 mMdNTPs, 0.7 mg ml-1 BSA and 1 U of Maxima HS Taq DNA polymerase (Fermentas). Pfu DNA polymerase (Fermentas) was added to the Maxima HS DNA polymerase in a 16:1 (unit to unit) ratio to increase the fidelity and accuracy of PCR amplification. The following thermal cycle was used for the PCR: [95°C for 5 minutes], 35 X [(95°C for 30 seconds), (annealing temperature for 60s) (72°C for 60s)], [72°C for 10m], [10°C∞]. Annealing temperatures were as described in published protocols.

To assess amplification success and primer specificity electrophoresis of PCR products was performed on 1% Agarose gels. Only loci that amplified across all eight samples of each species and that produced only one or two bands were kept for further analysis. Bands of similar sizes to the ones expected from the literature were then excised from gels and purified using the MG Gel Purification kit. Purified products were sent to Macrogen Europe (Netherlands) to be cycle-sequenced, in both directions, with respective forward and reverse primers on an ABI 3500 DNA Analyser. Sequence chromatograms were edited and aligned in Geneious Pro v5.6.2 (Biomatters, available from http://www.geneious.com). The resulting consensus sequences were checked against corresponding gene sequences using the BLAST tool (Madden 2002); in so doing, unspecific products were identified and the corresponding loci were discarded. These series of steps resulted in primer pairs for amplification of the *β-Tubulin* gene intron and *SRP54* discarded.

Design of species-specific EPIC-PCR primers

Although the EPIC primer sets previously available for the nuclear introns *Calm 2* and *ATPSα* successfully amplified all samples, they still produced non-optimal survey PCR amplifications. Therefore, new exon located reverse primers were designed for each species by identifying alternative nested exon-located conserved regions among the preliminary sequence alignments. Identified exon/intron boundaries were confirmed by comparison with sequences retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Reverse primer pairs were designed using the Primer3 function in Geneious Pro v5.6.2 with 60°C as the annealing temperature and were located at least 25 bp from the exon/intron boundary.

Genotyping and analysis of intron loci

The new species specific primer pairs (Table C.1) were used to amplify the entire tissue collection (i.e. 287 samples of *A. austera* and 311 of *P. daedalea*). PCR amplifications were carried out as described above except for the annealing temperature which was 56°C for both introns. PCR amplification was checked by Agarose electrophoresis. When no PCR product was observed, the failing sample was re-amplified from the initial PCR mixture (1ul as template). If unsuccessful, a new PCR mix was prepared and amplified following a touchdown PCR protocol. Successful PCR products were cycle-sequenced directly in the forward direction using Applied Biosystems BigDye Terminator v1.1 chemistry (Perkin-Elmer, Boston, MA) on an ABI 3500 DNA Analyser. When the forward sequence chromatogram had low HD scores (<20%) or mixture of DNA templates was evident, the corresponding PCR product was cycle-sequenced directly in the reverse direction.

Table C.1. Primers used for the PCR amplification of species specific sequences of two single copy nuclear DNA introns (i.e. Calmodulin 2 and ATPSα) in *A. austera* and *P. daedalea*. Data presented are forward (F) and reverse (R-) primers and analysed locus length (bp) for each species.

Locus	Species	Primer	Sequence (5'-3')	Length (bp)
Calm 2		F	GCT GAT CAA CTT ACA GAG GAA CAA	
	Acropora austera	R-Aa	TTG CCC AAG AGA TCG CAT CA	193
	Platygyra daedalea	R-Pd	TTG TCC AAG AGA TCG CAT CA	194
ATPSα		F	ACG AGA ACT TAT CAT TGG AGA CAG	
	Acropora austera	R-Aa	GGT GTC AAT AGC AAT TGC AG	156
	Platygyra daedalea	R-Pd	GGT GTC AAT TGC AAT GGC AG	158

Sequence alignment, editing and analysis

Sequences were aligned and edited in Geneious Pro v.5.62 and checked by eye. For both introns, PCR amplification products from several individuals contained mixed sequences of different length or were heterozygous for a deletion event. Mixed sequences were unravelled with the help of CHAMPURU v1.0 (Flot et al. 2007). For individuals with indels, the find heterozygotes plug-in for Geneious was used to assign ambiguity codes for positions downstream of the deletion event and then reconstructed the allelic sequences using Indelligent v1.2 (Dmitriev & Rakitov 2008). Haplotype allelic pairs were reconstructed for the remaining individuals using PHASE (Stephens et al. 2001; Stephens & Scheet 2005) in combination with SeqPhase (Flot 2010). All gaps were coded as a fifth character state. Ten replicate PHASE runs were used for each locus, using 100 iterations, a thinning interval of 1, and a burn-in of 100 per run. Pair probabilities of reconstructed haplotypes for individuals that were heterozygous for more than one site were above 90%. Descriptive statistics (e.g. number of haplotypes, haplotype diversity and nucleotide diversity) and neutrality tests for each nuclear intron for each species were calculated for the pooled samples using DnaSP version 4.50 (Rozas et al. 2003) and Arlequin v3.5 (Excoffier & Lischer 2010).

Results

Analysis of the direct sequencing of PCR products from the two nuclear introns was problematic for some samples. Phase reconstruction for samples with chromatograms showing sequencing mixtures of DNA templates were not straightforward. Mix DNA sequences resulting from putative length variations or heterozygous samples and not from poor DNA extraction or PCR conditions were assumed to be real when the same sample produced good quality chromatogram for at least one intron. Although the presence of more than two haplotypes per individual in introns of the *Calmodulin* and the *ATPSβ* genes in species of *Pocillopora* has been recorded before (Flot et al. 2008), samples with PCR products that contained more than one sequence for both introns were discarded from further analysis and assumed to be a consequence of poor preservation resulting in bad DNA templates for PCR amplification. The following results can then be an underestimation of the genetic diversity estimated from these two nuclear introns.

Acropora austera

Eight samples failed to amplify the *ATPSα* nuclear intron despite several PCR attempts. *ATPSα* sequences from 19 samples could not be resolved and were discarded. In total, seventy-six *ATPSα* alleles were reconstructed among 260 individuals from nine sampled reefs. The trimmed, aligned sequence length was 156 bp and the G + C content 35.7%. There were 78 variable sites, with an average of 10.07 nucleotide differences between sequences (Table C.2). The nucleotide diversity per site (π) was 0.081 (± 0.008). The overall haplotype diversity was 0.832 (± 0.014). Values calculated for Tajima's D and Fu's statistic were not significant and confirm that the *ATPSα* locus conforms to neutral expectations. Thirty-one InDels sites were found which yielded seven InDel haplotypes assuming a multiallelic gap model. A minimum of nine in recombination events was identified in DnaSP.

The success rate of *Calm 2* was lower when compared to *ATPSα*. *Calm 2* sequences from 53 samples could not be resolved either because they produced mix sequences (42 samples) or completely failed to amplify (11 samples). In total, twenty-nine *Calm 2* alleles were reconstructed among 235 individuals from nine sampled reefs. The trimmed, aligned sequence length was 193 bp and the G + C content 34.9%. There were 122 variable sites, with an average of 49.03 nucleotide differences between sequences. The nucleotide diversity per site (π) was 0.316 (± 0.007 SE). The overall haplotype diversity was 0.661 (± 0.017). Neutrality tests were significant ($P < 0.02$) and suggest that the *Calm 2* locus might be under selection. Thirty-eight InDels sites were found which yielded five InDel haplotypes. A minimum of six recombination events was identified in DnaSP.

Table C.2. Sequence statistics for each of the two intron loci screened in *Acropora austera* and *Platygyra daedalea* from various South African and Mozambican reefs. N = number of sequences; S = number of sites; Sv = number of variable sites; GC = % of GC content; *h* = number of unique haplotypes; *hd* = haplotype diversity (SE); *k* = average number of nucleotide differences; π = nucleotide diversity (SE); D = Tajima's D; Fu = Fu's D*. Values in bold indicate significance p< 0.02.

	N	S	Sv	GC	*h*	*hd*	π	D	Fu
Acropora austera									
ATPSα	260	156	78	35.7	76	0.832 (0.014)	0.081 (0.008)	-0.882	1.539
Calm 2	238	193	122	34.9	29	0.661 (0.017)	0.316 (0.007)	**3.228**	2.990
Platygyra daedalea									
ATPSα	306	158	61	34.0	54	0.676 (0.018)	0.026 (0.002)	-1.797	-0.139
Calm 2	306	194	40	39.1	38	0.380 (0.024)	0.004 (0.001)	**-2.479**	-1.553

Platygyra daedalea

All samples of *P. daedalea* amplified both nuclear introns. However, five samples produced mixed sequences for which phase determination could not be resolved. In total, fifty-four *ATPSα* alleles were reconstructed among 306 individuals from nine sampled reefs. The trimmed, aligned sequence length was 158 bp and the G + C content 34%. There were 61variable sites, with an average of 3.759 nucleotide differences between sequences. The overall haplotype diversity (0.676 ± 0.018) and nucleotide diversity per site (0.026 ± 0.002) were lower than in *A. austera*. Neutrality tests suggest that, as in *A. austera*, the *ATPSα* locus in *P. daedalea* is evolving as a neutral marker. Thirteen InDels sites were found which yielded six InDel haplotypes. A minimum of four recombination events was identified in DnaSP.

Calm 2 in *P. daedalea* was the least variable marker. Thirty-eight *Calm 2* alleles were reconstructed among 306 individuals from nine sampled reefs. The trimmed, aligned sequence length was 194 bp and the G + C content 39%. There were only 40 variable sites, with an average of 0.563 nucleotide differences between sequences. The nucleotide diversity per site (π) was 0.004 (± 0.001) while the overall haplotype diversity was 0.380 (± 0.024). Neutrality tests suggest that similarly to *Calm 2* in *A. austera*, this nuclear intron appears to be under selection in *P. daedalea*, although only for Tajima's D (Table C.2.). Thirty-three InDels sites were found which yielded four InDel haplotypes. A minimum of two recombination events was identified in DNASP.

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APPENDIX D. Estimates of genetic diversity

Table D.1 Large-scale (between-reef) genetic diversity estimates for *A. austera* on the south-east coast of Africa. N= number of samples; A_r= mean number of alleles; A_p = mean number of private alleles; H_e= expected heterozygosity; F_{IS}= Inbreeding coefficient (* = significant departures from Hardy-Weinberg equilibrium after FDR at 0.05, ** = significant departures from Hardy-Weinberg equilibrium at 0.01).

Pop		*Amil2_07*	*Amil2_10*	*Amil2_23*	*EST14*	*MS181*	*MS182*	*ATPs*	*Calm 2*	*CAH3550*	All loci
REU	N	6	6	6	6	6	6	6	6	6	6
	A_r	1.00	1.00	1.00	2.00	3.00	3.00	5.00	4.00	4.00	2.67
	A_p	0.00	0.00	0.00	1.00	0.00	0.01	3.63	0.00	0.76	0.60
	H_e	0.00	0.00	0.00	0.48	0.68	0.32	0.76	0.80	0.45	0.39
	F_{IS}	-	-	-	-0.50	-0.60	0.43	0.52	0.32	0.20	0.15
BAZ	N	20	20	20	18	19	17	18	13	20	20
	A_r	1.67	1.78	2.55	4.58	2.96	3.47	6.48	7.68	4.92	4.01
	A_p	0.11	0.20	0.50	1.12	0.00	1.57	3.41	2.73	2.37	1.33
	H_e	0.14	0.18	0.35	0.77	0.65	0.42	0.81	0.91	0.76	0.54
	F_{IS}	-0.08	1.00*	1.00*	0.04	0.33	0.71**	0.72**	0.21**	0.46**	0.47**
INH	N	13	13	13	13	12	9	13	8	13	13
	A_r	1.72	1.72	1.99	4.30	4.26	4.14	6.39	3.98	3.64	3.57
	A_p	0.14	0.15	0.00	0.46	1.17	0.58	1.90	0.00	1.07	0.61
	H_e	0.15	0.15	0.37	0.73	0.68	0.56	0.85	0.75	0.66	0.54
	F_{IS}	-0.08	1.00	1.00*	0.01	0.11	0.58	0.62*	0.29**	0.88**	0.50**
RAB	N	17	18	18	18	18	13	16	17	17	18
	A_r	1.00	1.00	1.33	2.82	4.63	3.16	7.99	3.65	2.71	3.14
	A_p	0.00	0.00	0.00	0.00	0.99	0.02	2.96	1.50	0.35	0.65
	H_e	0.00	0.00	0.06	0.60	0.75	0.41	0.91	0.65	0.57	0.44
	F_{IS}	-	-	-0.03	-0.04	0.08	0.21	0.28**	0.44*	0.36	0.25**
NMR	N	17	17	17	16	15	13	15	14	15	17
	A_r	1.94	2.55	3.16	5.19	4.51	4.29	6.00	4.86	6.16	4.22
	A_p	0.62	1.55	0.98	1.24	1.60	0.61	2.75	1.45	2.39	1.47
	H_e	0.17	0.32	0.51	0.79	0.70	0.52	0.82	0.69	0.82	0.59
	F_{IS}	0.64	0.42	0.29	0.10	0.21	0.39	0.83**	0.68**	0.33**	0.44**
FMR	N	15	16	16	16	15	12	16	14	16	16
	A_r	1.00	1.00	1.38	2.75	2.99	2.39	5.48	4.82	2.52	2.70
	A_p	0.00	0.00	0.00	0.00	0.00	0.06	2.86	0.80	0.15	0.43
	H_e	0.00	0.00	0.06	0.56	0.67	0.30	0.68	0.72	0.29	0.36
	F_{IS}	-	-	-0.03	-0.27	-0.54	0.42	0.24	0.38	0.32	0.07
TMR	N	85	86	86	86	68	75	72	71	85	86
	A_r	1.07	1.07	1.07	3.73	4.29	3.41	6.48	4.15	3.96	3.25
	A_p	0.00	0.00	0.00	0.22	0.27	0.06	2.73	1.33	1.05	0.63
	H_e	0.01	0.01	0.01	0.66	0.75	0.55	0.81	0.66	0.63	0.44
	F_{IS}	-0.01	-0.01	-0.01	-0.16	0.09	0.32	0.62**	0.63**	0.25*	0.29**
RED	N	21	21	21	20	20	18	21	18	21	21
	A_r	1.00	1.00	1.76	3.33	3.62	3.12	5.78	4.90	3.14	3.07
	A_p	0.00	0.00	0.00	0.10	0.02	0.57	1.81	1.32	0.70	0.50
	H_e	0.00	0.00	0.18	0.63	0.69	0.35	0.67	0.69	0.59	0.42
	F_{IS}	-	-	1.00*	0.10	-0.04	0.35	0.49**	0.26	0.59**	0.34**
LEA	N	22	22	22	22	21	15	21	17	22	22
	A_r	1.00	1.00	1.87	4.08	3.91	2.05	7.06	4.67	3.09	3.19
	A_p	0.00	0.00	0.00	0.15	0.08	0.03	2.76	1.69	0.60	0.59
	H_e	0.00	0.00	0.24	0.71	0.74	0.19	0.86	0.58	0.57	0.44
	F_{IS}	-	-	1.00**	0.48**	0.07	-0.08	0.37**	0.27	0.51*	0.39**

Table D.2 Small-scale (within-reef) genetic diversity estimates for *A. austera* on TMR. N= number of samples; A_r= mean number of alleles; A_p = mean number of private alleles; H_e= expected heterozygosity; F_{IS}= Inbreeding coefficient (* = significant departures from Hardy-Weinberg equilibrium after FDR at 0.05, ** = significant departures from Hardy-Weinberg equilibrium at 0.01).

Site		*Amil2_07*	*Amil2_10*	*Amil2_23*	*EST14*	*MS181*	*MS182*	*ATPsα*	*Calm 2*	*CAH 3550*	All loci
N_OFF	N	15	16	16	16	10	13	14	13	16	16
	A_r	1	1	1	3.1	3	2.59	4.47	3.42	2.41	2.45
	A_p	0.00	0.00	0.00	0.72	0.00	0.00	1.12	1.03	0.40	0.36
	H_e	0	0	0	0.72	0.7	0.54	0.86	0.74	0.52	0.45
	F_{IS}	-	-	-	0.05	-0.46	0.16	0.60*	0.70*	0.04	0.23
C_OFF	N	8	9	9	9	9	9	8	7	9	9
	A_r	1	1	1	2.97	3.6	1	5.07	1.86	2.85	2.26
	A_p	0.00	0.00	0.00	0.14	0.01	0.00	1.94	0.43	0.17	0.30
	H_e	0	0	0	0.68	0.79	0	0.93	0.27	0.66	0.37
	F_{IS}	-	-	-	-0.16	0.45		0.21	-0.04	0.34	0.21
SC_OFF	N	43	43	43	42	40	37	42	38	43	43
	A_r	1	1	1.27	2.46	3.42	1.31	3.92	2	2.48	2.1
	A_p	0.00	0.00	0.05	0.02	0.17	0.00	1.16	0.40	0.27	0.23
	H_e	0	0	0.09	0.56	0.75	0.11	0.8	0.33	0.57	0.36
	F_{IS}	-	-	0.49*	0.19	0.44*	0.49	0.65	0.44	0.31	0.43
S_OFF	N	6	6	6	6	6	6	6	5	6	6
	A_r	1.77	1	1	2.55	3.56	2	4.15	2.8	4.29	2.57
	A_p	0.23	0.00	0.00	0.00	0.00	0.00	0.53	0.00	1.43	0.24
	H_e	0.3	0	0	0.55	0.8	0.32	0.85	0.53	0.86	0.47
	F_{IS}	-0.11	-	-	0.41	-0.04	0.5	0.63*	0.27	0.04	0.25
N_IN	N	17	17	17	17	6	14	10	13	16	17
	A_r	1	1	1	2.29	3.23	3.25	3.68	2.11	3.62	2.35
	A_p	0.00	0.00	0.00	0.01	0.03	0.01	0.65	0.77	0.94	0.27
	H_e	0	0	0	0.56	0.74	0.75	0.78	0.35	0.75	0.44
	F_{IS}	-	-	-	-0.6	0.11	-0.25	0.88*	0.35	0.26	0.09
C_IN	N	24	24	24	24	18	21	19	19	24	24
	A_r	1	1.13	1	2.64	3.16	3.31	3.18	2.72	3.33	2.38
	A_p	0.00	0.04	0.00	0.04	0.02	0.20	1.18	0.64	0.27	0.26
	H_e	0	0.04	0	0.63	0.69	0.75	0.63	0.62	0.72	0.45
	F_{IS}	-	0	-	-0.54	0.2	0.24	0.59*	0.50*	0.02	0.14
SC_IN	N	28	28	28	28	21	23	22	22	28	28
	A_r	1	1	1	1.98	1.98	2.1	4.9	2.39	3.37	2.19
	A_p	0.00	0.00	0.00	0.00	0.00	0.00	2.28	0.93	0.31	0.39
	H_e	0	0	0	0.51	0.51	0.53	0.91	0.43	0.73	0.4
	F_{IS}	-	-	-	-1	-1	-0.76	0.56*	0.59*	0.22	-0.14
S_IN	N	4	4	4	4	4	4	4	3	4	4
	A_r	1	1	1	3.46	3.46	1	4.21	1	1.75	1.99
	A_p	0.00	0.00	0.00	0.00	0.08	0.00	1.51	0.00	0.61	0.24
	H_e	0	0	0	0.75	0.75	0	0.86	0	0.25	0.29
	F_{IS}	-	-	-	-0.41	0		0.45		0	0.05

Table D.3 Large-scale (between-reef) genetic diversity estimates for *P. daedalea* on the south-east coast of Africa. N= number of samples; A_r= mean number of alleles; A_p = mean number of private alleles; H_e= expected heterozygosity; F_{IS}= Inbreeding coefficient (* = significant departures from Hardy-Weinberg equilibrium after FDR at 0.05, ** = significant departures from Hardy-Weinberg equilibrium at 0.01).

Pop		*Pd29-2*	*Pd31*	*Pd48*	*Pd61*	*Pd62*	*ATPs*	*Calm 2*	All loci
CHA	N	10	7	9	10	10	10	10	10
	A_r	1.40	2.83	1.44	2.63	1.00	2.05	2.85	2.03
	A_p	0.09	0.00	0.00	0.00	0.00	0.66	1.95	0.39
	H_e	0.10	0.60	0.10	0.54	0.00	0.27	0.42	0.29
	F_{IS}	-0.05	-0.42	-0.06	-0.48	-	-0.13	0.52*	-0.10
KEN	N	3	3	3	1	3	3	3	3
	A_r	1.00	1.73	1.00	1.00	1.00	1.53	1.93	1.31
	A_p	0.00	0.00	0.00	0.00	0.00	0.02	1.06	0.15
	H_e	0.00	0.61	0.00	0.00	0.00	0.44	0.78	0.26
	F_{IS}		0.45				1.00	-0.29	0.39
TAN	N	8	7	4	6	8	8	8	8
	A_r	1.50	3.46	2.00	3.57	1.00	4.83	3.53	2.84
	A_p	0.50	0.29	0.03	0.00	0.00	1.14	1.31	0.47
	H_e	0.12	0.65	0.22	0.68	0.00	0.78	0.57	0.43
	F_{IS}	-0.07	0.13	-0.14	-0.22	-	1.00	0.78**	0.25**
BAZ	N	15	15	13	15	15	12	14	15
	A_r	1.00	3.18	2.45	2.00	1.27	4.83	2.33	2.44
	A_p	0.00	0.27	0.67	0.00	0.05	2.27	0.57	0.55
	H_e	0.00	0.68	0.34	0.29	0.06	0.78	0.36	0.36
	F_{IS}	-	-0.17	0.32	-0.16	-0.03	0.57**	0.60*	0.19**
PEM	N	11	11	10	10	12	12	12	12
	A_r	1.76	2.60	1.95	3.01	1.00	5.65	2.84	2.69
	A_p	0.46	0.00	0.00	0.29	0.00	1.64	1.12	0.50
	H_e	0.24	0.57	0.38	0.53	0.00	0.86	0.54	0.44
	F_{IS}	-0.16	0.05	0.73	0.06	-	0.51**	0.85**	0.34**
INH	N	12	12	12	10	12	12	12	12
	A_r	1.00	3.21	1.67	1.40	1.33	4.75	1.98	2.19
	A_p	0.00	0.11	0.35	0.00	0.07	1.79	0.01	0.33
	H_e	0.00	0.67	0.16	0.10	0.08	0.77	0.44	0.32
	F_{IS}	-	-0.11	-0.07	-0.05	-0.04	0.57**	1.00	0.21**
RAB	N	18	17	17	18	18	18	18	18
	A_r	1.54	2.40	2.33	2.42	1.62	5.53	4.16	2.86
	A_p	0.54	0.00	0.34	0.00	0.54	1.85	1.60	0.70
	H_e	0.15	0.53	0.44	0.38	0.16	0.85	0.64	0.45
	F_{IS}	-0.09	0.22	-0.33	0.11	0.64	0.28**	0.31	0.16**
NMR	N	15	14	15	14	14	15	15	15
	A_r	1.47	3.16	2.27	2.28	1.50	4.82	4.07	2.80
	A_p	0.47	0.20	0.58	0.17	0.14	1.51	1.87	0.71
	H_e	0.12	0.67	0.30	0.31	0.13	0.77	0.65	0.42
	F_{IS}	1.00	0.04	0.10	0.32	-0.08	0.65**	0.28*	0.33**
FMR	N	11	11	11	11	11	11	11	11
	A_r	1.00	2.76	1.00	2.71	1.00	3.91	3.67	2.29
	A_p	0.00	0.00	0.00	0.34	0.00	0.57	1.72	0.38
	H_e	0.00	0.61	0.00	0.55	0.00	0.62	0.57	0.33
	F_{IS}	-	0.10	-	0.00	-	0.41*	0.04	0.14
TMR	N	118	116	116	116	118	118	118	118
	A_r	1.13	3.06	1.48	2.50	1.26	4.88	3.12	2.49
	A_p	0.13	0.28	0.04	0.08	0.15	1.75	0.99	0.49
	H_e	0.03	0.65	0.14	0.41	0.07	0.79	0.52	0.37
	F_{IS}	0.24	-0.07	0.16	0.29*	0.49*	0.43**	0.36	0.27**
RED	N	20	20	17	20	20	20	19	20
	A_r	1.00	3.14	1.24	2.84	1.20	4.94	3.41	2.54
	A_p	0.00	0.18	0.01	0.00	0.04	1.23	1.37	0.40
	H_e	0.00	0.66	0.06	0.55	0.05	0.81	0.52	0.38
	F_{IS}	-	0.08	-0.03	0.09	-0.03	0.50**	0.09	0.12**

Pop		*Pd29-2*	*Pd31*	*Pd48*	*Pd61*	*Pd62*	*ATPs*	*Calm 2*	All loci
LEA	N	16	16	14	17	17	16	15	17
	A_r	1.70	2.23	1.29	2.72	1.24	4.37	3.74	2.47
	A_p	0.70	0.00	0.02	0.00	0.04	2.01	1.92	0.67
	H_e	0.22	0.49	0.07	0.49	0.06	0.71	0.58	0.37
	F_{IS}	1.00*	-0.40	-0.04	0.16	-0.03	0.47**	0.08	0.18**

Table D.4 Small-scale (within-reef) genetic diversity estimates for *P. daedalea* on TMR. N= number of samples; A_r= mean number of alleles; A_p = mean number of private alleles; H_e= expected heterozygosity; F_{IS}= Inbreeding coefficient (* = significant departures from Hardy-Weinberg equilibrium after FDR at 0.05, ** = significant departures from Hardy-Weinberg equilibrium at 0.01).

Site		*Pd29-2*	*Pd31*	*Pd48*	*Pd61*	*Pd62*	*ATPs*	*Calm 2*	All loci
N_OFF	N	14	14	14	14	14	14	14	14
	A_r	1.00	4.92	2.00	2.93	1.93	9.63	2.00	3.49
	A_p	0.00	0.01	0.00	0.12	0.12	2.38	0.00	0.38
	H_e	0.00	0.67	0.20	0.26	0.07	0.82	0.25	0.33
	F_{IS}	-	-0.22	-0.12	-0.13	-0.04	0.46*	1.00*	0.16**
C_OFF	N	16	16	15	15	16	16	16	16
	A_r	1.00	4.62	2.00	3.87	1.81	11.72	3.62	4.09
	A_p	0.00	0.00	0.00	0.00	0.81	3.26	0.81	0.70
	H_e	0.00	0.65	0.19	0.49	0.06	0.86	0.33	0.37
	F_{IS}	-	0.01	-0.11	0.30	-0.03	0.40**	0.61*	0.20**
SC_OFF	N	15	15	15	15	15	15	15	15
	A_r	1.00	3.87	1.00	2.87	1.87	7.56	8.32	3.78
	A_p	0.00	0.00	0.00	0.06	0.00	1.32	2.81	0.60
	H_e	0.00	0.71	0.00	0.34	0.07	0.76	0.67	0.36
	F_{IS}	-	-0.37	-	0.19	-0.03	0.73**	0.18	0.14**
S_OFF	N	17	17	17	17	17	17	17	17
	A_r	1.95	4.53	1.76	3.52	1.00	6.59	7.49	3.83
	A_p	0.95	0.00	0.00	0.76	0.00	1.54	3.16	0.92
	H_e	0.11	0.67	0.06	0.27	0.00	0.59	0.50	0.32
	F_{IS}	-0.06	-0.09	-0.03	0.33	-	0.48**	0.04	0.11*
N_IN	N	20	20	20	20	20	20	20	20
	A_r	2.30	3.53	1.99	3.65	1.00	10.85	7.19	4.36
	A_p	1.30	0.00	0.00	0.00	0.00	3.74	3.34	1.20
	H_e	0.10	0.58	0.18	0.51	0.00	0.85	0.51	0.39
	F_{IS}	0.48	-0.05	0.44	0.30	-	0.33*	0.39**	0.32**
C_IN	N	14	14	14	14	14	14	14	14
	A_r	1.00	3.93	1.93	3.93	2.00	9.43	3.86	3.72
	A_p	0.00	0.93	0.00	0.00	0.13	4.65	0.00	0.82
	H_e	0.00	0.68	0.07	0.52	0.25	0.63	0.32	0.35
	F_{IS}	-	-0.09	-0.04	0.28	1.00	0.30	0.54*	0.32**
SC_IN	N	16	16	15	15	16	16	16	16
	A_r	1.00	4.44	1.99	3.99	1.00	8.99	5.41	3.83
	A_p	0.00	0.00	0.00	0.00	0.00	2.89	1.78	0.67
	H_e	0.00	0.60	0.13	0.53	0.00	0.71	0.57	0.36
	F_{IS}	-	0.14	-0.07	0.35	-	0.36**	0.44	0.24**
S_IN	N	15	13	15	15	15	15	15	15
	A_r	1.00	4.00	2.00	2.87	1.87	10.40	6.85	4.14
	A_p	0.00	0.07	0.00	0.00	0.06	2.07	0.33	0.36
	H_e	0.00	0.70	0.24	0.30	0.07	0.85	0.73	0.41
	F_{IS}	-	-0.14	0.42	0.30	-0.03	0.43**	0.05	0.17**

Table D.5 Genetic diversity estimates (mean and standard error) for each size class (i.e. temporal genetic diversity) of *A. austera* and *P. daedalea* on TMR. N= number of samples; A_r= mean number of alleles; A_p = mean number of private alleles; H_e= expected heterozygosity; F_{IS}= Inbreeding coefficient (* = significant departures from Hardy-Weinberg equilibrium after FDR at 0.05, ** = significant departures from Hardy-Weinberg equilibrium at 0.01).

	Acropora austera				***Platygyra daedalea***		
Population	**Size-class 0**	**Size-class 1**	**Size-class 2**		**Size-class 0**	**Size-class 1**	**Size-class 2**
N	60	61	24	N	7	22	91
$A_{r(36)}$	5.14 (1.56)	4.98 (1.29)	5.01 (1.14)	$A_{r(14)}$	2.29 (0.56)	2.95 (0.77)	2.96 (0.66)
$A_{p(36)}$	1.85 (0.98)	1.68 (0.89)	1.74 (0.79)	$A_{p(14)}$	0.32 (0.20)	0.96 (0.58)	0.93 (0.45)
H_e	0.46 (0.12)	0.45 (0.12)	0.47 (0.11)	H_e	0.26 (0.11)	0.35 (0.12)	0.38 (0.11)
F_{IS}	0.26* (0.12)	0.26* (0.09)	0.18* (0.12)	F_{IS}	0.31 (0.23)	0.32* (0.17)	0.20* (0.07)

APPENDIX E. Null allele effects on estimates of genetic variation

Table E.1. Estimation of global F_{ST} of Weir (1996) both using and without using the ENA correction described in Chapuis and Estoup (2007). 95% Confidence Interval (CI) obtained by bootstrap resampling over loci.

Acropora austera – Large-scale

All loci

F_{ST} not using ENA	F_{ST} using ENA
0.032575	0.050522

Per locus

Locus	F_{ST} not using ENA	F_{ST} using ENA
Amil2_07	0.034607	0.08227
Amil2_10	0.069322	0.162167
Amil2_23	0.094816	0.184335
MS181	0.037501	0.041921
MS182	0.018776	0.019145
EST14	0.014332	0.021595

Bootstrap resampling over loci
95% Confidence Interval

F_{ST} not using ENA	F_{ST} using ENA
0.019435	0.025485
0.060001	0.113465

Friedman's Test χ^2= 7, *df* = 1, *P*-value = 0.008151

Platygyra daedalea – Large scale

All loci

F_{ST} not using ENA	F_{ST} using ENA
0.036293	0.041334

Per locus

Locus	F_{ST} not using ENA	F_{ST} using ENA
Pd29-2	0.046523	0.108128
Pd-31	0.033956	0.032684
Pd-48	0.064257	0.069492
Pd_61	0.03407	0.033721
Pd_62	-0.01327	0.018654

Bootstrap resampling over loci
95% Confidence Interval

F_{ST} not using ENA	F_{ST} using ENA
0.024696	0.031479
0.050465	0.069048

Friedman's Test χ^2= 0.6667, *df* = 1, *P*-value = 0.4142

Acropora austera - TMR

All loci

F_{ST} not using ENA	F_{ST} using ENA
0.212859	0.212159

Per locus

Locus	F_{ST} not using ENA	F_{ST} using ENA
Amil2_07	0.169963	0.15636
Amil2_10	-0.00818	-0.01153
Amil2_23	-0.01392	0.058703
MS182	0.052978	0.046225
EST14	0.396832	0.402812

Bootstrap resampling over loci
95% Confidence Interval

F_{ST} not using ENA	F_{ST} using ENA
0.018457	0.045743
0.389530	0.394391

Friedman's Test χ^2= 0.6667, *df* = 1, *P*-value = 0.4142

Platygyra daedalea - TMR

All loci

F_{ST} not using ENA	F_{ST} using ENA
0.004576	0.016247

Per locus

Locus	F_{ST} not using ENA	F_{ST} using ENA
Pd29-2	0.003918	0.002535
Pd-31	0.005761	0.006152
Pd-48	-0.00656	0.019977
Pd_61	0.00242	0.012156
Pd_62	0.030267	0.124064

Bootstrap resampling over loci
95% Confidence Interval

F_{ST} not using ENA	F_{ST} using ENA
-0.000697	0.007136
0.010988	0.064785

Friedman's Test χ^2= 2.6667, *df* = 1, *P*-value = 0.1025

Table E.2 Pairwise F_{ST} by all loci estimated both without using (a) and with using (b) ENA-corrected allele frequencies.

Acropora austera – Large-scale

Pairwise F_{ST} not using ENA

pop	REU	BAZ	INH	RAB	NMR	FMR	TMR	RED
BAZ	0.075							
INH	0.120	-0.001						
RAB	0.165	0.043	0.030					
NMR	0.112	0.020	0.001	0.023				
FMR	0.159	0.038	0.061	0.014	0.050			
TMR	0.138	0.056	0.033	-0.004	0.054	0.029		
RED	0.150	0.033	0.001	-0.011	0.019	0.018	0.008	
LEA	0.125	0.003	0.004	-0.001	0.026	0.014	0.022	0.000

Pairwise F_{ST} using ENA

pop	REU	BAZ	INH	RAB	NMR	FMR	TMR	RED
BAZ	0.124							
INH	0.157	0.002						
RAB	0.163	0.077	0.068					
NMR	0.134	0.014	0.003	0.048				
FMR	0.159	0.076	0.095	0.013	0.074			
TMR	0.146	0.084	0.065	0.000	0.078	0.028		
RED	0.159	0.047	0.019	0.003	0.028	0.029	0.020	
LEA	0.139	0.029	0.034	0.025	0.043	0.046	0.049	0.013

Acropora austera – TMR

Pairwise F_{ST} not using ENA

pop	N_OFF	C_OFF	SC_OFF	S_OFF	N_IN	C_IN	SC_IN
C_OFF	0.027						
SC_OFF	0.056	0.086					
S_OFF	0.320	0.158	0.463				
N_IN	0.137	0.064	0.269	0.068			
C_IN	0.346	0.185	0.470	-0.057	0.108		
SC_IN	0.310	0.180	0.416	-0.009	0.095	0.026	
S_IN	0.258	0.130	0.381	0.124	0.076	0.161	0.168

Pairwise F_{ST} using ENA

pop	N_OFF	C_OFF	SC_OFF	S_OFF	N_IN	C_IN	SC_IN
C_OFF	0.023						
SC_OFF	0.056	0.083					
S_OFF	0.319	0.177	0.462				
N_IN	0.130	0.065	0.261	0.072			
C_IN	0.345	0.202	0.469	-0.057	0.109		
SC_IN	0.304	0.193	0.409	0.001	0.088	0.033	
S_IN	0.248	0.136	0.374	0.111	0.053	0.143	0.130

Platygyra daedalea – Large-scale

Pairwise F_{ST} not using ENA

pop	CHA	TAN	PEM	BAZ	INH	RAB	NMR	FMR	TMR	RED
TAN	0.079									
PEM	0.042	0.063								
BAZ	0.016	0.051	0.039							
INH	0.084	0.102	-0.018	0.081						
RAB	0.102	0.047	0.028	0.116	0.043					
NMR	0.030	0.039	-0.021	0.038	-0.024	0.013				
FMR	0.023	0.076	0.042	0.018	0.089	0.152	0.061			
TMR	0.052	0.043	0.009	0.100	-0.001	0.035	-0.005	0.087		
RED	0.029	0.000	0.027	0.081	0.029	0.038	0.002	0.077	-0.003	
LEA	0.061	0.066	0.056	0.072	0.056	0.062	0.022	0.103	0.032	0.018

Pairwise F_{ST} using ENA

pop	CHA	TAN	PEM	BAZ	INH	RAB	NMR	FMR	TMR	RED
TAN	0.080									
PEM	0.052	0.069								
BAZ	0.035	0.065	0.038							
INH	0.084	0.104	-0.008	0.099						
RAB	0.100	0.052	0.033	0.112	0.051					
NMR	0.026	0.035	-0.002	0.040	0.000	0.018				
FMR	0.023	0.073	0.054	0.040	0.088	0.145	0.061			
TMR	0.047	0.038	0.018	0.101	0.011	0.029	0.005	0.079		
RED	0.028	0.003	0.037	0.097	0.029	0.041	0.008	0.076	-0.001	
LEA	0.075	0.083	0.084	0.093	0.079	0.077	0.018	0.118	0.051	0.039

Platygyra daedalea – TMR

Pairwise F_{ST} not using ENA

pop	N_OFF	C_OFF	SC_OFF	S_OFF	N_IN	C_IN	SC_IN
C_OFF	0.023						
SC_OFF	-0.004	-0.006					
S_OFF	-0.005	0.004	0.004				
N_IN	0.011	0.023	0.023	-0.029			
C_IN	0.019	-0.005	0.021	-0.020	-0.009		
SC_IN	0.001	0.026	0.013	-0.033	-0.029	-0.009	
S_IN	0.018	0.006	0.006	0.011	0.036	0.014	0.034

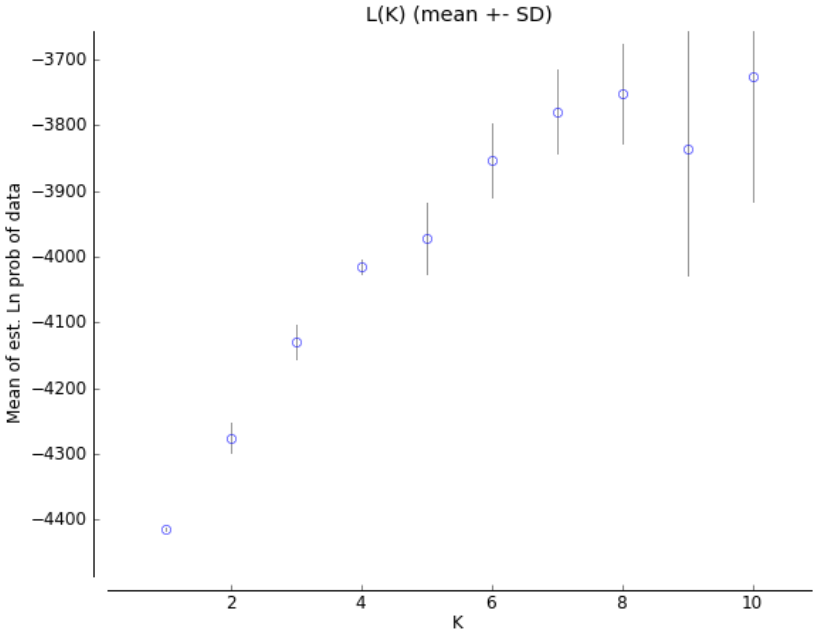
Pairwise F_{ST} using ENA

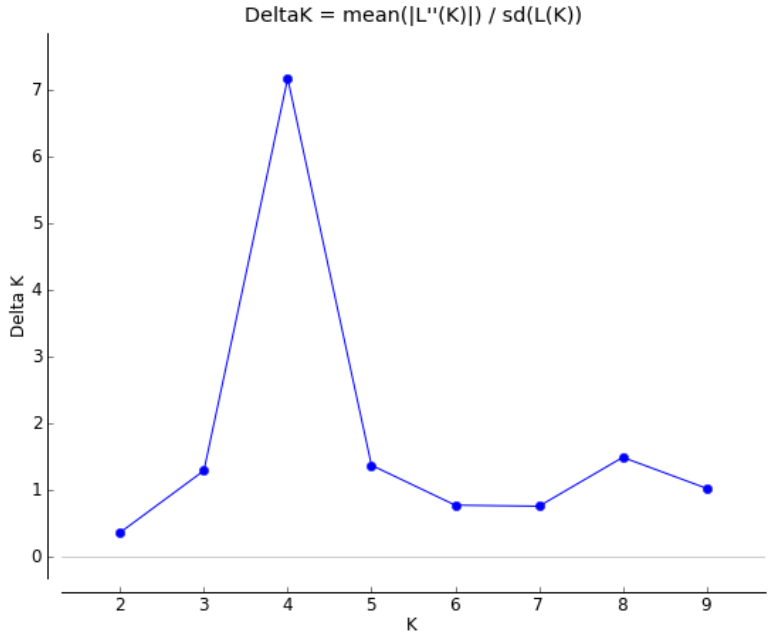
pop	N_OFF	C_OFF	SC_OFF	S_OFF	N_IN	C_IN	SC_IN
C_OFF	0.028						
SC_OFF	0.000	-0.005					
S_OFF	0.005	0.011	0.005				
N_IN	0.023	0.035	0.028	-0.022			
C_IN	0.050	0.025	0.046	0.005	0.024		
SC_IN	0.014	0.029	0.012	-0.030	-0.020	0.019	
S_IN	0.028	0.014	0.014	0.016	0.034	0.042	0.041

APPENDIX F. Supporting plots for Structure's results

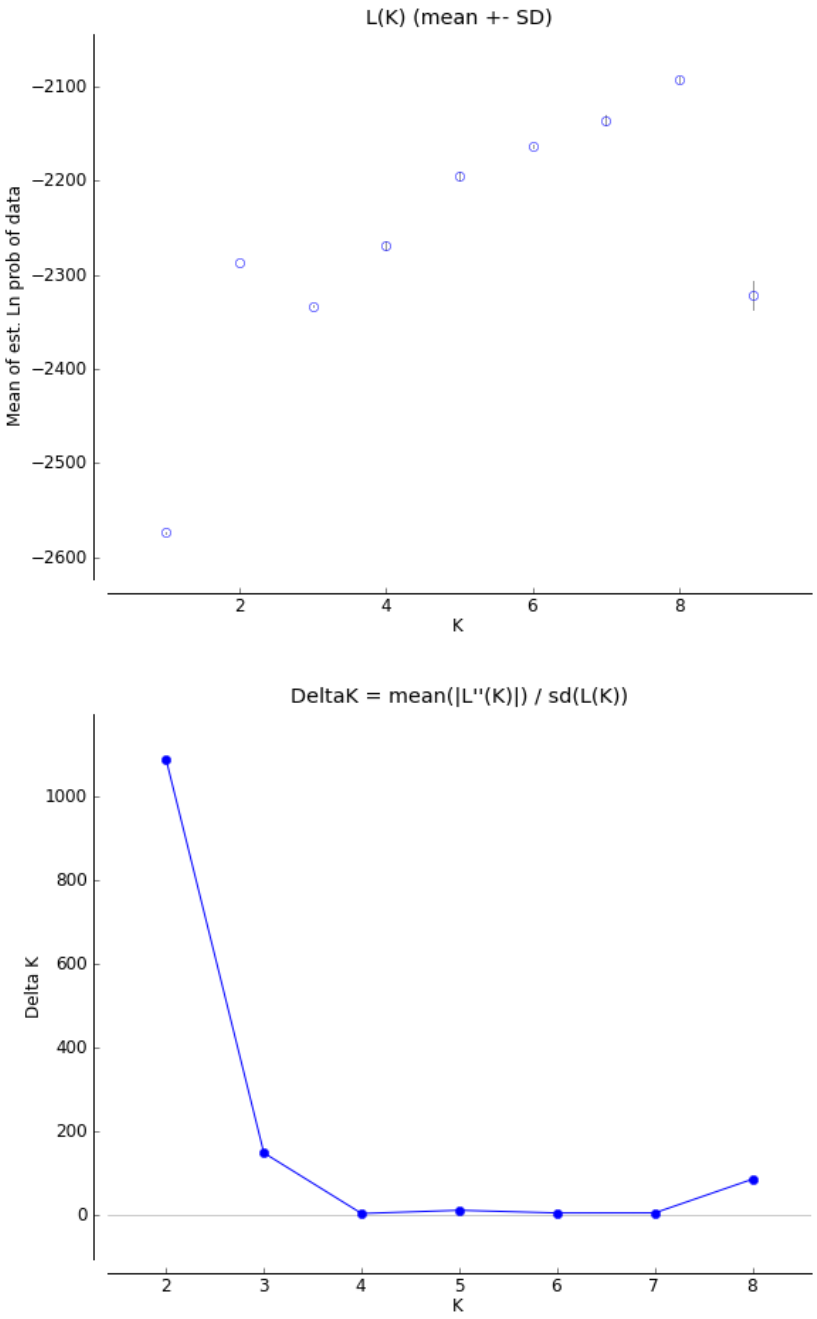
The following are all supporting plots for Structure results as implemented in Structure Harvester (Earl & VonHoldt 2012).

a. Between-reef genetic subdivision in *Acropora austera*, $K = 4$

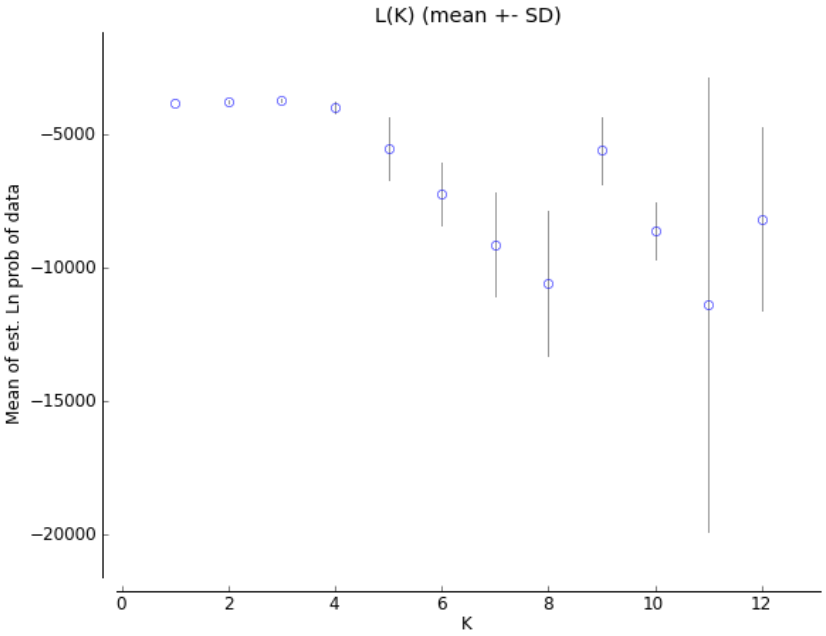


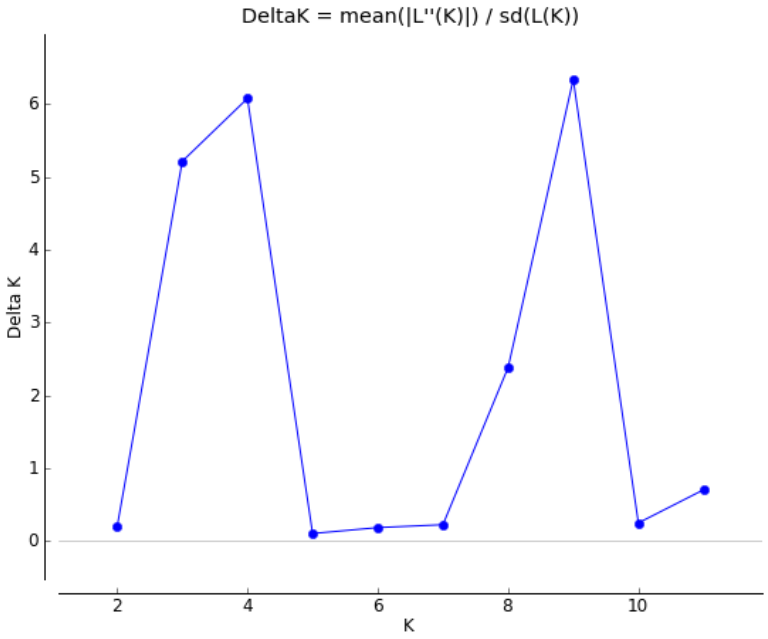


b. Within-reef genetic subdivision in *Acropora austera*, $K = 2$

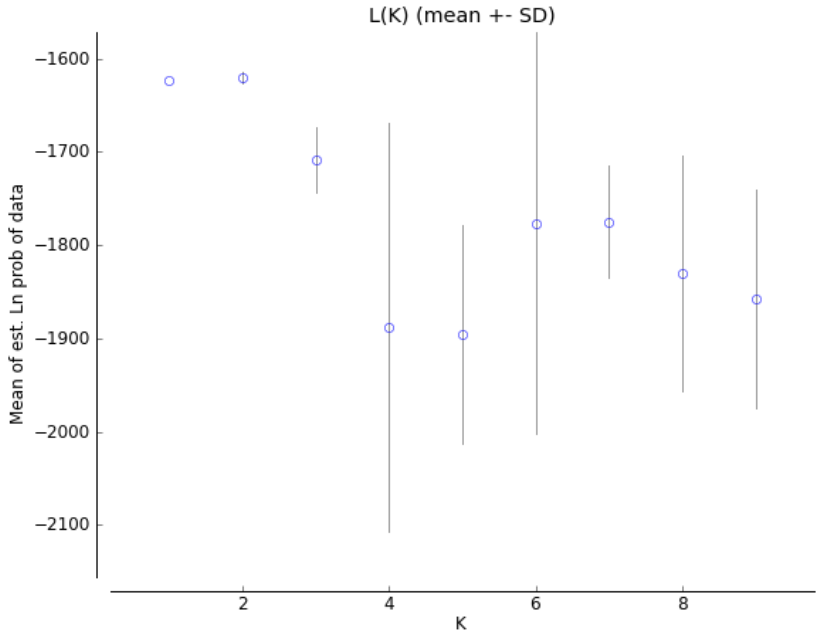


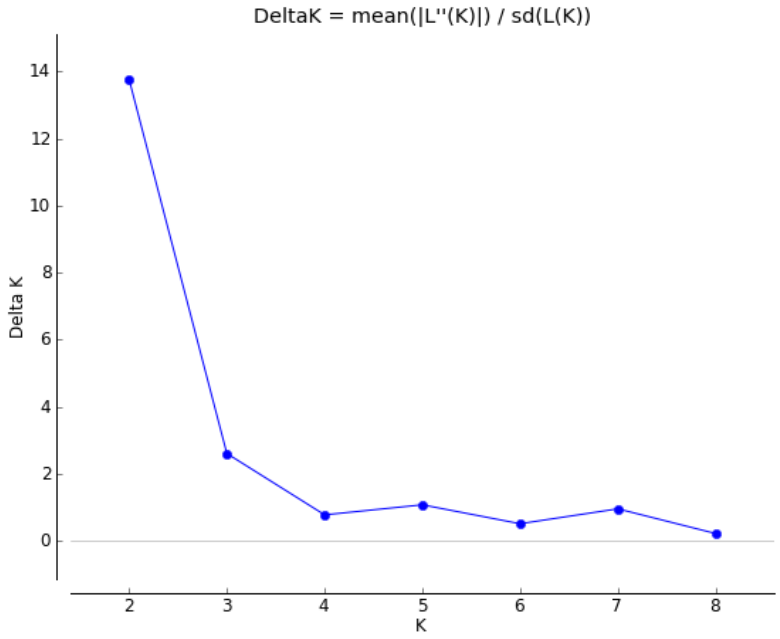
c. Between-reef genetic subdivision in *Platygyra daedalea*, *K* = 4





d. Within-reef genetic subdivision in *Platygyra daedalea*, $K = 2$





APPENDIX G. Correlograms by sites

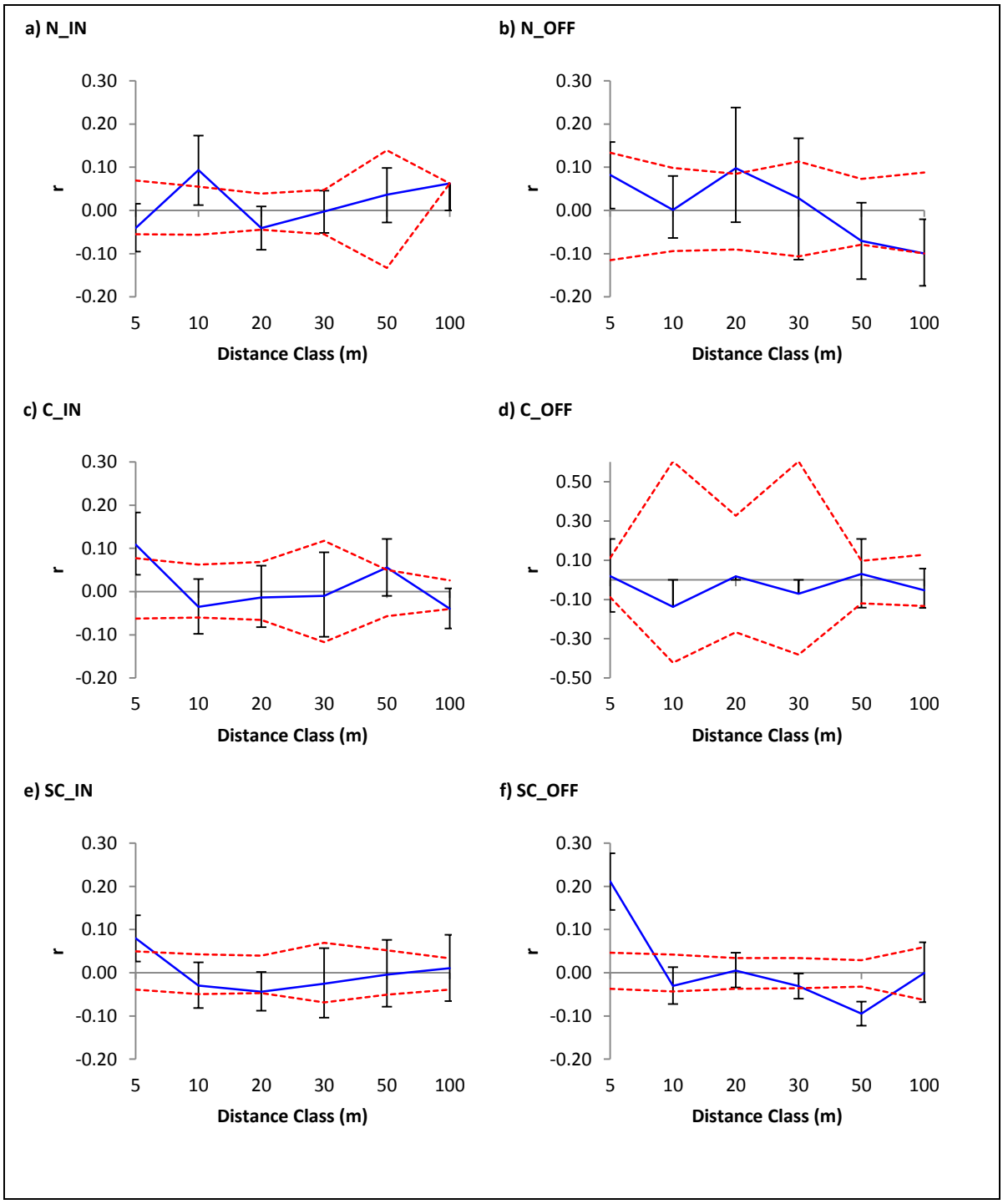


Figure G.1 Spatial autocorrelation analysis of *A. austera* on TMR using GenAlEx. Dashed lines represent the 95% confidence intervals of a null hypothesis of no autocorrelation in 9999 permutations, and error bars delineate standard errors from jack-knifed estimates.

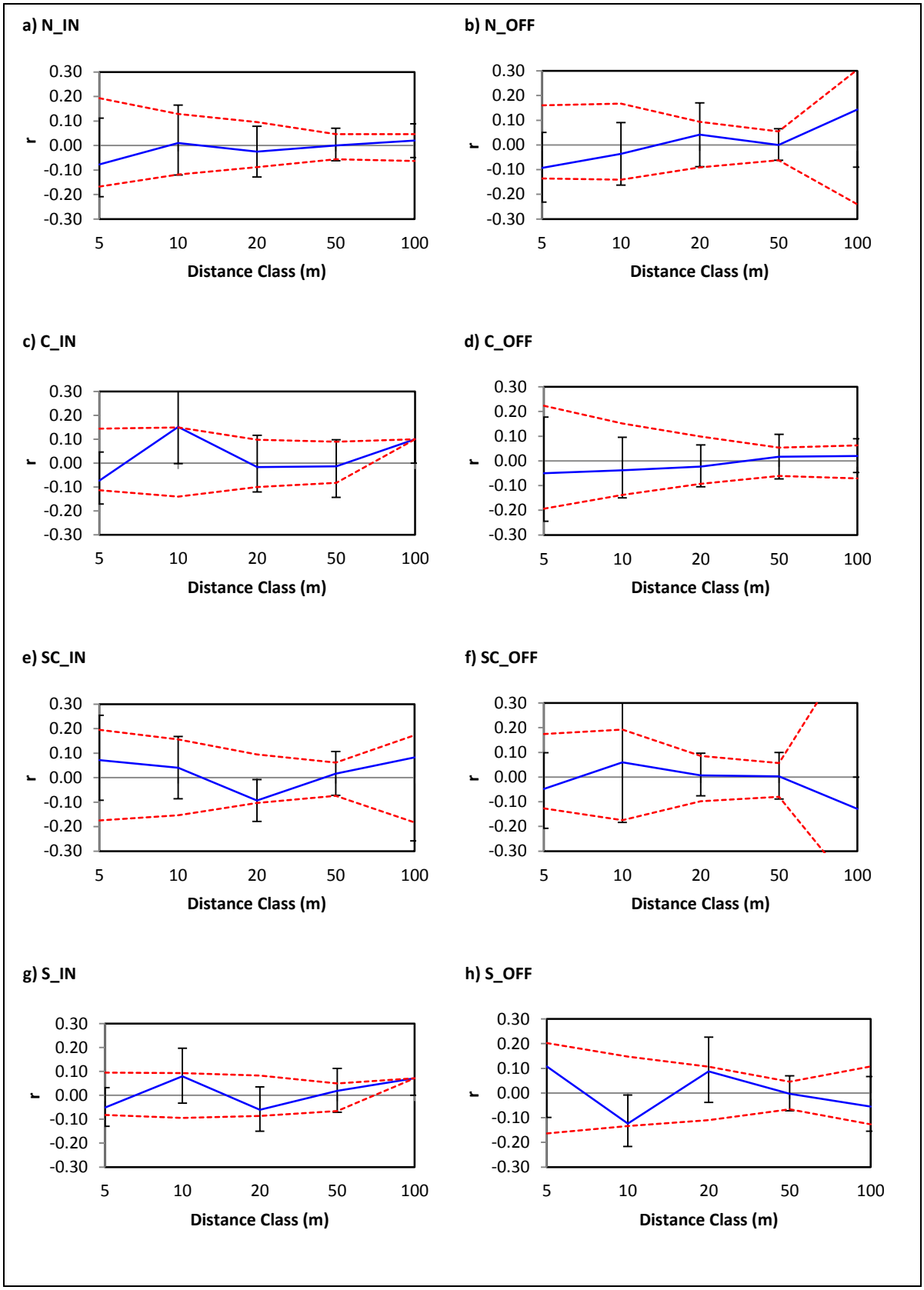
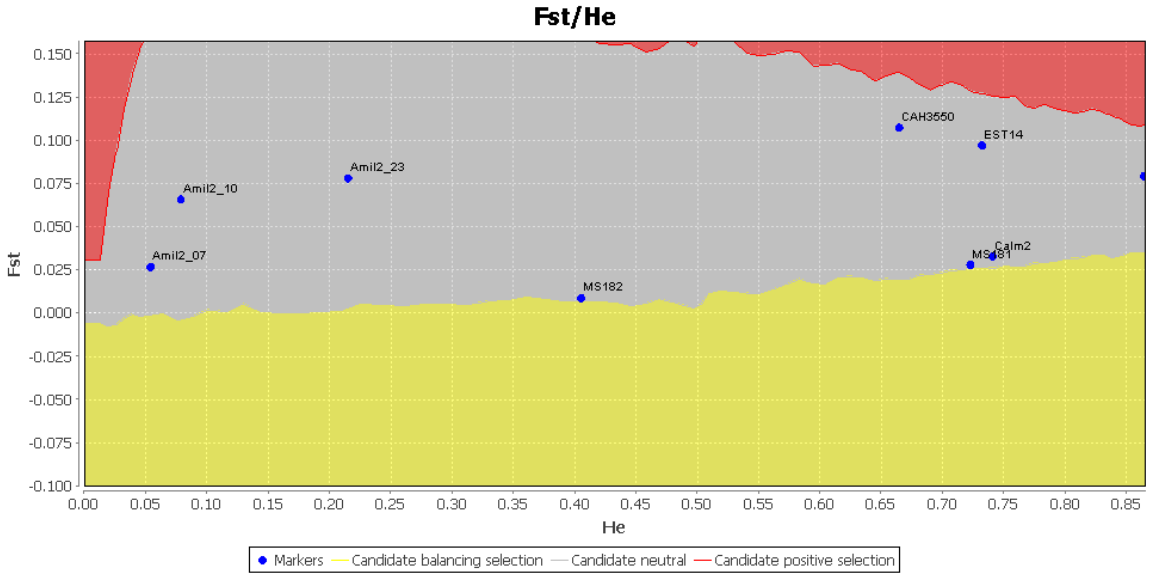


Figure G.2 Spatial autocorrelation analysis of *P. daedalea* on TMR using GenAlEx. Dashed lines represent the 95% confidence intervals of a null hypothesis of no autocorrelation in 9999 permutations, and error bars delineate standard errors from jack-knifed estimates.

APPENDIX H. Scatter plots for analysis of selection on genetic markers

Scatter plot of F_{ST} vs. expected heterozygosity (H_e) for the loci in the analysis of between reef genetic connectivity in *Acropora austera* (a) and *Platygyra daedalea* (b) populations along the south-east coast of Africa. Shaded boundaries indicate the 95% confidence intervals obtained through simulations in LOSITAN. Red region indicates candidates for positive selection, and yellow region candidates for balancing selection.

a.



b.