Rarity in the Coral Genus *Acropora*: Implications for Biodiversity Conservation

Thesis submitted by

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Statement on the Contribution of Others

Publications arising from Thesis

At the time of thesis submission, two manuscripts were published. A third manuscript describing the research findings of Chapter 6 was accepted for publication in Systematics and Biodiversity. A fourth manuscript relating to species-level monitoring to detect change (Chapter 7) was sent back for revision by Conservation Biology. Three additional manuscripts are in preparation. Details of each manuscript are provided below:

1. *Acropora rongelapensis* sp. nov., a new species of *Acropora* from the Marshall Islands (Scleractinia: Astrocoeniina: Acroporidae). Richards, Z.T. & Wallace, C.C. (2004). *Zootaxa*, Magnolia Press. 590: 1-5. www.mapress.com/zootaxa

2. Some rare Indo-Pacific coral species are probable hybrids. Richards, Z.T., van Oppen, M.J.H., Wallace, C.C., Willis, B.L., and Miller D.J. (2008). *PLoS ONE* 3(9): e3240. Doi:10.1371/journal.pone.0003240. Arises from Chapter 4.

3. Archetypal 'elkhorn' coral discovered in the Pacific Ocean. Richards, Z.T., Wallace, C.C., and Miller D.J. Accepted with *Systematics and Biodiversity* 7th October 2009 – in print March 2010. Arises from Chapter 6.

4. Manage ecosystems, monitor species: Detecting change in coral reef biodiversity requires species-level monitoring and evaluation. Z. Richards, M. Beger, H.S. Grantham, S.A. McKenna, D. Ceccarelli, L. Mc Cook, H. Possingham. Currently undergoing revision for *Conservation Biology*. Arises from Chapter 7.

5. Multiple types of rarity in the coral genera *Acropora* and *Isopora*. Richards, Z.T., Wallace, CC, Syms, C., Muir, PR, Willis, BL. *In advanced stage of prep*aration December, 2009. Arises from Chapter 2.

6. Rarity in the coral genus *Acropora* – a molecular phylogenetic perspective. Richards, Z.T., van Oppen, M.J.H., Wallace, C.C., Willis, B.L., and Miller D.J. *In advanced stage of preparation* for Molecular Phylogenetics and Evolution, December 2009. Arises from Chapter 3.

7. Low levels of genetic diversity in Indo-Pacific *Acropora*. Richards, Z.T., and van Oppen, M.J.H. *In prep.* Arises from Chapter 5.

Publications arising during my Candidature not directly related to Thesis.

During my candidature, I first authored a research paper in Marine Pollution Bulletin and co-authored three other peer-reviewed publications, one book chapter and five reports. Details of each manuscript are provided below.

1. Richards, Z. T., M. Beger, S. Pinca, and C. C. Wallace. (2008). Bikini Atoll coral biodiversity resilience revealed; five decades after nuclear testing. *Marine Pollution Bulletin*. 56, 503-515.

2. Carpenter, K.E., M. Abrar, G. Aeby, R.B. Aronson, S. Banks, A. Bruckner, A. Chiriboga, J. Cortés, J.C. Delbeek, L. DeVantier, G.J. Edgar, A.J. Edwards, D. Fenner, H.M. Guzmán, B.W. Hoeksema, G.Hodgson, O. Johan, W.Y. Licuanan, S.R. Livingstone, E.R. Lovell, J.A. Moore, D.O. Obura, D. Ochavillo, B.A. Polidoro, W.F. Precht, M.C. Quibilan, C. Reboton, Z.T. Richards, A.D. Rogers, J. Sanciangco, A. Sheppard, C. Sheppard, J. Smith, S. Stuart, E. Turak, J.E.N. Veron, C.C. Wallace, E. Weil, and E. Wood. (2008). One-Third of Reef-Building Corals Face Elevated Extinction Risk from Climate Change and Local Impacts. *Science*. 321(5888):560-563.

3. Peterson, E. L., M., Beger, Z.T. Richards. (2008). Thinking Outside the Reef. *Science*, 319:1759.

4. Peterson E.L., M., Beger and Z.T. Richards. (2007). Hydrodynamics and biodiversity used to propose MPAs at Rongelap Atoll. pp361-362 In: Day JC, Senior J, Monk S, and Neal W (eds.) First International Marine Protected Areas Congress, 23-27 October 2005, Conference Proceedings:IMPAC1 2005, Geelong, Victoria, Australia. http://www.impacongress.org

Book Chapters

1. Beger, M., Jacobson, D., Pinca, S., Richards, Z., Hess, D., Harris, F., Page, C., Peterson, E., Baker, N. (2008). "The State of Coral Reef Ecosystems of the Republic of the Marshall Islands" In: The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008, Waddell, J.E. and A.M. Clarke (eds.), NOAA.

Reports

1. Richards, Z.T., Beger, M., Hobbs, J-P, Bowling, T, Chong-Seng, K., and Pratchett, M. (2009). Ashmore Reef National Marine Reserve and Cartier Island Marine Reserve Marine Survey 2009. Prepared for DEWHA by ARC Centre of Excellence for Coral Reef Studies, James Cook University.

2. Cecarelli, D. Choat, H, Ayling, T., Richards, Z., Van Herwerden, L., Ayling, A., Ewels, G and Hobbs, JP and Cuff, B. (2008). Coringa-Herald National Nature Reserve Marine Survey 2007. Prepared for the Department of Environment, Heritage, Water and the Arts, by C&R Consulting and James Cook University.

3. Cecarelli, D., Kospartov, M., Beger, M, Richards, Z and Birrell, C. (2007). An assessment of the impacts of illegal fishing on invertebrate stocks at Ashmore Reef National Nature Reserve. 2006. C & R Consulting, for the Department of Environment and Heritage.

4. Beger, M., and Richards, Z. (2007). Finding Napo: Conservation of Napoleon Wrasse and Rare Corals. An assessment of rare coral reef organisms from Rongelap and Rongelap Atolls in the Marshall Islands prepared for BO-Conservation Programme.

5. Kospartov, M., Beger, M., Cecarelli, D., and Richards, Z. (2006). An assessment of the distribution and abundance of sea cucumbers, trochus, giant clams, coral, fish and invasive marine species at Ashmore Reef National Nature Reserve and Cartier Island Marine Reserve: 2005. UniQuest Pty Ltd. For the Department of Environment and Heritage.

Acknowledgements

Abstract

The processes that determine why some species are rare and others common are of major ecological and evolutionary significance. While theories concerning the causes and consequences of rarity have been developed for terrestrial systems, it is not clear to what extent they apply to marine invertebrates such as corals. In this study, data for the genera *Acropora* were used to test key ecological, phylogenetic and population genetic hypotheses about rarity and the results are interpreted in the context of biodiversity conservation. Overall, my goal is to improve information about rare coral species to enable better decisions about their threatened status, and to provide quality data to pilot fresh evaluations of marine invertebrate conservation action.

A new multi-scale model of coral rarity was developed, which shows that 54% of *Acropora* species examined in the NW Pacific Ocean are characterised by one of five different types of rarity. I found that thirteen species of *Acropora* are vulnerable to global extinction because they are rare and restricted across all scales of distribution and abundance. Twenty-two species were found to have a widespread global distribution but to occur in low abundance at a small number of sites across that range – these species are vulnerable to local extinction. I demonstrate that signals of rare species can be captured in multivariate analysis with inverse transformation. I show the inclusion of rare species in ecological data reveals more about community structure than examining common species alone.

Bayesian analyses of nuclear and mitochondrial sequence data imply that, although all of the rare species examined evolved relatively recently, phylogeny alone cannot account for the rarity of these corals because many common species have the same relative age. Members of the *horrida* and *echinata* groups occur in basal positions in both mitochondrial and nuclear topologies suggesting these lineages are far older than currently understood. My results show complex patterns of allele sharing along with mitochondrial monophyly coupled with nuclear polyphyly. I infer that in rare species, these patterns provide the first unequivocal evidence of hybridization in Indo-Pacific corals. A case study of a new population of elkhorn coral discovered in the Pacific Ocean is used to highlight the challenges corals pose to the conservation and management of endangered species.

Nine highly polymorphic microsatellite loci were used to examine the amount of genetic diversity and level of inbreeding in rare corals and closely related congeners.

Overall, rare species of *Acropora* did not have significantly lower levels of genetic diversity or higher levels of inbreeding than common congeners. However, species-specific microsatellite data suggests that at least 3 rare corals are genetically depleted. Another has 100% observed heterozygosity and I infer this species is a F1 hybrid*.* My findings of non-depletion in some rare species and depletion in some populations of common species, in conjunction with the lack of significantly greater levels of inbreeding in common species, means general conservation genetic hypotheses about the genetic resilience of rare/common species are not always relevant for species in the coral genus *Acropora*

Alarmingly, my population genetic results suggest that over 90% of the *Acropora* species examined here have lower mean allelic diversity at individual loci than a 'conservative mean' published in a recent review of scleractinian coral genetic diversity. Estimates presented here of numbers of alleles per locus are likely to be underestimates of genetic diversity due to the relatively small sizes of populations sampled. Despite this, my startling results suggest that even though Indo-Pacific *Acropora* are the most species rich group of corals, as a group, they may be far less genetically diverse than first thought.

Results of this research provide new insights into ecological and genetic theories about the relationship between rarity, phylogeny and diversity for marine, modular organisms. I show how an examination of occupancy types can help prioritise conservation action by informing management which species have the highest and lowest extinction risk in their region. I suggest that given the poor knowledge of the responses of coral reef species to environmental change and management actions, further targeted species-specific monitoring and research is needed to detect change and gauge the success of conservation action. Overall, the results of this research show that rare *Acropora* corals do not always conform to the expections of conservation genetic theory. Further the ecological, and likely evolutionary, significance of hybrid corals poses a major challenge to conservation legislation, and I suggest that an urgent re-evaluation of conservation policies for marine invertebrates is warranted.

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CHAPTER 1: General Introduction

1.1 Background

The majority of species in ecological communities are rare (Magurran and Henderson, 2003), however, rarity remains one of the most enigmatic aspects of ecology. Rare species, particularly habitat specialists, are highly vulnerable to extinction (Munday, 2004) because natural fluctuations due to variable environmental conditions can readily reduce population sizes below critical thresholds (Gaston, 1994; Brooks *et al.,* 2006). In highly diverse ecosystems such as coral reefs, there is a critical shortage of rigorous baseline data on levels of marine biodiversity (Balmford *et al.,* 2005). There is little detailed information in most reef regions, at any scale, about population size, population dynamics, and ecological roles of species or the impact management practices and environmental change have on marine biodiversity.   Thus, the biological and genetic consequences of rarity on coral reefs are largely unknown. In light of the major impact that natural variability can have on population sizes and the persistence of rare species, understanding how assemblages of rare marine species are structured across spatio-temporal scales and developing tools to help document biodiversity in threatened coral reef environments is of critical importance.

Coral reefs are globally significant but seriously threatened repositories of marine biodiversity, hence there is growing impetus to forecast, detect and mitigate the effects of stressors on coral reef biodiversity (Hughes *et al.,* 2003). Securing the health and resilience of coral reef ecosystems is imperative, not only because of their diversity and unique geological structure (Kleypas *et al.,* 2001), but also because of their socio-economic value (Moberg and Folke, 1999; Access Economics, 2008).   Scleractinian corals are critical components of the coral reef ecosystem, providing the structural framework of reefs and contributing to primary production and nutrient recycling (Done, 1996). Coral colonies provide microhabitat and food for a wide diversity of coral reef species (Knowlton 1991; Paulay, 1997; Knowlton and Rohwer, 2003). Coral mortality is known to cause declines in the richness and abundance of certain groups of reef fish (Sano *et al.,* 1987; Jones *et al.,* 2004; Munday, 2004; Berumen and Pratchett, 2008; Feary, 2007; Pratchett *et al.,* 2008; Graham *et al.,* 2009) but the full scale of trophic level impacts following coral mortality are not known. The global decline of reef quality (Jackson *et al.,* 2001; Harvell *et al.,* 2002; Hughes *et al.,* 2003; Pandolfi *et al.,* 2003; Wilkinson, 2004; Bruno and Selig, 2007) has led to declines in the accretion potential and productivity of reefs (Hoegh-Guldbeg *et al.,* 2007).   With 231 species of

scleractinian coral now included on the red list of threatened species (Carpenter *et al.,* 2008), a major crisis for coral reef biodiversity is looming.

Lack of knowledge about the population dynamics, life history patterns, reproductive potential, genetic characteristics and environmental factors constraining the distribution and abundance of the majority of species listed in an elevated category of threat by IUCN, suggests that many of these species may go extinct before we even begin to document population characteristics critical for their management. This is particularly true for rare species of coral, which typically have not been studied other than through occasional collecting.   Despite these grave predictions for the future, in the past 200 years, no hard coral is thought to have gone extinct and only the hydrocoral, *Millepora boschmai,* is thought to have been extirpated from the eastern Pacific following severe thermal anomalies and bleaching (Glynn and de Weerdt, 1991). Although there is no evidence that coral species have gone extinct in the recent past, all evidence points to the overall decline of reef habitat. A rising number of threats is likely to force local population declines (particularly in isolated locations; Ayre and Hughes, 2004), global range contractions (Gaston and Fuller, 2007), ecological extinctions (Estes, Duggins and Rathburn, 1989) and ultimately global extinctions.

Rare species are sometimes overlooked in ecosystem management because they are not thought to be functionally important or keystone members of communities. As a consequence of such oversights, the impact that rare species have on coral community structure is rarely considered in management decision-making (Chapman, 1999). Numerical representation of rare species is a challenge, both logistically and analytically, hence most marine biodiversity research has focused on distribution patterns (e.g. studies of centres of diversity and endemicity hotspots by Myers et al. 2000; Bellwood and Hughes 2001; Robertson et al. 2001; Roberts et al. 2002), with little consideration of population size.   In ecological studies that examine local abundance and distribution patterns; rare species are excluded, either implicitly by the use of restricted sampling designs (i.e. insufficient searching, inappropriate search strategy or sampling within a limited subset of habitat types) or explicitly during analyses. In some cases, rare species are described as contributing little other than 'noise' to a statistical solution (Gauch 1982) or are thought to increase computational time while lowering confidence scores (Marchant 2002). These perceived difficulties in sampling and analysing rare species data have hindered representation of rare species in the numerical abundance and structure data upon which ecological theory is based (Gaston, 1994; Chapman, 1999), especially in coral communities.

It is particularly important to test key hypotheses about rare corals because there is such a small margin for error in threatened species conservation. Untimely or inaccurate information about the status of rare species or in the worst case, absence of information, is a very real challenge for the conservation of marine biodiversity. At present, the data used in coral reef conservation planning are largely based on broad-scale biological or environmental parameters, or surrogates of biodiversity including reef health indicators or the presence of functional components of communities (e.g. coral cover estimates and herbivore/predator densities - Bellwood *et al.,* 2003; Bacompte *et al.,* 2005). These broad-scale biodiversity data are valuable given the logistical and funding constraints on large-scale reef monitoring and management, but none of these surrogates provide adequate ongoing proxies for biodiversity (Rodrigues and Brooks, 2007). Surrogate data for biodiversity provide no information about rare species and those most threatened with extinction. In order to understand biodiversity and hence conserve it, rare species must be explicitly examined.

1.2 Definition of rare species

Rarity is an intuitive concept (Usher, 1986) that is scale-dependent (Kunin and Gaston, 1997). In the literature, the term 'rare' has been variously applied to species with diverse patterns of distribution and abundance (Harper, 1981; Rabinowitz, 1981; Hanski, 1991; Fieldler and Ahouse, 1992; Kunin and Gaston, 1993; Gaston, 1994; Jones *et al.,* 2002). The definition used by the IUCN Red List is: "rare species are those taxa with small world populations that are localized within a restricted geographical area or habitats that are thinly scattered over a more extensive range" (IUCN 2002). Another pertinent definition is that of Revel (1981), which states "rarity is the state of an extant organism that by combination of factors is restricted either in numbers or area to a level that is significantly less than the majority of other related organisms". What these definitions share is the idea that rarity can be measured in terms of both abundance and distribution. Rarity has also been described in the context of habitat specialization. The '7 forms of rarity' model proposed for grasses (Rabinowitz, 1981) measures rarity using three different characteristics: range size, population size and level of specialization (Figure 1).

Large range	Large range	Small range	Small range
Habitat generalist	Habitat specialist	Habitat generalist	Habitat specialist
Large population size	Small population size	Large population size	Small population size
Large range	Large range	Small range	Small range
Habitat generalist	Habitat specialist	Habitat specialist	Habitat specialist
Small population size	Small population size	Large population size	Small population size

Figure 1. Seven forms of rarity model (and one of commonness, as defined in the top left square) as proposed by Rabinowitz (1981).

In practice, the definition of rarity depends on the particular taxa being considered and the ecological attributes considered most relevant. In this thesis, I define rare coral species as those with a restricted range (on global and local scales) and/or a small population size using defined cut-off points (see Chapter 2 for more detail). In the seminal text "Rarity", Gaston (1994) argued that the 25th percentile should be used as the standard criterion to designate a species as rare (in terms of abundance or distribution). In contrast, Hughes *et al.* (2002) used a 10% cut-off point to delineate geographically restricted coral species (Hughes *et al.* 2002). I consider the 10th percentile more appropriate for corals because, in comparison to terrestrial or freshwater organisms, corals in the lowest 25th percentile have ranges too large to be classified as rare. Therefore, in this thesis I will also use the 10th percentile to represent rarity in terms of abundance.

1.3 Causal factors of rarity

The factors constraining the distribution and abundance of species have long been the subject of ecological research (Darwin, 1859; Mayr 1963; Krebs, 1978; Begon *et al.,* 1990). Even in the absence of anthropogenic disturbances, biological communities remain characterized by an excess of rare species (Magurran and Henderson, 2003). Generally, rarity is shaped by both intrinsic (e.g. physiological capabilities, life histories and dispersal characteristics) and extrinsic factors, the latter including interactions with other species (e.g. diseases and parasites, predators, competitors) and with aspects of the physical environment that limit population expansion (e.g. specialist niche requirements) (Rosenzweig, 1995; Angel *et al.,* 2006). The present day occupancy of a species is also influenced by historical factors such as species age (Johnson, 1998) and historical biogeography (Benzie, 1999; Nelson *et al.,* 2000; Wallace, 2001; Barber *et al.,* 2000; 2002; Nyryanto and Kochzius, 2006; Timm *et al.,* 2008).

Understanding the causes of rarity is very important for conservation biology; however, freshwater and marine communities are under-represented in published studies of rarity (Chapman, 1999). Most of our current understanding of rarity has come from studies of terrestrial plants, birds, mammals and insects. From studies of these groups, it is clear that rarity can be both a natural phenomenon in ecological communities and an ecological state forced upon a species by anthropogenic pressures. On modern reefs, it can be very difficult to differentiate between underlying natural versus anthropogenic causes of rarity unless intensive long-term monitoring is conducted. Furthermore, the distinction between natural and human impacts is now blurred, for example human-related CO_2 emissions are contributing to climate change and this is thought to be increasing the frequency and intensity of storm events that would have previously been considered natural.

Whether natural and / or anthropogenic in origin, a species' disturbance history plays a major role in determining its pattern of occupancy (Connell, 1978; Connell and Sousa, 1983; Ajeroud *et al.,* 2009). The threat of disturbance is particularly high on coral reefs because threats can be both land-based and marine-based, including over-fishing (commercial, recreational and subsistence), harmful collecting practices (e.g. destructive fishing using blast/poison/cyanide/muro-ami), harvesting for the aquarium trade, coral mining for construction material, dredging activities and consequent increased sedimentation, habitat conversion, eutrophication, hypoxia, pesticides and herbicides causing declining water quality, oil spills and ballast water discharge, outbreaks of predators and disease/viruses, dust storms and algal blooms, and climate change-related threats including acidification, ocean warming, sea-level rise, and increased intensity and frequency of storms (Bryant *et al.,* 1998; Wilkinson, 2004). As a result of these cumulative threats, there are many examples of marine species that have undergone substantial population declines (for examples see Dulvey *et al.,* 2003).

Recruitment is variable on coral reefs; hence recruitment limitation is considered one of the main causes of rarity in populations of coral reef fish (Jones *et al.,* 2002). Recruitment-limitation assumes that there are not enough larvae to saturate the available habitat (Doherty and Fowler, 1994; Hughes and Tanner, 2000) and could result from a lack of external migrants and / or a lack of locally produced larvae. In broadcast spawning corals, where mature individuals can produce thousands of gametes, recruitment limitation may at first seem improbable. However, recruitment limitation could occur if a species has low fecundity, or if there is a lack of mature breeding individuals in the community or if dispersal is patchy. Fecundity is an

expression of reproductive effort and can be highly variable in corals because gametogenesis typically has a narrower tolerance to stress than other life functions (Harrison and Wallace 1991). For example, gamete production is affected by environmental factors such as food quantity (Llodra 2002), temperature (Kojis and Quinn 1981; Hoegh-Guldberg 1999), nutrient availability (Rinkevich and Loya 1977; Guzman and Holst 1993) and turbidity (Kojis and Quinn 1984). Hence in disturbed habitats, for example after storm or bleaching events, corals either do not spawn or have a lower reproductive output (Wallace, 1985), which could lead to recruitment limitation.

In some marine species, population size is related to constraints on body size, thus a small population can be explained by a lack of large breeding individuals (e.g. skates, mantis shrimps, coral reef fish and rocky intertidal communities - Marquet, 1990; Dulvey *et al.,* 2000; Munday and Jones, 1998; Reaka, 1980). This also applies to corals and other clonal organisms where fecundity increases as a function of colony size (Hughes, 1989; Hall and Hughes, 1996; Harrison & Wallace, 1990; Sakai, 1998). Hence, if there is an absence of breeding individuals, there will be negative population growth and recruitment limitation for successive years. In addition to the role that reproductive life history traits may play in the spatio-temporal dynamics of populations, rarity may also be caused by a species' colonization ability, both in terms of its dispersal and its establishment ability. There are numerous examples of rare species having poorer dispersal or lower establishment ability than common species (Gaston, 1994). Colonization ability is particularly relevant in degraded coral reef systems that experience phase shifts to macroalgal dominated communities, because studies of coral-algal interactions show a negative correlation between macroalgal cover and coral recruitment in the field (Birkeland 1977, Wittenberg and Hunte 1992, Mumby et al. 2007). It is important to understand, however, that life history traits such as dispersal ability are often traded off against other traits, so without a thorough comparison of traits between rare and closely related common species, it is difficult to generalize about the specific causes of rarity.

The coral genus, *Acropora,* is the most species-rich group of scleractinians and provides an ideal model group for testing hypotheses concerning rarity. A trend for rare species of *Acropora* to occur in deep or sheltered lagoonal sites or in unusual habitats (e.g. *A. russeli* on deep sandy slopes and A. *torihalimeda* on submerged *Halimeda* algal banks; Wallace, 1999) led Wallace (2001) to suggest that habitat specialization of these species may reflect the persistence of these habitats during glacial falls in sea level

(see also Paulay, 1990, 1996). The tendency for rare species to be habitat specialists or to occur in marginal niches may also indicate that rare species have been excluded from preferred habitats by more competitive species. Alternatively, they may be species with hybrid origins that have greatest fitness in non-parental niches (Vollmer and Palumbi, 2002). In corals, endemic species in the regional Indo-Pacific and putative subspecies' are hypothesized to be morphogically unique hybrid species (Veron, 1995). The likelihood that hybridisation is a cause of rarity in Indo-Pacific corals remains to be tested here.

1.4 Expectations about the consequence of rarity

Rarity theory predicts that small and isolated populations will be vulnerable to local extinction (Darwin 1846; Gaston, 1994; Ricketts *et al.,* 2005; Brooks *et al.,* 2006). This is because rare species are less able to respond to changing environmental circumstances compared to common species, given their lower genetic diversity and higher levels of inbreeding and/or high degree of population structure (Kimura and Ohta, 1971; Soule 1986; Simberloff 1988; Frankham, *et al.,* 2002). In general, the greater the genetic diversity in a population, the greater its adaptive potential (Briggs, 2005), hence there is a highly significant correlation between heterozygosity and population fitness (Reed and Frankham, 2003). Because rare species are prone to loss of genetic variation through genetic drift, founder effects, directional selection and high levels of inbreeding (Wright, 1931; Kimura and Ohta, 1971; Avise, 1994, Frankham *et al.,* 2002; Willi *et al.,* 2006), they are expected to have reduced reproductive fitness. Furthermore, rare species are thought to be vulnerable to the fixation of deleterious mutations at fitness loci. These predictions are based on theories that have been developed for terrestrial systems (plants, birds, mammals and insects) but corals possess a number of traits that may buffer them from the evolutionary constraints of small population size, such as the potential for high gene flow, overlapping generations and the prevalence of both inbreeding and outbreeding. In marine invertebrates such as corals, little is known about the extent, causes or consequences of rarity. Coral reef communities represent a fertile system for research aimed at testing the relevance of ecological theories developed from terrestrial systems for marine systems.

1.5 Detecting rare species

Even in common marine species, population densities are extremely variable spatio-temporally because of natural stochasticity and patch dynamics (Picket and White,

1985; Levin *et al.,* 2002; Wu and Loucks, 1995). Therefore in heterogeneous marine populations, an interpretation of the extent, causes and consequences of rarity is likely to be confounded by high detection error (Chapman, 1999). Variability in recruitment and breeding population size can obscure long-term trends in marine systems and for this reason, imminent population crashes have proven difficult to forecast (e.g. the collapse of many fisheries – see Hutchings and Reynolds, 2004). Patchy distributions make it extremely difficult to obtain reliable estimates of density or population size; hence only quantum changes in numbers may be detected (Schroener et al. 1993). In addition to this, methodologies for detecting and determining trends in the abundance of rare species are scarce (but see Green & Young 1993). In corals, the traditional way to examine biodiversity is through line-intercept or point-intercept transects. While these techniques are optimal for examining coral cover, whether they provide the coverage necessary to detect coral biodiversity is not known. The most common way to examine coral biodiversity and capture rare and cryptic species in numerical data is through rapid visual assessments covering all local microhabitats with thorough searching within a defined area (Devantier 1998; 2006). However due to the high level of expertise needed to identify scleractinian corals in-situ, there is a severe shortage of reliable species-level data available.

1.6 Aims of the thesis

Overall, my goals are to improve information available on the status of rare species in the genus, *Acropora,* to enable better decisions about the threatened status of rare corals and also to provide quality data to pilot new direction for the conservation of marine invertebrates. To achieve these goals, I use ecological, phylogenetic and population genetic tools to examine the extent, causes and consequences of rarity in the scleractinian genus *Acropora* and the implications of rarity for biodiversity conservation. More specifically I aim to:

1. **Examine patterns of rarity and restriction in species of the coral genus, *Acropora* and *Isopora* (Chapter 2).**

In this chapter, I examine local patterns of abundance and distribution for 87 species of *Acropora* at 100 sites in the Northwest Pacific and compare these with global distribution patterns (from the World Wide *Acropora* Database - Museum of Tropical Queensland). I trial new analytical technique to examine the impact that rare species have on community structure and develop a new multiscale model of

coral rarity that enables species to be examined in the context of three outcomes – extinction, persistence or compensation.

2.   To examine the relationship between rarity and phylogenetic position for selected species within the *genus Acropora* (Chapter 3).

Phylogenetic studies are used to investigate the age, mode of speciation and nucleotide diversity of rare and closely related common species in the scleractinian coral genus, *Acropora.* I compare new DNA sequence data for the *Pax-C* 46/47 single-copy nuclear intron and the mitochondrial DNA (mtDNA) control region (*rns-cox3*), for 16 *Acropora* species that have geographically restricted distributions in the Indo-Pacific, with new and published sequences (van Oppen *et al.,* 2001) for 22 widespread species.

3.   To determine if hybridisation plays a role in the evolutionary history of rare species of *Acropora* (Chapter 4).

DNA sequence data from nuclear and mitochondrial loci were analysed for evidence of reticulate evolution for 14 rare and 8 common Indo-Pacific species of *Acropora* collected from the Great Barrier Reef (Palm Island Group), the Marshall Islands (Rongelap Atoll) and Papua New Guinea (Kimbe Bay).   Global census sizes and effective population sizes were estimated so that coalescence times could be infered and further insights gained into the likelihood that hybridisation has contributed to *Acropora* diversification.

4.   To examine genetic diversity, inbreeding and the population structure of rare and common species of *Acropora* (Chapter 5).

The extent of genetic variation within populations of rare corals was analysed and compared to similar data for multiple populations of common corals sampled across a broad geographic range.   I determine the level of allelic diversity and expected heterozygosity as metrics of genetic diversity in rare and common corals. I investigate the extent of inbreeding and the level of structure within and between populations.

5.   To explore the challenges facing coral conservation (Chapter 6).

Morphological and phylogenetic techniques were used to explore the identity of a new population of rare 'elkhorn' coral found in the Pacific Ocean. I use this case study to highlight the challenges that corals pose with respect to the conservation and management of endangered species.

6. To discuss the research findings for conservation purposes (Chapter 7).

Conservation implications of the different types of rarity, based on ecological, phylogenetic and population genetic results for species in the genus *Acropora,* are discussed. Links between rarity and persistence are identified for rare species of *Acropora* and lastly I discuss the associate uncertainty and challenges facing the conservation of biodiversity in complex ecosystems such as coral reefs.

1.7 Model taxa: Genus Acropora

The genus *Acropora* (commonly known as staghorn corals) is an ideal model group for studies of rarity and commonness in sessile marine invertebrates. Species in this genus display complex spatio-temporal patterns and an extensive literature exists on their global ranges (Veron and Wallace, 1984; Wallace, 1999; Veron 1993, 2000). Many species of *Acropora* are described as occurring in small, restricted, isolated and / or disjunct populations (Wallace, 1999, Veron, 2000; Wallace et al. 2001) suggesting that they are rare. However, most of these species have not been studied in any way other than occasional collecting for systematic studies. In general, species of *Acropora* are extremely susceptible to coral bleaching (Marshall and Baird, 2000), changes in water quality, disease (Sutherland, *et al.,* 2004; Bruno *et al.,* 2007) and predation (e.g. the starfish *Acanthaster planci:* Pearson, 1981; and the gastropods *Coralliophila abbreviata* and Drupella spp.). Given that up to 50% of *Acropora* species are predicted to face an elevated risk of extinction this century under IUCN categories and criteria (Carpenter et al. 2008), greater understanding of the implications of rarity for the conservation of species in this genus is paramount.

Today, *Acropora* is the largest extant genus of reef-building corals. The genus *Acropora* was formally known as *Madrepora* (Linnaeus 1758). The name *Acropora* was introduced by Oken in 1815 and reintroduced by Verrill in 1901. In 1999, Wallace published the first full monograph of the genus since Brook (1893). This fundamental revision described 114 species, revised hundreds of synonymies (after re-examination of type species) and updated species ranges based on 15,500 specimen-based records from 800 sites around the world. Approximately 400 *Acropora* species have been described – however some are represented by fossil material only, and others are *nomina nuda,* meaning they are not officially validated (including many of the new species described in Veron 2000). The systematics of the genus *Acropora* is traditionally based on morphology but more recently, has used insights derived from

breeding experiments and phylogenetics (e.g. Willis et al. 1997; Wolstenholme *et al.,* 2003, 2004). New genetic findings from this project that are of relevance to the systematics of the genus are clearly defined in terms of the traditional morphological scheme. All specimens used in all phylogenetic and population genetic studies have been identified using the coral collection held at the Museum of Tropical Queensland as reference material and verified by Dr Carden Wallace. All skeletal material collected in the project is available upon request at the Museum of Tropical Queensland.

Various markers are available to assess phylogenetic relationships within the *Acropora,* including ribosomal DNA (rDNA) and ITS (internal transcribed spacer) sequences (Ordrico and Miller, 1997; van Oppen et al. 2002; Marquez et al. 2003). However, rDNA is now considered unsuitable to test evolutionary relationships in the *Acropora* because it is a fast evolving genus (Wei, et al. 2006) and extremely high rDNA diversity can predate species divergence (Vollmer and Palumbi, 2004). Mitochondrial markers such as *Cytochrome b* have also been examined. However, in *Acropora*, *Cytochrome b* is highly conserved (van Oppen *et al.,* 1999) and for this reason is also not ideal for phylogenetic purposes. I use a single-copy nuclear intron in this study because single-copy introns are the most phylogenetically informative markers for the *Acropora* because they accumulate mutations relatively rapidly and provide many potentially phylogenetically informative characters that occur with a frequency of one per haploid genome unlike repetitive markers (such as rDNA) that occur in multiple copies. Single-copy markers available for use in *Acropora* phylogenetics include: *Mini-C* (Wang *et al*. 1995), *Cnox2* (Hayward *et al*. 2001) and *Calmodulin* (Vollmer and Palumbi 2002). In this study I use *Pax-C 46/47* nuclear intron (van Oppen *et al*, 2001) and the putative mitochondrial control region *rns-cox3* which is also considered phylogenetically informative (van Oppen *et al*, 2001). Microsatellites (or simple sequence repeats) are particularly informative for understanding genetic resilience and the pathways of coral connectivity (Pritchard *et al.,* 2000; van Oppen and Gates, 2006). Five microsatellites were developed for *A. palmata* (Baums *et al.,* 2005) and 10 markers have been developed for *A. millepora* (Underwood *et al.,* 2006; van Oppen *et al.,* 2007). More recently, data mining of public DNA databases has led to the characterization of another 40 microsatellites in *A. millepora* (Wang *et al.,* 2008), of which 25 were transferable to *A. hyacinthus*. Here I use microsatellites of (van Oppen *et al.,* 2007).

1.8 Study sites and species

Species selected for ecological and genetic studies were chosen to represent a range of categories of rarity and commonness (see Plate 1). The Northwest Pacific region was chosen as the general location for the ecological studies in this project to take advantage of fieldwork opportunities presented by international collaborative projects that I participated in from 2002-2005 [i.e. The Natural Resource Assessment Survey of the Marshall Islands (NRAS - Bikini, Rongelap, Ailinginae, Mili Atolls), and the Rapid Visual Assessment of Kimbe Bay (The Nature Conservancy, PNG)]. Phylogenetic and population genetic samples were collected from the NW Pacific Region and also from Orpheus Island, GBR. Additional population genetic collections were donated by: Dr CC Wallace and Dr Paul Muir (Seychelles and Maldives,) Dr Akira Iguichi (Okinawa Japan); Mrs Natalie Rosser (Ningaloo Reef); Mrs Maria Beger (Chuuk Atoll, Micronesia), and Mr David Abrego (Heron Island) (See Figure 2). The species examined and category of research undertaken on each species is listed in Table 1.

Figure 2. Map showing locations of surveys and collections used for ecological and genetic studies.

Plate 1. A selection of *Acropora* coral species included in this study that represent different categories of rarity and commonness.

Table 1. Species included in this study and category of investigation undertaken. # Indicates species recognized in Veron 2000 but not Wallace 1999. Species in grey bold are examined across all three disiplines.

Species	Ecology	Phylogenetics	Population Genetics
Acropora abrotanoides	YES		
Acropora aculeus	YES		
Acropora acuminata	YES		
Acropora anthocercis	YES		
Acropora aspera	YES		
Acropora austera	YES	YES	YES
Acropora awi	YES		
Acropora batunai	YES	YES	
Acropora bifurcata #	YES		
Acropora bushyensis	YES		
Acropora carduus	YES		
Acropora caroliniana	YES		
Acropora cerealis	YES	YES	
Acropora chesterfieldensis	YES	YES	
Acropora clathrata	YES		
Acropora cytherea	YES		
Acropora derawanensis	YES	YES	
Acropora desalwii	YES		
Acropora digitifera	YES	YES	
Acropora divaricata	YES		
Acropora donei	YES		
Acropora echinata	YES		
Acropora elseyi	YES	YES	
Acropora exquisita #	YES		
Acropora florida	YES		
Acropora gemmifera	YES	YES	
Acropora glauca	YES		
Acropora gomezi	YES		
Acropora grandis	YES		
Acropora granulosa	YES	YES	
Acropora horrida	YES	YES	YES
Acropora humilis	YES	YES	
Acropora hyacinthus	YES		
Acropora insignis #	YES		
Acropora intermedia	YES		
Acropora jacquelineae	YES	YES	YES
Acropora kimbeensis	YES	YES	YES
Acropora kirstyae	YES	YES	YES
Acropora latistella	YES		
Acropora listeri	YES		
Acropora loisetteae	YES	YES	
Acropora lokani	YES	YES	
Acropora longicyathus	YES	YES	
Acropora loripes	YES	YES	
Acropora lovelli	YES		
Acropora lutkeni	YES		

Acropora microclados	YES		
Acropora microphthalma	YES	YES	YES
Acropora millepora	YES	YES	YES
Acropora monticulosa	YES		
Acropora muricata	YES		
Acropora nana	YES		
Acropora nasuta	YES	YES	
Acropora paniculata	YES		
Acropora papillare		YES	YES
Acropora pichoni	YES	YES	YES
Acropora plana #	YES		
Acropora plumosa	YES		
Acropora prostrata #	YES		
Acropora robusta	YES		
Acropora rongelapensis	YES	YES	YES
Acropora sameonsis	YES		
Acropora sarmentosa	YES		
Acropora secale	YES		
Acropora selago	YES		
Acropora solitaryensis	YES		
Acropora spathulata	YES	YES	YES
Acropora speciosa	YES	YES	
Acropora striata	YES		
Acropora subglabra	YES		
Acropora subulata	YES		
Acropora tenella	YES	YES	
Acropora tenuis	YES	YES	
Acropora tortuosa	YES	YES	YES
Acropora valida	YES	YES	YES
Acropora valiencinessi	YES		
Acropora vaughani	YES	YES	
Acropora verweyi	YES		
Acropora walindii	YES	YES	YES
Isopora brueggmanni	YES		
Isopora crateriformis	YES		
Isopora cuneata	YES		
Isopora palifera	YES		

CHAPTER 2 – Multiscale patterns of coral rarity and restriction

2.1 Introduction

In complex marine systems like coral reefs, there is limited understanding of the population dynamics of rare species. Basic research into demographic characteristics of rare species would improve decisions that rely on the identification of species that are most threatened and provide the basis for fresh evaluations of optimal actions to conserve rare species. For corals, a recent IUCN assessment of conservation status suggests that 231 species of shallow-water scleractinian species have an elevated risk of extinction (Carpenter et al. 2008). Against a backdrop of human-mediated ecosystem declines (Gaston et al. 2000) and forecasts of unprecedented rates of biodiversity loss (Pimm et al. 1995), investigating rare species is of critical importance for biodiversity conservation (Gaston, 1994; Ricketts et al. 2005; Brooks et al. 2006).

If a species is endemic or has a restricted range, documenting its abundance within that range is particularly important for management purposes. However, as discussed in Chapter 1, examining the abundance of rare marine species in heterogeneous coral reef communities is a challenge for reasons both logistical and analytical. Rare species are excluded from ecological studies either implicitly by the use of restricted sampling designs or explicitly during data analysis. These perceived difficulties in sampling and analysing data on rare species have hindered representation of rare species in the numerical abundance and structure data upon which ecological theory is based (Gaston, 1994; Chapman, 1999).

Rarity cannot be defined easily, it can relate to patterns of abundance (relative/absolute), distribution (range size, endemism, occurrence) and specialization (habitat/niche space) (Gaston, 1994). Rabinowitz (1981) proposed a model that identified seven forms of rarity by rating species as either 'rare' or 'common' on these three scales (abundance, distribution, habitat specialization). Of the resulting eight possible combinations, seven represent rarity on at least one scale, and one represents commonness on all scales. This approach has been successfully adapted for the study of mammals (Yu and Dobson, 2000). The Rabinowitz model provides a platform from which to examine rarity in complex coral reef communities. Like in Rabinowitz's grasses, habitat specialization is an important driver of rarity in coral reef communities (Munday, 2004). As noted by Hartley and Kunin (2003), rarity, in particular the way that it relates to extinction risk requires a multi-scale approach because focusing on global range size

reveals nothing about local population size or the extent of population fragmentation. This is particularly true for corals for which we know little about spatio-temporal patterns.

One of the most critical gaps in marine biodiversity conservation is the lack of knowledge that currently exists on local population sizes of species, particularly among those with restricted global distribution patterns. Developing tools that improve the documentation of rare species in threatened ecosystems is critical for marine biodiversity conservation. Here I apply a simple field survey method to determine levels of biodiversity that include rare coral species and use a novel analytical technique to obtain a multivariate signal from rare species distributions. These data are used to examine how the high diversity coral genus, *Acropora*, is structured. Because little quantitative data is available about habitat specialization for the majority of coral species, I modify the Rabinowitz rarity model to generate a new multi-scale rarity model for corals. I test the model on a dataset pertaining to 87 *Acropora* species collected from 100 sites in the North West Pacific Ocean. I synthesize rarity patterns and use the rarity model to forecast which species have the highest and lowest extinction risk in the NW Pacific Region.

2.2 Methods

2.2.1 Definition of rarity

As described in chapter 1, I adopt the definition of rarity proposed by Revel (1981), which interprets rarity as "the state of an extant organism that by combination of factors is restricted either in numbers or area to a level that is significantly less than the majority of other related organisms". Here I use 10% as the cut-off to designate rarity in terms of both abundance and distribution.

2.2.2 Global distribution patterns

The global distribution patterns of 114 species from the genus *Acropora* (sensu Wallace 1999) were first examined using the WorldWide *Acropora* database (www.qm.qld.gov.au) in two ways. First, global range sizes were estimated by calculating the surface area of an ellipse (r1 and r2 = r1/2 * r2/2 * pi) encompassing the maximum latitudinal and longitudinal range in km (calculated from the lat/longs) as the axes. Species were described as globally range restricted if their range was one-tenth or less that of the most widespread species, *A. valida* (see figure 3). Secondly, specimen-based distribution records in the WorldWide *Acropora* database (n = 1523 sites; Wallace and Muir pers comm.; plus *Acropora rongelapensis* from Richards and

Wallace, 2004) were used to determine global distribution patterns. I examine the relationship between these two variables using a linear regression to investigate if range size strongly represents the number of records in the WW *Acropora* database. If a species had one-tenth the range size of *A. valida* (which has the greatest range) it was classified as globally range restricted. Species not falling within the globally range restricted category are described as widespread in this study. The five species described in Veron (2000) for which there is no distribution data in the WW *Acropora* database were classified as globally restricted in this study.

Figure 3. a). The species of *Acropora* with the largest range is *Acropora valida*. The global range size was calculated from WW *Acropora* database records. The range size of *A. valida* is used to determine which species have ranges that are restricted in comparison b). *Acropora pichoni* is an example of a globally restricted species. 4

2.2.3 Local distribution and abundance patterns

The local distribution and abundance patterns of 83 species of *Acropora* were recorded at 100 sites in the North-west Pacific Ocean: 87 sites in the Marshall Islands (Rongelap Atoll, Mili Atoll, Ailinginae Atoll, Bikini Atoll) and 13 sites in Kimbe Bay, Papua New Guinea (Figure 4). These locations were chosen because they represent the reef types available in the NW Pacific – i.e. atoll environments with steep slopes and lagoons, along with fringing continental reefs and submerged patch reefs. Sites were randomly chosen within 2 habitats (exposed wall and protected lagoon) to a maximum depth of 30m. Exposed sites included fringing reef flat, reef crest and both shallow and deep reef slope microhabitats. Lagoon sites included sandy inter-reefal areas and submerged patch reefs featuring deep vertical and shallow reef top microhabitats. Over half of the recording time was spent in shallow habitats (<12m) that feature the greatest abundance of corals, however we included the 12-30 m depth range because many *Acropora* species extend their range into deeper habitats (Wallace, 1999; Veron 2000).

The diversity and relative abundance of corals in the genus, *Acropora*, were surveyed at each site by rapid visual assessment during a 60 minute timed SCUBA swim or until

species accumulation reached a plateau. The widest possible variety of microhabitats was searched at each site. Each site represents approximately 3000m^2. All *Acropora* species encountered were identified and relative abundance was ranked on a 5-point scale that is analogous to a logarithmic scale (1-2 colonies = rare; 3-5 colonies = infrequent; 6-20 colonies = frequent; 21-50 colonies = common; 51+ colonies = dominant). If individuals could not be identified confidently in situ, skeletal specimens were taken to enable microscopic analyses of morphological features. Skeletal specimens are registered in the Museum of Tropical Queensland. When large stands were encountered, every 1m^2 was treated as a separate colony. Local distribution is quantified by determining the percentage of sites a species occurs in, if a species occurs in <10% of sites, it is described as having a restricted local distribution.

Figure 4. The local distribution and abundance patterns of 83 species of *Acropora* were recorded at 100 sites in the North West Pacific Ocean (Marshall Islands and Papua New Guinea). A star denotes locations where surveys were undertaken.

2.2.4 Analysis

The relationship between local abundance and local distribution was analysed using descriptive statistics, non-parametric tests and linear regression. Principal co-ordinates analysis (PCO) was initially considered to examine community structure but the large number of negative eigenvectors prevented the utility of this analytical technique. Thus multivariate assemblage structure was analysed by SIMPER (in PRIMER 6 Beta

version) and by non-metric multidimensional scaling (nm-MDS in SAS). Two features of the data provided challenges - firstly, the data consisted of many species relative to the number of samples and secondly the data contained many structural zeroes (reflecting true absences). A range of power transforms were considered to reduce the effect of dominant species, and visual examination of species-frequencies plots indicated that the $x^{0.25}$ transformation best balanced the potential influence of abundant, mid-range, and rare species in the analysis. Considering the large number of zeros in the dataset, a range of multivariate distance measures was considered. The Bray-Curtis measure was chosen because it weights co-occurrences of species in samples (Legendre and Legendre 1998; Krebs, 1989). MDS's were run for a range of number of axes to identify the dimensionality of the data, and Stress 1 used as a measure of goodness of fit. For final presentation the ordination axes were centred and rotated by Principal Components Analysis (implicit in the software). Scaling analyses were carried out in the Statistical Analysis System (SAS) MDS procedure, using custom macros to calculate the Bray-Curtis distance.

In the first multivariate analysis, common species dominated the analysis, and so information on rare species was not represented. To address this problem, we reversed the weighting of abundant and rare species. Data were transformed by their inverse: $(x+0.5)^{-1}$, making large values small, and small values large. The addition of the constant was required because the inverse of 0 is infinity. The value of 0.5 was chosen by after examining a range of values, and selecting the one that gave the best 'spread' of values across species and did not over-weight 0's relative to 1's and other low values. As above, nm-MDS with the Bray-Curtis measure was found to be the best ordination approach and the same procedures for examining dimensionality and final display were used. To test whether location, exposure, and their interaction were statistically significant we ran Multivariate Analysis of Variance on the sample scores of the set of nm-MDS axes that accounted for Stress values of <0.05 for each the $x^{0.25}$ and $(x+0.5)^{-1}$ analyses. This Stress value was chosen arbitrarily to represent the number of axes that explained the variation well, yet still was analysable given the ratio of species to observations. Similar approaches have been used elsewhere to deal with problems of too few samples for full analysis (eg. Syms & Jones 2000) or to preserve a particular multivariate distance measure (eg. Anderson & Willis 2003).

2.2.5 Coral rarity model

I modify the Rabinowitz's (1981) rarity model to replace the habitat specificity parameter with that of local distribution to optimise the relevance of the rarity model for understanding coral biodiversity. The new multi-scale coral rarity model for corals describes eight patterns of distribution and abundance, herein called 'occupancy types' (Figure 5). The parameter 'local distribution' is defined as the number of sites at which a species occurs within a location (e.g. Rongelap Atoll). I hypothesize that the eight different types of occupancy can lead to 3 general consequences for a species' ecological trajectory – persistence, compensation or extinction. Species classified as displaying occupancy type 1 are rare across all scales and are vulnerable to global extinction. Species with occupancy type 2 are locally rare in terms of distribution and abundance however they have a large global distribution. These taxa are vulnerable to insidious local population extinctions. Occupancy types 3, 4 and 5 represent species that have a restricted global distribution but may potentially compensate for their restricted range by having a large local abundance or by occurring at a large number of sites locally.

Figure 5. Multi-scale rarity model depicting 8 types of occupancy that are proposed to describe the patterns in the distribution and abundance of corals in the genus *Acropora*. Occupancy types 1-7 represent different types of rarity and occupancy type 8 represents commonness. Large circles represent species that are not range-restricted or have a large local abundance. Small circles represent species that are range-restricted or have a low abundance. Adapted from Rabinowitz, 1981.

Species with occupancy types 6, 7 or 8 describe species that have widespread global distributions and a high likelihood of long-term population persistence. In the case of occupancy type 6, species occur in low abundance at a large number of sites and in occupancy type 7 species have widespread distributions but occur at a small number of sites in high numbers. Species displaying occupancy type 6 are characterized here as persistent, at least partly because their small local populations would minimize density dependant processes (Gaston, 2004). Species displaying occupancy type 7 are also characterized as persistent because they could avoid density dependant processes by occurring in high numbers at only a small number of sites dispersed over a wide range. Occupancy type 8 describes species that are not rare according to any of the 3 measures of rarity. Overall, species occupying positions 6, 7 and 8 would be of least conservation concern and species displaying occupancy types 1 and 2 represent those species with the highest risks of extinction.

While the concepts of extinction and persistence are well described in the literature, the compensatory category is not. The concept of compensatory mechanisms to enable local permanence has recently been suggested to explain the permanence of rare frogs through time (Williams *et al.,* 2006). In this case, the generalist dietary habits of rare frog species enable them to sustain a large local population size (Williams et al. 2006). The relative contributions of compensatory mechanisms to the projected survival of marine populations have not been tested. I use the term compensation here to represent the potential for species that have restricted global ranges to offset the disadvantages of global rarity by having a large local distribution or large local population size or both.

2.3 Results

2.3.1 Global distribution patterns

A moderate strength significant positive relationship exists between the number of records in the WW *Acropora* database and range size estimates (Figure 6). Hence for the remainder of the project I use range size estimates to represent global distribution patterns. From range size estimates 41 species of *Acropora* (i.e. 36% of species in the genus) are geographically restricted, that is their range sizes were ≤10% of the range of the most widespread species, *A. valida*, which had a range of 142,761,832 km^2 (Figure 7) (see Table 2 for species list). Twelve species occurring in the NW Pacific have globally restricted ranges.

Figure 6. Regression of number of records in WW *Acropora* database against range size estimates (km^2) of 114 species. A moderate strength significant relationship exists between these two variables. (Regression Analysis r^2= 0.722, df=113, p=0.000). The remainder of the results I present relate to ranze size only.

Figure 7. Global range size estimates of 114 species of *Acropora*. Depicted in red are the 36% of species that have a globally restricted distribution (meaning their range is one tenth that of *A. valida*). 64% of species have a widespread distribution.

Table 2. List of globally restricted *Acropora* species, region of occurrence and habitat preference. Species with asterix and bold occur within the NW Pacific.

Species	Region of Occurrence	Habitat Preference
A. bushyensis*	**GBR, Pacific**	**reef flat, shallow coastal shoals**
A. chesterfieldensis*	**Chesterfield Is., Micronesia**	**not well characterized**
A. loisetteae*	**Malaysia, W. Australia, Micronesia**	**protected lagoons**
A. kimbeensis*	**PNG, Micronesia**	**submerged reef flat, 3-12m**
A. willisae	GBR, PNG, Japan	submerged reef flat and slopes
A. russelli	Timor Sea, Indonesia	deep sandy reef slopes
A. sukarnoi	Indonesia, Timor Sea	subtidal, submerged reefs
A. elegans	Indonesia	protected steeply sloping reef edges
A. awi	SE Asia, Micronesia	submerged, shallow reef flat, slope
A. hoeksemai	SE Asia	subtidal, slopes or walls of outer reefs
A. lokani*	**SE Asia**	**fringing reefs, reef flat**
A. pichoni*	**PNG, Micronesia**	**submerged shelfs 25-70m, shipwrecks**
A. halmaherae	Indo, PNG	protected sandy slopes
A. globiceps	Central Pacific Ocean	intertidal reef flats
A. indonesia	Thailand, Indonesia	submerged reef flats, slopes
A. simplex	Indonesia, Phillippines	deep reef slopes, 20-60m
A. rudis	Indian Ocean, Thailand, W. Indonesia	fringing reef
A. plumosa*	**Indonesia, PNG**	**reef slopes and submerged reefs**
A. jacquelineae*	**Indonesia, PNG**	**reef slopes and submerged reefs, 10-35m**
A. cervicornis	Atlantic Ocean	submerged reefs, slopes
A. palmata	Atlantic Ocean	submerged reef tops
A. downingi	Red Sea, Arabian Gulf	shallow margins of fringing reefs
A. prolifera	Atlantic Ocean	submerged reef flats, slopes
A. batunai*	**Indonesia, PNG**	**submerged reefs, slopes, 10-40m**
A. eurystoma	Red Sea	upper reef flopes
A. spathulata	GBR, PNG	reef flat and slope to 5m
A. pharaonis	Red Sea	sheltered reef slopes
A. derawanensis*	**SE Asia**	**protected deep sandy slopes, submerged reefs**
A. desalwii	Indonesia	shallow protected areas
A. variolosa	Red Sea	shallow reef flat/crest
A. squarrosa	Red Sea	upper reef slopes
A. arabensis	Arabian Gulf	upper reef slopes and lagoons
A. suharsonoi	Indonesia	submerged walls, 15-25m
A. hemprichii	Red Sea	submerged patch reefs
A. walindii*	**PNG**	**deep sandy reef slopes, fringing reefs**
A. cardenae	GBR	deep intereefal habitat, 55 - 130m
A. rongelapensis*	**Marshall Islands, Irian Jaya**	**protected deep sandy slopes, submerged reefs**
A. roseni	Mauritius, Central Pacific	shallow exposed reef slopes
A. tanegashimensis	Japan	shallow exposed rocky reef
A. torihalimeda	WA, GBR	Halimeda banks

A plot of the number of geographically restricted species occurring in different biogeographic regions shows that thirty-five percent of globally range restricted *Acropora* occur in the NW Pacific Ocean (Figure 8) and thirty percent of globally restricted species occur in South East Asia only. An additional 20% of globally restricted species occur in SE Asia with connections to Pacific Ocean or Indian Ocean locations. Location records of geographically restricted *Acropora* species were plotted to locate rarity hotspots (Figure 9). I find most reef locations provide important habitat for globally range-restricted rare *Acropora* species. I also recognize an accumulation of rare species along the West Pacific rim and along an extended 'Wallaces Line' (Wallace, 2001).

Figure 8. Percentage of globally range restricted species occurring within different biogeographic regions showing the NW Pacific has the highest percentage of globally range restricted species * Note regions are not of equal size.

Figure 9. Globally range restricted *Acropora* species are dispersed across the globe. Collated from the Worldwide *Acropora* Database – Museum of Tropical Queensland, C.C. Wallace and P Muir pers comm.   Dots represent locations where globally restricted *Acropora* species listed in Table 2 occur.

2.3.2 Local distribution and abundance patterns

Surveys at 100 sites in the NW Pacific revealed that more than 80% of *Acropora* species are rare (1-2 colonies per 3000 m^2) or occur infrequently (3-5 colonies per 2500m^2), as highlighted by the skewed frequency distribution of local abundance compiled from counts of each species at each site (Figure 10). The majority (55%) of species were locally restricted, as indicated by their occurrence at 10 or less of the 100 sites surveyed (Figure 11a). Similarly, when the total abundance of each species was summed across sites, the majority (55%) of species were found to be numerically sparse across their NW Pacific ranges (i.e. <20 colonies recorded across all 100 sites Figure 11b). Frequency distributions of species abundances were bimodal with one peak capturing frequent/common species and the other capturing the large number of rare species (Figure 12).

Figure 10. Frequency of occurrence of local abundance categories indicating the largest proportion of *Acropora* in the NW Pacific occur at a density of 3-5 colonies per 3000m^2).

a).

b).

Figure 11. Frequency histograms for 83 species of *Acropora* in the NW Pacific showing: a) The largest proportion of species display a restricted local range (present at 10% or less sites); b) The largest proportion of species have a small total sum of relative abundance across the 100 sites. Normal distribution curves are plotted on histograms to show non-conformity.

Figure 12. A bimodal species-abundance distribution is hypothesized to explain the species abundance distribution of *Acropora* corals whereby a hollow curve captures the rare species and a normal curve captures those species with an intermediate distribution.

When *Acropora* biodiversity is pooled across the 87 sites in the NW Pacific, there is a significant linear relationship between the total sum of local relative abundance of species and the total number of sites they occupy (Figure 13, Table 3). This result supports the theory of Lawton (1999) that hypothesizes a positive relationship exists between abundance and distribution which suggests an increase in the number of sites a species occupies will lead to a comparative increase in its abundance. If locally restricted species were locally common, this would lead to departure from linearity however I find species that locally rare and restricted species (i.e. occupancy type 1 - see Section 2.3.4 for further explanation) also display a significant linear relationship (Table 3). The only species that display a non-significant linear relationship between abundance and distribution are those that fall within occupancy type 6 meaning they are locally rare but not locally restricted (Table 3). If these species conformed to the theory of positive abundance distribution relationship then they would be expected to have a larger summed relative abundance than observed.

Figure 13. Linear regression showing the relationship between the sum of relative abundance and local distribution of 83 species of *Acropora* that occur in the NW Pacific.

Table 3. Regression statistics for a test of linearity between abundance and distribution across the pooled dataset and and within different occupancy types (see section 2.3.4).

	R	R Square	df	Sig.
Full dataset	0.974[a]	0.949	83	0.000[a]
Occupancy type 1	0.928[a]	0.861	14	0.000[a]
Occupancy type 4	0.999[a]	0.998	3	0.030[a]
Occupancy type 5	0.903[a]	0.815	22	0.000[a]
Occupancy type 6	0.468[a]	0.219	8	0.242[a]
Occupancy type 8	0.952[a]	0.903	35	0.000[a]

[Predictors]: (Constant), abundance

Determining the local population size of globally restricted species is particularly important for conservation purposes because if globally restricted species have small population sizes that makes them more vulnerable to extinction. All of the globally restricted species (with the exception of one species) occur at a mean density of less than 0.5 individuals per $2500m^2$ (Figure 14). A Mann-Whitney test confirmed that globally range-restricted species have a significantly smaller mean relative abundance

than globally widespread species (U=305.5, p=0.005). However, this result was driven by the 14 most common species because when these 14 common species were excluded from the test, there is no difference in the mean relative abundance of globally widespread and globally restricted species ((U=305.5, p=0.105). Species that have widespread global distributions show considerable variation in their mean relative abundance

Figure 14. Mean (+SE) local abundance per 3000m^2 globally restricted and widespread species of *Acropora* in the NW Pacific. Mean local abundance of restricted species is not significantly different from widespread species when the 12 most common species are excluded. Names of species are given in Table 1.

2.3.3 Community structure

Average similarity within locations was relatively low and average dissimilarity between locations was high especially between Alligninae Atoll, Bikini Atoll and Kimbe Bay (Table 4). A MANOVA confirmed that *Acropora* assemblages differed between sites, irrespective of exposure (Table 5a). These differences were not strong however, and the dimensionality of the data was very high with 14 dimensions required to obtain a Stress value of <0.05. In the 2-dimensional scaling solution Marshall Island (Alinginae, Kimbe, Mili and Bikini) assemblages were relatively distinct from each other (Fig. 15a). The Rongelap assemblage in the Marshall Islands shared features with all other locations. When rare species were weighted more highly than common ones by inverse transformation the patterns in *Acropora* assemblages changed and a strong

location*exposure interaction with associated main effects was revealed (Table 5b). This result was driven primarily by Kimbe Bay (Fig. 15b) where the exposed sites were distinct from the sheltered sites.

Table 4. Average similarity within locations and average dissimilarity between locations.

	Rongelap	Mili	Bikini	Ailinginae	Kimbe
Rongelap	37.04	64.64	76.66	78.92	79.19
Mili		47.86	81.67	83.15	78.46
Bikini			29.5	85.9	84.58
Ailinginae				34.01	85.5
Kimbe					32.81

Table 5a. Multivariate Analysis of Variance of the first 14 dimensions (Stress 1 0.0481) from nm-MDS of the Bray-Curtis distance of $x^{0.25}$ transformed data. This analysis tests differences between assemblages weighted by dominant species. Pillai's criterion is used because it is robust to violations of assumptions.

Source	Pillai's Trace	F	DF	Significance
Location	2.292	7.58	56,316	<0.0001
Exposure	1.730	1.14	14,76	0.3427
Location*Exposure	0.694	1.18	56,218	0.1793

Table 5b. Multivariate Analysis of Variance of the first 12 dimensions (Stress 1 0.0491) from nm-MDS of the Bray-Curtis distance of $(x+0.5)^{-1}$ transformed data. This analysis tests differences between assemblages weighted by rare species.

Source	Pillai's Trace	F	DF	Significance
Location	2.092	7.49	48,306	<0.0001
Exposure	0.250	2.19	12,79	0.0196
Location*Exposure	0.765	1.62	48,328	0.0087

A).

Dimension 1

B).

Dimension 1

Figure 15. Two-dimensional nm-MDS of *Acropora* assemblages, where the distance measure was the Bray-Curtis index calculated for: a) x0.25 transformed data; and b) (x+0.5)-1 transformed data, which weighted rare species over abundant ones. ◆ Alinginae ■ Mili ▲Rongelap ● Bikini Atoll ⬢Kimbe Bay. Open symbols are sheltered locations, shaded symbols are exposed locations.

Plotting the log regression of the mean relative abundance of *Acropora* species at different locations revealed important differences between locations (Figure 16). The Kimbe bay *Acropora* community is characterized by high evenness whereby all species reach a relatively high local abundance. Species that dominate the Marshall Island locations are less abundant at Kimbe Bay. As opposed to this, the Mili atoll and Rongelap atoll communities are dominated by a small number of species and the majority of species reach a low mean relative abundance. Overall, the Bikini Atoll and Ailinginae coral communities feature low mean relative abundance of individuals.

A plot of the median relative abundance of species at the five locations illustrates which common species drive the observed differences between locations (Figure 17). Mili Atoll features a large local abundance *Isopora palifera, A. nasuta, A. gemmifera and A. loripes*. Bikini features a large population of *A. hyacinthus*. Ailinginae atoll features large local populations of *A. intermedia, A. nasuta, A. nana, A. tortuosa and A. florida*. Rongelap Atoll has a shared affinity with Mili Atoll by featuring large populations of *Isopora palifera, Acropora nausta, Acropora speciosa and Acropora cytherea*. Kimbe Bay is distinct because if it's populations of *A. jacquelineae*, *A. plumosa* and *A. walindii*. The mean abundance and standard error of approximately half of the species is too small to obtain a median value. The only species that occurs in abundance at all location is *A. nasuta*.

Figure 16. Log regression of mean relative abundance with species ranked from most widespread (*A. valida*) to most geographically restricted. This figure shows that all species have relatively high abundance at Kimbe Bay and that a small number of species are extremely common at Mili Atolls. Generally the Bikini Atoll and Ailinginae Atoll communities have the lowest abundance of individuals of each species.

Figure 17. Median abundance of *Acropora* spp. species at five NW Pacific Ocean locations. Depicted are those species whose population size drives differences between locations.

2.3.4 Coral rarity model

Six of the eight possible occupancy types are present in the NW Pacific and five of these occupancy types relate to a type of rarity (types 1-3, 5, 6; Figure 18, Table 6). The greatest proportion of species (38 of 83) has a widespread global distribution with large local distributions and large population sizes (occupancy type 8). The most common type of rarity was represented by species that had widespread global distributions but restricted local distributions and small local abundance (occupancy type 2; 22 out of the 83 species). 13 species were found to be restricted on both global and local distribution scales and had a small local population size (occupancy type 1).

Figure 18. Multi-scale rarity model depicting 6 patterns of occupancy exist in corals in genus *Acropora*. Occupancy type 8 concerning commonness has the highest number of species followed by occupancy type 5 relating to species that have a widespread global distribution and small local distribution and local abundance. No species display occupancy types 3 or 7.

8 species had widespread distribution patterns on both scales but were locally rare (occupancy type 6). One species had restricted global distributions but was locally widespread and common (*A. plumosa* - occupancy type 5). A single species, *Acropora chesterfieldensis*, had a restricted global distribution, was locally widespread but locally rare (occupancy type 5). No *Acropora* species in this analysis displayed occupancy type 3 or 7. Overall, 44 of the *Acropora* species examined here were characterised by permanence occupancy types, 35 had extinction occupancy types, and 2 species followed a compensatory occupancy types.

Table 6.   Category of occupancy exhibited by each *Acropora* species examined in this study.

Occupancy type 1	Occupancy type 2	Occupancy type 3	Occupancy type 4	Occupancy type 5	Occupancy type 6	Occupancy type 7	Occupancy type 8
Restricted Restricted Rare	Widespread Restricted Rare	Restricted Restricted Common	Restricted Widespread Common	Restricted Widespread Rare	Widespread Widespread Rare	Widespread Restricted Common	Widespread Widespread Common
A. rongelapensis *A. lokani* *A. derawanensis* *A. bushyensis* *A. walindii* *A. spathulata* *A. pichoni* *A. loisetteae* *A. jacquelineae* *A. batunai* *A. kimbeensis* *A. awi* *A. plana*	*I. crateriformis* *A. prostrata* *A. spicifera* *A. caroliniana* *A. anthocercis* *A. abrolhosensis* *A. desalwi* *A. glauca* *A. carduus* *A. aspera* *A. insignis* *A. listeri* *A. clathrata* *A. valiencinessi* *I. brueggemanni* *A. solitaryensis* *A. donei* *A. microclados* *A. sarmentosa* *A. kirstyae* *A. tenella* *A. exquisita*	-	*A. plumosa*	*A. chesterfieldensis*	*A. latistella* *A. paniculata* *A. horrida* *A. subulata* *A. divaricata* *A. abrotanoides* *A. lovelli* *A. grandis*	-	*A. subglabra* *A. microphthalma* *A. vaughani* *A. longicyathus* *A. tenuis* *A. intermedia* *A. echinata* *A. acuminata* *A. selago* *I. cuneata* *A. millepora* *A. verweyi* *A. aculeus* *A. elseyi* *A. lutkeni* *A. nana* *A. humilis* *A. robusta* *A. granulosa* *A. monticulosa* *A. muricata* *A. florida* *A. secale* *A. cerealis* *A. samoensis* *A. austera* *A. loripes* *A. hyacinthus* *A. gemmifera* *A. striata* *A. cytherea* *A. valida* *A. digitifera* *I. palifera* *A. nasuta* *A. tortuosa* *A. speciosa*

2.4 Discussion

Acropora species in the NW Pacific display five of seven possible types of rarity according to this analysis of global and local distribution patterns in combination with local abundance patterns.   Overall, 54% of *Acropora* species examined (n= 45 species) displayed a type of rarity, whereas the other 46% had widespread distributions across both global and local scales and were locally common.   Two factors contributed to the identification of a larger number of rare species than previously represented in the coral literature: 1) I recognize multiple types of rarity, and 2) rare species were explicitly targeted in rapid, visual assessment methodology.   Line-intercept transects are often used in coral population ecology studies (e.g. Hughes 2002; Dornelas *et al.,* 2006), however, these methods sample a small area within a restricted subset of habitat types. Because most species occur in low numbers and are sparsely distributed, extensive searching in all available reef habitats is required to accurately represent patterns of *Acropora* biodiversity.   Rapid visual assessment minimizes sampling or statistical zeros that occur from simply not finding a species, and maximizes the structural zeros that are a true measure of absence (Gaston 1994).

Detecting rare species and estimating their population sizes is important for conservation purposes, particularly in a functionally important coral genus like the *Acropora*.   Part of the challenge in detecting rare species is that rarity comes in many forms. I found that thirteen species of *Acropora* are rare and restricted across all scales of distribution and abundance (occupancy type 1). These species are particularly vulnerable to global extinction.   The most common type of rarity, however, is local rarity (Occupancy type 2).   Local rarity occurs when a species occurs in low abundance (low local abundance) at a small number of sites (narrow local distribution) but is sparsely distributed across a large global range (widespread global distribution).   This combined distribution and abundance pattern is also called suffusive rarity (Schoener, 1987). These species are vulnerable to population fragmentation and each individual population is vulnerable to the deleterious effects of small population size (Frankham *et al.,* 1995).

Approximately 10% of *Acropora* species have a widespread global distribution and occur at a large number of sites across that distribution but in very small numbers at each site (Occupancy type 6).   One species had a restricted global distribution but was locally widespread and common (*A. plumosa* - Occupancy type 4).   This latter pattern has been given a variety of names, including diffusive rarity (Schoener, 1987), 'pseudo-

rarity' (Rabinowitz, 1981), and 'abundant centre' distribution (Sagarin & Gaines, 2002), and would typically be synonymous with endemicity. Lastly, a single species, *A.chesterfieldensis* had a restricted global distribution but occurred at a large proportion of NW Pacific sites in low abundance (Occupancy type 5).

Previous studies of coral community structure showed that scleractinian coral assemblages' display truncated log-normal distributions (Connolly et al. 2005), which indicates that rare species are underrepresented in the numerical data. One study rectified this by examining a larger sample (Dornelas and Connolly, 2008) and unveiled a multimodal log-normal distribution for scleractinian coral assemblages. However, the impact that rare species had on the species-abundance distribution was not well represented because, although species were sampled intensively, the study focused on a single habitat type (the exposed reef crest), which features only 'hardy' species or those that are typically common. Numerical data presented here arises from 2 genera of coral across a broad range of habitats and supports the premise that coral communities do not show a unimodal normal distribution, moreover their species abundance distribution is more likely to be bimodal.

By using numerical data to capture rare species, we show that not all species of *Acropora* follow the predicted ecological relationship of a positive correlation between a species' abundance and its distribution (Gaston, 1996; Lawton, 1999). Linear regression confirmed that a strong linear relationship generally exists between the abundance and distribution of *Acropora* species, but the significance of this relationship was driven by common species. Important deviations from this pattern were observed for species displaying rarity type 6. In these cases, species are locally rare despite being widespread across local and global scales, which leads to departure from linearity. This finding is not unprecedented, with studies of plants (Boecken & Shachak 1998), fish (Marshall & Frank, 1994), and birds (Blackburn et al 1998; Gaston and Curnutt 1998) showing that abundance and distribution patterns are not always positively correlated.

There is an excess of rare species in nature (Magurran and Henderson, 2003). The excess of rare species was evident in the isolated Marshall Island coral communities that were dominated by a small number of species while the majority of species occur in low abundance. In Kimbe Bay communities however, the hollow curve distribution (i.e. a few abundant species with widespread distributions and many rare species with narrow distributions) was not evident. Here, most species reached a similar local

abundance, thus the assemblage is characterised by high evenness and lack of community domination. The former pattern is in accord with MacArthur *et al.*'s (1972) hypothesis of density compensation in island communities. Density compensation refers to the lower overall species richness on islands enabling a small number of species to capitalize on available resources and reach higher densities than they would in a more species rich (mainland) community. In island communities, the loss of a dominant species would have a lasting impact at the community level, whereas in a community with higher evenness, the loss of a single species is typically of lesser significance unless that species plays an irreplaceable functional role.

Some rare species of *Acropora* are reported to occur only in protected habitats (Wallace *et al.,* 2001), consistent with Rabinowitz's (1981) finding that habitat specialization is often associated with rare species. Consequently, the lack of an exposure effect in our data using conventional data transformations in MDS analyses was intriguing. However the poor MDS solution and high dimensionality of the 0.25 transformed data indicated that the structure was weak. Moreover, common species, which show the greatest amount of variance, were over-represented in the dataset and rare species were under-represented, even though rare species were targeted in field surveys and included in analysis. The inverse transformation, which weights rare species more heavily and enables them to be represented across multiple dimensions, provided a solution to the challenge of adequately representing rare species in community analyses and a significant exposure effect was revealed. Similarly, I found a high level of similarity among locations when *Acropora* assemblages were compared across the 100 sites in two dimensions. However, following inverse transformation, *Acropora* assemblages at Kimbe Bay sites were found to be significantly different from those at Micronesian sites. A previous study showed that when rare species are excluded from data, the trends remain the same; giving rise to the conclusion that rare species data are redundant (Marchant 1999). By including rare species I reveal differences in assemblage structure between habitats and among locations. This result highlights the importance of maintaining rare species in datasets and in using appropriate analyses, like inverse transformation, to capture their signal.

It is clear that the large number of rare species in the genus *Acropora* contributes to the high diversity of the genus. But the question remains, why are patterns of abundance and distribution so diverse in *Acropora*? Generally, environmental stochasticity is thought to be the major driver of diversity patterns on coral reefs (Chesson and Huntly, 1989; Dornelas et al. 2006). Moreover, spatial heterogeneity in the structure of coral

communities has been shown to result from fluctuations in species composition as a consequence of environmental change (Bak and Nieuwland, 1995; Pandolfi, 2002; Connell et al, 2004).   However, it is interesting to note that the Pleistocene fossil record suggests that environmental variability is not an exclusive force in governing coral communities because coral community structure appears to have remained stable through multiple episodes of climate fluctuations (Pandolfi and Jackson, 2006). However, rare species are not well preserved in the fossil record and thus it would be premature to rule out environmental variability as an explanation for the high diversity patterns observed in the genus.

Alternative biological explanation for the high proportion of rare species in *Acropora* communities is that the broadcast spawning mode of reproduction of *Acropora* corals increases their dispersal potential (Graham et al. 2008) and opportunities for speciation (Willis *et al.,* 2006). In broadcast spawning corals there can be a high level of gene flow and population connectivity (Underwood *et al.,* 2007).   However through time, ecological factors such as environmental change and competition drive temporary isolations and pulses of range expansion and contraction (Erwin, 1981).   During phases of range contraction, new moderately differentiated lineages can evolve (Ricklefs and Bermingham, 2002) and hybridization via introgression has also been argued to contribute to coral diversification (see review by Willis *et al.,* 2006).

Traditionally, only species that are rare across multiple axes would be thought of as threatened, however each of the patterns of rarity described here correspond to an elevated extinction risk. For example, even if a species has a widespread global distribution, if it occurs sparsely across that distribution, it is vulnerable to local extinctions and population fragmentation because the permanence of a species is related to its local population size (Magurran and Henderson, 2003). Hence regardless of a species' overall range, if local populations are small, insidious losses can have devastating effects on species survival (Ehrlich and Daily 1993; Hughes et al 1997). However, there are always exceptions to almost all broad correlates of extinction risk, and in as much, some rare species survive through time (Pianka, 1986; Stacey and Taper, 1992; Willi *et al.,* 2006).

In terrestrial systems, compensation is suggested to be the mechanism enabling the permanence of rare frogs (Williams, et al. 2006). It has been shown that despite having a restricted distribution, some rare frogs have a broad niche breadth that confers a large local population size and high ecological resilience that compensates for their

restricted range. Species following compensation occupancy types are taken to be less vulnerable than would be expected from their global distribution patterns alone. We found little evidence that NW Pacific species of *Acropora* use compensation tactics to minimise extinction risk, with only two species exhibiting compensatory occupancy types.

The coral rarity model proposed here provides a framework within which species can be examined in diverse communities. It identifies those species following 'persistence' pathways, which is important because these species are likely to play critical functional roles and be the most resistant and/or resilient to the current environmental conditions. I assume persistent species to have a strong chance of surviving through population fluctuations by virtue of their large population sizes and ability to reverse local losses by recolonization. One would surmise however that a species position in a permanence occupancy type is by no means fixed. Disturbances could potentially cause species to shift into another (less desirable) occupancy type. Such shifts could be traced through time by conducting the repetitive biodiversity surveys and interpreting the results in the context of the rarity model. If a species in a compensatory or permanence occupancy type falls into an extinction occupancy type this could trigger management response.

Given that *Acropora* corals are among the most highly susceptible to climate change and local disturbances (Carpenter et al. 2008), examining the permanence of rare species is a critical future direction for biodiversity conservation. Here I examine ecological occupancy types from global and local assessments of relative abundance and distribution and demonstrate how this can help prioritise conservation action by informing management which species are of highest and lowest extinction risk in their region. Such knowledge is imperative for setting conservation priorities and will be invaluable for management agencies. While this ecological study was conducted in the NW Pacific, it provides a benchmark for similar studies to be conducted in other regions. I show that together, the large number of species with rare and restricted abundance and distribution patterns contribute significantly to the local biodiversity and overall complexity and integrity of coral communities.

CHAPTER 3   Phylogenetics of rare *Acropora* species

3.1 Introduction

Rare species may occur at both ends of a phylogenetic spectrum. A rare species may be a "living-fossil" or a relict of an ancestral lineage which may have formerly been common but whose populations have declined and/or fragmented through time or are approaching the end of their life span (Ricklefs and Cox, 1972; Blackburn & Gaston, 1998, Segarra-Moragues & Catalan, 2002; Byrne *et al.,* 2001).   Alternatively, a rare species may be newly evolved and still expanding their distribution ranges (Blackburn & Gaston, 1998; Givnish and Sytsma 1997; Knowlton, 1993; Knowlton and Jackson, 1994; Schluter, 2000; Thomas *et al.,* 1997; Young and Brown, 1996; Mora *et al.,* 2003).   Both relictual and newly evolved rare species have high conservation value because they retain either ancient genetic characteristics, or new characters or character states.

The current systematic system of the genus *Acropora* is based on morphological characters (skeletal form and structure, Wallace 1999).   Under this system, 1114 species are described (Wallace 1999; Richards and Wallace, 2004) in 20 distinct lineages (also called 'species groups'). The evolution of *Acropora* depicted in the morphological phylogeny of Wallace (1999) can be seen as a transformation from heavy, simple thick branched corals in which the axial corallite forms the major skeletal component of the branch towards light, complex narrow branching corals in which the axial corallite is slender but repeated more often giving slender branches with more porus skeletons. Some of the terminal clades repeat patterns seen in the basal clades, most notably returning to a dense skeleton, but by this stage, the dense skeleton occurs within a light and complex skeletal architecture. It is apparent some morphological characters (such as radial corallites) are much more informative for tracing evolutionary relationships in *Acropora* than others (Wallace, 1999).

While the family Acroporidae dates back to the late Cretaceous (144-65 mya) and crossed the K-T boundary (Baron-Szabo 2006), paleontological records suggest the first *Acropora* diversification events occurred in the Eocene (39 – 49 mya) (Wallace and Rosen 2006, Wallace 2008).   This initial diversification facilitated later post-Miocene diversifications in the central Indo West Pacific Ocean when low sea level led to the isolation of submerged calm-water habitats (Wallace, 1999).   It is at this time when many rare *Acropora* species are thought to have evolved.   The fossil record provides

additional evidence in support of this view because endemic corals exhibit relatively derived character states (Pandolfi, 1992).

Currently, the morphological phylogeny portrays the *rudis* group to be the oldest living lineage and the *echinata* group to be the youngest (Wallace, 1999). However some fundamental differences are apparent with the published molecular phylogenies. For example, mitochondrial DNA suggests the Atlantic species - *A. cervicornis* and *A. palmata* are the basal lineage (sensu van Oppen *et al.,* 2001; Marquez, *et al.,* 2002) rather than members of the *rudis* group. *A. longicyathus* occurs in a near basal position in the molecular phylogenies of van Oppen *et al.,* 2001 despite belonging to the 'most recently evolved' echinata group (Wallace, 1999). Lastly, *A. humilis* is scattered through the derived clades in the molecular phylogeny (van Oppen et al. 2001) but is placed in a basal clade in the morphological phylogeny.

So far, only common *Acropora* species have been examined using molecular phylogenetic tools (Odorico and Miller, 1997; Hatta *et al.,* 1999; Márquez *et al.,* 2002; van Oppen *et al.,* 2001, 2002; Wolstenholme, 2004). Rare species are notably absent from the existing molecular phylogenies and this is why they form the focus of this study. Here I use the highly polymorphic and phylogenetically informative single-copy nuclear *Pax-C* 46/47 intron and the mitochondrial DNA (mtDNA) control region (located between *rns* and *cox3*) to examine the evolutionary history of rare and common species in the genus *Acropora*. I construct new phylogenetic topologies based on sequence data for 38 *Acropora* species (16 of which are rare). I also examine intraspecific and interspecific patterns, and discuss the evolutionary processes that sustain *Acropora* biodiversity.

3.2 Methods

3.2.1 Species examined and tissues collected

Thirty-eight of the 114 species of *Acropora* recognized in Wallace (1999) were examined (Table 7). Tissue samples were collected from ~1 cm of branch from individual colonies and placed immediately in absolute ethanol. Ethanol was changed twice (24hrs after collection and after one week) to optimise the quality and stability of DNA.

Samples were collected from the Palm Island Group (S 18° 36.77' E 146° 29.426'), Rongelap Atoll, Marshall Islands (N 11° 09.207' E 166° 50.189') and Kimbe Bay, PNG

(N 5° 25.186' E 150° 05.353').   Sequences from 38 species (102 specimens) were
included in this study (Table 7). The majority of geographically restricted species
included in this study display diffusive rarity whereby they have a patchy distribution
and high abundance within some patches (See Table 1 for distribution/abundance and
habitat descriptions of species included in this study). Voucher specimens are available
on request from the Museum of Tropical Queensland (www.mtq.qld.gov.au). Samples
included in this study are 'representative' of each species and individuals with
intermediate morphologies were purposefully excluded from the study.

3.2.2 DNA extraction, PCR conditions

DNA was extracted from approx. 20 mg of coral branch. Branches were air dried briefly
on paper towel to remove ethanol. The coral skeleton was crushed in a sterile mortar
and pestle with 750 µl of grinding buffer (100 mM Tris pH 9.0, 100 mM EDTA, 1% SDS,
100mM NaCl, Filtered H_2O) to enable maximum disruption the cell membrane and
release of DNA. Contents were vortexed for 30 sec and incubated for 3 hours at 65ºC,
vortexing occasionally. When removed from the water bath, tubes were thoroughly
vortexed and cooled on ice. To precipitate the proteins, 187.5 µl of ice-cold 5 M KOA
(Potassium Acetate) was added, mixed well and incubated on ice for 10 min.   The
samples were spun for 10 min in a centrifuge (12000 rpm) to remove proteins and cell
debris. The supernatant was then transferred to a new eppendorf tube.   Six-hundred
µl isopropanol was added to each tube to precipitate the DNA. After mixing, the
samples were left to stand at room temperature for 5 min. Samples were then spun in a
centrifuge for 15 min (12000 rpm), and the supernatant was removed. 150 µl of 70%
ethanol was then added to the DNA pellets, mixed well and centrifuged for 5 min.   The
supernatant was removed again and the pellets were air-dried for 5 min. The purified
DNA pellet was then resuspended in 100 µl 0.1 M Tris pH=9 and stored at -20°C.
Target segments were amplified in a Polymerase Chain Reaction (PCR) using the
primer pairs in Table 8.

3.2.3 Pax-C Intron

For the Pax-C intron, Pax-C-intron-FP1 and Pax-C-intron-RP1 primers were used. The
forward primer was located at 112-90 bp upstream of the intron. The reverse primer
annealed to the 3' end of the intron. Conditions for the PCR reaction included using
150-200 ng of DNA template and 0.13 µ l *Taq* polymerase in a 25 µl reaction in the
presence of a 10x reaction buffer (Fisher Biotech), 2 µl $MgCl_2$ (25 mM), 1.5 µ l dNTPs
(2 mM), 2 µ l forward and reverse primers and 14.87 µl H_2O.

The PCR profile consisted of an initial denaturation step of 95º for 3 mins followed by 35 cycles of 30 sec at 94ºC, 30 sec at 55ºC and 1 min at 72ºC. The mix was incubated at 72ºC for 10 min. Three µl of the PCR product was electrophoresed in a 1% TAE-agarose gel in 1xTAE buffer to assess the yield. Successful products were then cleaned using MO BIO UltraClean DNA Purification Kit. Alternately, the whole PCR product was electrophoresed and successful segments were excised and purified by spinning in a microcentrifuge for 5 min at 12 00rpm.

3.2.4 Cloning

Pax-C PCR products were cloned using the ligation kit, pGEM T easy (Promega) (5 µl ligation buffer, 1 µl pGEM-T Easy Vector, 3 µl PCR product, 1 µl DNA ligase) and left to incubate for 1-4 hrs at room temperature or overnight at 4°C. The ligation reaction was transformed by adding 60 µl of NM522 competent cells. Following 20 min on ice, the transformation was heat shocked at 42°C for 90 seconds. One ml LB Broth was added and the transformation was incubated and shaken at 37°C for 1 hr. The transformation was transferred to an eppendorf tube and centrifuged for 30 sec. Eight hundred µl of supernatant was removed and the remaining pellet was resuspended by pipetting. The transformation was added to warm LB Agar Plates composed of X-Gal, IPG and Ampicillin and spread with a sterile glass stirrer. The plates were then placed overnight in a 37°C incubator.

Ten positive (white) colonies were picked from each plate and put into a PCR tube with 3 µl of PCR grade H_2O. Ten sectors were drawn on a separate AIX plates and swiped by each positive clone. Plates were place overnight in a 37°C incubator. PCR tubes are heated at 95°C for 5 mins to denature the DNA and transferred to ice. The PCR reaction was set up as previously described and the product was electrophoresed to verify if colonies have an insert. Up to 10 positive clones were transferred to 50 ml falcon tubes with 5 ml of LB media and 5 µl Ampicillin (50 mg/ml). Clones were

Table 7. Collection location, habitat and mean depth of species included in this study. * Denotes rare species. Depth records were obtained from the Worldwide Acropora database (www.mtq.qld.gov.au).

Group	Species	Collection Location and Source	Habitat	Mean depth (meters) (+/-SD)	Range
aspera	A. aspera	Palm Islands (van Oppen eta l., 2001)	intertidal/shallow subtidal	3 (+/-2.1)	Indo-Pacific
	A. millepora	Palm Islands (van Oppen eta l., 2001)	intertidal/shallow subtidal	4 (+/-3.4)	Indo-Pacific
	A. pulchra	Heron Island (van Oppen eta l., 2001)	intertidal/shallow subtidal	2.6 (+/-2.3)	Indo-Pacific
	A. palillare*	Palm Islands (This study)	intertidal/shallow subtidal	1.8 (+/-3.3)	SE Asia-Pacific-WA
	A. spathulata*	Palm Islands (This study)	intertidal/shallow subtidal	3.5 (+/-2)	SE Asia-Pacific
echinata	A. carduus	Davies Reef (van Oppen eta l., 2001)	protected sandy slope	10.4 (+/-5)	SE Asia-Pacific
	A. elseyi	Davies Reef (van Oppen eta l., 2001)	shallow subtidal	6.5 (+/-4.9)	Indo-Pacific
	A. longicyathus	Palm Islands (van Oppen eta l., 2001)	subtidal	8.4 (+/-5.7)	SE Asia-Pacific
	A. batunai*	Kimbe Bay, PNG (This study)	deep reef slopes	15.3 (+/-11)	SE Asia
elegans	A. pichoni*	Kimbe Bay, PNG (This study)	deep reef slopes	27.4 (+/-8.3)	PNG
	A. tenella*	Kimbe Bay, PNG (This study)	deep reef slopes	28.6 (+/-11.8)	SE Asia-Pacific
	A. walindii*	Kimbe Bay, PNG (This study)	deep sandy slopes	21.5 (+/-11.4)	PNG
horrida	A. horrida	Big Broadhurst Reef (Fleury unpublished)	shallow subtidal	10.1 (+/-6.1)	Indo-Pacific
	A. microphthalma	Palm Islands (This study)	reef slopes	6.7 (+/-4.4)	Indo-Pacific
	A. vaughani	Palm Islands (This study)	protected subtidal	8.9 (+/-5.9)	Indo-Pacific
	A. tortuosa*	Rongelap Atoll, RMI	subtidal sandy	14.9 (+/-9.6)	Central Pacific
	A. derawanensis*	Kimbe Bay, PNG	protected reef slopes	19.6 (+/-6.6)	SE Asia
	A. kirstyae*	Palm Islands	protected subtidal sandy	14.6 (+/-5.6)	SE Asia-Pacific
humilis	A. digitifera	Magnetic Island	intertidal/shallow subtidal	3 (+/-3)	Indo-Pacific
	A. gemmifera	Trunk Reef (van Oppen eta l., 2001)	intertidal/shallow subtidal	5 (+/-3.8)	Indo-Pacific
	A. humilis	Trunk Reef (van Oppen eta l., 2001)	intertidal/shallow subtidal	5 (+/-4.1)	Indo-Pacific
loripes	A. granulosa	Rongelap Atoll, RMI (This study)	reef slopes	18 (+/-8.9)	Indo-Pacific
	A. loripes	Rongelap Atoll, RMI (This study)	subtidal	10.8 (+/-6.3)	Indo-Pacific
	A. speciosa	Rongelap Atoll, RMI (This study)	reef slopes	19.6 (+/-9.3)	SE Asia-Pacific
	A. caroliniana*	Palm Islands (This study)	shallow subtidal	13.9 (+/-7)	SE Asia/Pacific
	A .chesterfieldensis*	Rongelap Atoll, RMI (This study)	subtidal	19.5 (+/-6)	Pacific Ocean
	A. jacquelineae*	Kimbe Bay PNG (This study)	subtidal reef slopes	18 (+/-7.6)	SE Asia
	A .lokani*	Kimbe Bay PNG (This study)	shallow subtidal	11 (+/-3.3)	SE Asia
	A. rongelapensis*	Rongelap Atoll, RMI (This study)	deep reef slopes	23.8 (+/-4.8)	Micronesia
nasuta	A. cerealis	Trunk Reef (van Oppen eta l., 2001)	shallow subtidal	9 (+/-6.6)	Indo-Pacific
	A. nasuta	Trunk Reef (van Oppen eta l., 2001)	shallow subtidal	4.8 (+/-4.4)	Indo-Pacific
	A. valida	Magnetic Island (van Oppen eta l., 2001)	shallow subtidal	7.1 (+/-6.3)	Indo-Pacific
	A. kimbeensis*	Kimbe Bay PNG (This study)	shallow subtidal	10 (+/-3.4)	SE Asia-Pacific
selago	A. loisetteae *	Rongelap Atoll, RMI (This study)	protected subtidal sandy	17 (+/-3.2)	SE Asia-WA
cervicornis	A. cervicornis*	SBI (van Oppen, 2000)	subtidal	6 (+/-6.7)	Caribbean
	A. palmate*	SBI (van Oppen, 2000)	subtidal	2 (+/-.6)	Caribbean
	A. prolifera*	SBI (van Oppen, 2000)	subtidal	2 (+/-1)	Caribbean

incubated overnight in a shaker at 37°C.   Cell stocks were made by mixing 900 µl of the overnight culture and 300 µl of 80% glycerol, followed by freezing in liquid nitrogen. Cell stocks were kept at -80°C. The remaining overnight cultures were spun in a in a benchtop centrifuge for 5 min at 4000 rpm. The supernatant was removed and DNA isolated using the plasmid isolation protocol in the RBC Hyfield Plasmid Mini Kit. The concentration of DNA was determined using a spectrometer and a sufficient quantity of purified DNA was dried down to reach a final concentration of approximately 100ng /µl.

3.2.5 Mitochondrial Control Region

The same PCR conditions as for the *PaxC* intron were used to target the mtDNA segment, however, an additional 10 cycles were used to amplify the large segments. In some cases, DNA had become unstable and the large mtDNA segments proved difficult to amplify. A number of trials were therefore conducted varying the PCR conditions and master mix composition.   In some cases, an initial denaturation step of 30 sec at 94°C, followed by 35 cycles of 10 sec at 94°C, 60 sec at 54°C and 60 sec at 68°C and 5 min at 68°C proved optimal. Increasing the amount of Taq polymerase assisted the amplification of some segments. The most degraded samples required the use of a Qiagen Core PCR Kit (CAT 201223) or Platinum Taq (Invitrogen) for amplification.

Table 8. Target Primer Pairs

Primer Name	Primer Sequence (5' – 3')
PaxC_Intron_FP1	TCCAGAGCAGTTAGAGATGCTGG
PaxC_Intron_RP1	GGCGATTTGAGAACCAAACCTGTA
Rns_FP1	GGTTTCTAATACCTCCGAGG
Cox3_RP1	TACATAACACTGCCCACAGT
CR_FP1	TCTGATGAGACCCTTGTC
CR_RP1	AATTCTTAGGCAACCCCC

3.3 Data Analysis

3.3.1 Phylogenetics

Nexus files were created in Se-Al 2.0a11 (Rambaut, 2002) from sequences aligned manually in Sequencher 4.5. Mitochondrial and nuclear sequence data were analysed separately in PAUP* 4.0b10 (Swofford 2002) and Mr Bayes 3.1.2 (Huelsenbeck & Ronquist 2001). The optimal model of sequence evolution was identified using hierarchical likelihood ratio tests in Modeltest 3.7 (Posada 2005 – for PAUP*) and Mr

Modeltest 2.2 (Nylander 2004 - for Mr Bayes 3.1.2). Phylogenetic trees were constructed using Neighbour-joining (NJ), Maximum-parsimony (MP) (heuristic search, 1000 bootstrap replicates); Maximum-likelihood (ML) (heuristic search, 100 bootstrap replicates) in PAUP* 4.0B10 (a beta version; Swofford 2002); and Bayesian methods (BI) using Mr Bayes 3.1.2 (Huelsenbeck & Ronquist 2001)). Likelihood settings are listed below. Trees were rooted using sequences of *Isopora (*van Oppen *et al.,* 1999; Fukami et al. 2000) which is a sister genus to the Genus *Acropora* in the family Acroporidae (Wallace *et al.,* 2007). The sequence alignment data is available from GeneBank {accession numbers - EU918202-918288 (mitochondrial data) and EU918771-918925 (nuclear intron data)}. Analyses were conducted on the full alignments without the exclusion of indels or repeat regions because I found the overall topology remained the same when large indels or repeat regions were included, excluded or weighed down (see also: van Oppen *et al.,* 2001). Analyses were also conducted at the species group level however results are not shown as they provide little additional information to that provided in the full trees.

3.3.2 Likelihood and Bayesian settings

nDNA

ML settings from best fit model HKY+G selected via hLRT in Modeltest 3.7: Lset Base=(0.2907 0.2216 0.2105) Nst=2 TRatio=1.3856 Rates=gamma Shape=1.7474 Pinvar=0. BI analyses used likelihood settings from best-fit model HKY+G selected by hLRT in MrModeltest 2.0: Prset statefreqpr – dirichlet (1,1,1,1); Lset nst=2 rates = gamma; burnin=30,000.

mtDNA

ML likelihood settings from best-fit model HKY+I+G selected by hLRT in Modeltest 3.7: Lset Base=(0.2418 0.1958 0.2604) Nst=2 TRatio=0.7400 Rates=gamma Shape=0.3209 Pinvar=0.1591. BI analyses used likelihood settings from best-fit model HKY+I+G selected by hLRT in MrModeltest 2.0: Prset statefreqpr – dirichlet (1,1,1,1); Lset nst=2 rates = invgamma; burnin= 50, 000.

3.3.3 Genetic diversity from pairwise distances

Genetic distances were calculated as Kimura 2-parameter distances (Kimura, 1980) because it allows for unequal substitution rates. The distribution of genetic variation among species was compared using the mean haplotype and allele diversities +/- 95% confidence intervals. The significance of the difference in nucleotide diversity between rare and common species was tested using a Mann-Whitney test. Mann-Whitney tests

were also used to examine the significance of differences between genetic diversity in nDNA versus mtDNA.

3.4 Results

3.4.1 Pax-C Nuclear Intron

A total of 116 new sequences were derived from cloned PCR products of three individuals of each of 22 species. Thirty-seven nuclear sequences are included from previous studies (van Oppen *et al.,* 2001; Marquez *et al.,* 2002}. New sequences were obtained from closely related species within five species groups (A. *loripes, A. pichoni, A. nasuta, A. aspera* and *A. horrida* groups - sensu Wallace, 1999). Additional common species (e.g. *A. millepora, A. tenuis*) were added to the analysis because these species belonged to separate clades in the existing phylogenies. The complete *Pax-C* alignment consisted of 153 sequences from 38 species. *Pax-C* intron sizes ranged from 545 bp – 965 bp and the alignment contained several large indels.

The total *Pax-C* intron sequence alignment consisted of 1681 positions including four large insertions in *Isopora cuneata* (position 231-370), *Acropora millepora* and *A. spathulata** (position 370-760), A. *horrida* (position 871 – 1284) and *A. aspera* (position 1403-1546). Insertions were blast searched (www.ncbi.nlm.nih.gov) and significant matches were found between the *A. horrida* insertion and *Pax-C* intron sequences from *A. latistella* and *A. tenuis* (sequences not included in analysis) while the *A. aspera* insertion matched insertion sequences of *A. florida* and *A. sarmentosa* (sequences not included in this analysis). The *A. millepora* and *A. spathulata** indels were not located in any other species when blast-searched. While this insertion was present in all *A. millepora* sequences, it was only present in half of the *A. spathulata** sequences. A unique large insertion and several unique deletions were also present in *A. horrida* sequences. Individuals of *A. rongelapensis*, A. pichoni** and *A. tenella** had the shortest sequences (544 bp). Seven other species (*A. nasuta, A. kimbeensis*, A. caroliniana*, A. austera, A. longicyathus, A. digitifera, A. gemmifera*) had sequences between 13-25 bp shorter than the most common sequence length of 574 bp.

Phylogenetic analyses of *Pax-C* intron data were broadly consistent with published results (van Oppen *et al.,*2001; Márquez, 2002), but some details differ due to the selection of taxa. To facilitate comparison with previous analyses, clades were labelled according to published trees (van Oppen *et al.,* 2001; Márquez, 2002). The BI tree (shown) distinguished five main clades (Figure 19). As in previous analyses, basal

clade I contains *A. longicyathus*, and, in the present case, *A. austera*. Clade II comprises all *A. horrida* sequences with 100% support, making this species monophyletic.   Given significant matches to *A. latistella* and *A. tenuis* sequences on the NCBI database, we consider *A. horrida* to belong to Clade II which comprises these species in previous analysis.   The strongly supported clade IV includes two species, *A. aspera* and *A. digitifera*, and corresponds to clade IVB in previous analyses. The next clade comprises two species with 100% posterior probability (*A. millepora* and *A. spathulata**) and corresponds to clade IIIA of the previous analyses.   A polytomy then gives rise to three strongly supported clades corresponding to III and IV of previous studies (van Oppen *et al.,* 2001).   Strongly supported subclade IIID corresponds to the Atlantic species. Subclades IIIE, F and G form the large terminal clade and contain 31 species and many small clusters with varying resolution at their base.   A major difference in the present tree is the novel subclade V that is composed exclusively of alleles belonging to rare species (*A. loisetteae**, *A. batunai**, *A. derawanensis**, *A. jacquelineae**, *A. spathulata**, *A. papillare**, *A. tortuosa*) with the exception of a single allele of *A. valida*.   The present tree also distinguishes two novel subclades (III F + G) within the large terminal clade.   These terminal subclades were composed largely of rare species, with the exception of *A. valida*, *A. loripes*, *A. granulosa* and *A. microphthalma*.

Parsimony analysis revealed the same five clades as the BI analysis (not shown). Parsimony analysis revealed the 583 most parsimonious trees. Eighty-two percent (1377) of positions were constant, 8% (39) of positions were parsimony uninformative and 10% (165) of positions were parsimony informative (CI = 0.6501, RI = 0.7466). Maximum Likelihood analysis conducted on the same dataset (not shown) yielded 140 equally likely trees (Shimodaira-Hasegawa probability ≥ 0.05).   Trees constructed in ML yielded were very similar to the BI trees, but with lower support.   Generally, higher resolution was revealed as we moved from MP to ML to BI Analysis. Groupings within clades I, II, and III were preserved throughout all trees.

All rare species fell within clades III and V that form part of the large, derived cluster comprising most *Acropora* species, indicating they are relatively recently evolved species. *A. spathulata** is the only rare Indo-Pacific species that has alleles occurring in the basal part of the tree (clade IIIA in Figure 19).   The only monophyletic rare species in Clade V is *A. jacquelineae**.   Generally, all sequences from clones obtained from the same PCR product fell within a single clade. However on eight occasions, unexpected patterns were observed whereby sequences from a single species fell within two or

Figure 19. *Pax-C* Bayesian topology with posterior probabilities >50%. Rare species are denoted by red font. Clade nomenclature on left in black reflects van Oppen *et al.,* 2001; clade nomenclature on right in blue is new to this study.

more clades (Figure 19, Table 4). For example in a single individual of *A. tortuosa* three different alleles occurred in divergent positions within clades III & V.

3.4.2 Mitochondrial DNA Phylogeny

Sixty-one new mitochondrial sequences were obtained from the same individuals as nuclear sequences and 33 mitochondrial sequences were included from previous studies (van Oppen *et al.,* 2001; Marquez *et al.,* 2002}. The mtDNA control region data set included 94 sequences that varied from 1081-1268 bp in length. *Acropora* species have previously been shown to contain several repeat sequences within the mtDNA control region (van Oppen *et al.,* 2002). Whilst containing the same conserved blocks, the new sequences added to the phylogeny lacked the majority of repeat regions previously described; hence analyses were conducted on the full alignment. There are three general size classes – some individuals from six species have sequences up to 400 bp shorter than the longest and most common sequence length (*A. cervicornis*, A. loisetteae*, A. millepora, A. longicyathus, A. tenuis, A. cerealis* – from 860-863 bp). Four other species have individuals with sequences 141-188 bp shorter than the longest sequence (*A. horrida, A. caroliniana*, A. austera, A. valida* – from 1070-1127 bp). The remainder of species have sequences in the largest size category >1268 bp.

BI analysis identified four clades (Figure 20). All *A. horrida* haplotypes clustered in a highly supported basal clade (II). The next clade contained the Caribbean species along with three Indo-Pacific species, *A. tenuis, A. austera* and *A. longicyathus* and corresponds to clades IIID, IA and IB of previous analysis (van Oppen *et al.,* 2001; Marquez, 2002). The positioning of *A. longicyathus* and *A. horrida* in basal clades is interesting because these two species belong to species groups that are predicted by cladistic analysis of morphological characters to have evolved recently. The next clade to diverge corresponds to clade IV of previous analyses, and is represented by three subclades (IVB, C and D). Subclade IVB contains two species, *A. aspera* and *A. humilis*. Subclade IVC is composed entirely of rare species with the exception of *A. aspera*. Some of the rare species occurring within this clade have very long-branch lengths. Subclade IVD contains three common species: *A. microphthalma, A. vaughnai, and A. cerealis*. The large terminal clade corresponds to clade III of former analysis. It contains 6 subclades, the most divergent of which corresponds to subclade IIIA of former analyses and contains ten species, only one of which is rare (*A. lokani**). The remainder of the terminal clade relates to sublades III E, F, and G and contains twenty species, twelve of which are rare.

ML and MP trees (not shown) were generally consistent with the BI results. As in the case of the nuclear intron, BI analysis provided additional resolution within clades. Notable difference was observed in the mtDNA phylogeny within clade III, as the BI analysis distinguished 6 general subclades that were not resolved in MP/ML. A posterior probability of 100% supports monophyly for six species, *A. horrida. A. papillare*, *A. kimbeensis* A. pichoni*, A. tortuosa*, A. tenuis*. Five species are polyphyletic. For example, *A. jacquelineae** has one haplotype within clade IV and two in a single cluster within clade III. *A. longicyathus* has one haplotype in clade I and one in clade III (Table 4 and Figure 20). Four cases of haplotype sharing were observed in the following species pairs: *A. millepora* and *A. pulchra*, *A. cerealis* and *A. nasuta, A. kirstyae** and *A. walindii**, *A. longicyathus* and *A. carduus*.

Figure 20. Mitochondrial Bayesian 'best tree' with posterior probabilities >50%. Rare species are denoted by red font. Clade nomenclature in black on left reflects van Oppen *et al.,* 2001; clade nomenclature on the right in blue is new to this study.

3.4.3 Genetic diversity from pairwise distances

The Kruskal-Wallis test showed no significant difference between the mean nucleotide diversities of rare and common species $(x^2(2) = 0.887, p = 0.346)$. There was a large amount of variation of haplotype and allelic diversity in some species (Figures 21 a, b). *A. horrida* has high allelic diversity and low haplotype diversity whilst *A. lokani** shows the opposite pattern by having high haplotype diversity and low allelic diversity. *A. kirstyae* has low diversity in both haplotypes and alleles whilst *A. longicyathus* has high diversity in both haplotypes and alleles.

Eight of the 27 species included in this analysis had higher mean mtDNA diversity than nDNA diversity but no significant difference in diversity was detected (Mann-Whitney test, *U = 224, p = 0.15* - see Figure 22). Six of these eight species (*A. walindii**, *A.lokani**, *A. derawanensis**, *A. chesterfieldesis, A. speciosa, A. jacquelineae**) have geographically restricted distributions. The highest mean diversity was found in the geographically restricted coral *A. derawanensis** followed by the widespread species *A. granulosa* and *A. longicyathus*. The highest mean nDNA diversity was observed in the widespread species *A. longicyathus,* while *A. horrida* (which also has a widespread distribution) has high but highly variable nDNA diversity and geographically restricted *A. batunai* also has high nDNA diversity. Limited or no mtDNA diversity was detected among three species (*A. kirstyae, A. papillare** and *A. tortuosa)*; however, considerable levels of nDNA diversity were detected among these species indicating that these individuals were not clonemates.

a).

b).

Figure 21. a). Mean haplotype diversities (+/- 95% confidence intervals). b). Mean allelic diversities (+/- 95% confidence intervals).

Figure 22. Mean pairwise K2P distances + SE for combined nDNA and mtDNA calculated in PAUP* 4.0B10 (a beta version; Swofford 2002). Species are listed in order of decreasing nuclear diversity. * Indicates rare species.

3.4.4 Genetic Overlap between species

Thirty-two percent of species included in this study share an mtDNA haplotype with another species and 57% share a nuclear allele with at least one other species (Table 10). I found nine cases of nuclear polyphyly (alleles/haplotypes phylogenetically interspersed with those of other species, Funk and Omland, 2003) and nine cases of mtDNA polyphyly. Some species are polyphyletic in nuclear DNA and monophyletic in mtDNA (e.g. *A. spathulata* and *A. papillare**), while some show the reverse pattern and are polyphyletic in mtDNA and monophyletic in nuclear DNA (e.g. *A. jacquelineae*. A. vaughani, A. walindii*, A. granulosa, A. microphthalma* and *A. cerealis*). One species is polyphyletic for both loci (*A. longicyathus*).

3.5 Discussion

Most of the rare species included in this analysis are recently evolved. However, phylogeny alone does not explain rarity in *Acropora* corals because some comparatively recently evolved species have wide distributions and large population sizes (e.g. *A. microphthalma*). The results concur with Wallace (1999) in that most of the rare species examined here diverged from their most recent ancestor after the Miocene (approx 5.32 mya). There is little evidence to support rare species being especially young (with the exception of *A. loisetteae* which is suggested to belong to the

selago lineage that arose 10,000 years ago - see Table 9). However my ability to interpret the age of rare corals is hampered by the lack of fossil records for the species included in this study and this prevented molecular clock calibration.

Results from this study suggest that the *horrida* group which was thought to have originated in the Pliocene (1.81 my – 10,000 yrs), respectively (sensu Wallace, 1999; Wallace and Rosen, 2006), is likely to also have been part of the Eocene diversification because *A. horrida* occurs in basal positions in both mitochondrial and nuclear topologies. Similarly, *A. longicyathus* a member of the echinata group (supposedly the most recently evolved lineage in Wallace, 1999); shows a similar evolutionary pattern in nDNA. This discrepancy may be due to the fragility of these species precluding long-term preservation of fossils or it may be a reflection of how difficult it is to identify fossil corals. I consider it likely that these two species are also products of the Eocene diversification events. This result increases the number of *Acropora* lineages arising in the Eocene from 10 to 12.

Some of the most important results of my analysis are the 14 cases of allele sharing (Table 10). Surprisingly, alleles were shared not only between species within species groups but also between species belonging to different species groups. The globally widespread species *A. valida* (*nasuta* group) shares alleles with three members of the *horrida* group *(A. tortuosa*, A. vaughnai and A. microphthalma*). One of these species *A. tortuosa*;* shares identical alleles with two members of the *loripes* group (*A. loripes* and *A. granulosa*). Together these 10 species form a complex syngameon (group of intermittently interbreeding species) (Figure 23). It is likely that other species not included in this analysis would also feature in this syngameon. This syngameon provides convincing evidence that *Acropora* species follow complex evolutionary pathways, and that suggests reticulate processes have played a role in the proliferation of *Acropora* species since the Palaeocene (discussed further in Chapter 4).

Figure 23. Potential syngameon showing allele sharing between species from five different species groups.

Another intriguing example of allele sharing that warrants further discussion is that of *A. tenella** and *A. chesterfieldensis**, these two species belong to separate but closely related species groups (*elegans* and *loripes* groups). Their modern geographic distribution and habitat preferences do not overlap. *A. tenella** is a deepwater specialist occurring in the central Indo-Pacific, *A. chesterfieldensis** is a shallow species occurring in the Coral Sea. The most likely explanation for the allele sharing is that these two species have a shared common ancestry indicating these two species are more closely related than currently recognized. Similarly, I show that *A. kirstyae** and *A. walindii** share both nuclear and mitochondrial DNA indicating that they may be ecomorphs of a single species. *A. kirstyae** is currently placed in the *horrida* species group and *A. walindii** in the *elegans* group. *A. kirstyae** occurs in isolated patches throughout SE Asia, *A. walindii** is known only from its type locality in Kimbe Bay, PNG. If these species are not synonomized as *A. kirstyae** (Veron & Wallace, 1984) they should at least be reorganized to belong in the same species group (the horrida group).

Table 9: Fossil record of species included in this analysis. * Indicates rare species.

Species	Oldest Fossil Record for species	Fossil record for species group lineage	Source
A. walindii*	None	Elegans group - none	
A. rongelapensis*	None	Loripes group - none	
A. loisetteae*	None	Selago group – Holocene – 10, 000 years to present	Wallace 1999
A. pichoni*	None	Elegans group - none	
A .lokani*	None	Loripes group - none	
A. derawanensis*	None	Horrida group - none	
A. tenella*	None	Elegans group - none	
A. batunai*	None	Echinata group - none	
A. chesterfieldensis	None	Loripes group - none	
A. kimbeensis*	None	Nasuta group - none	
A. prolifera	None	Cervicornis group– Lutenian (49-41.3my) – Bartonian (41.4 – 37my)	Budd et al 1999; Wallace 2008
A.spathulata*	None	Aspera group - Bartonian 41.4-37.5 my	Wallace 2008
A. palmata*	Pleistocene - 1.81 –10, 000 years Holocene – 10,000 years to present	Cervicornis group – Lutenian (49-41.3my) – Bartonian (41.4 – 37.5 my)	Wallace, 1999 Wallace 2008
A. kirstyae*	None	Horrida group - none	
A. cervicornis*	Pleistocene - 1.81 – 10, 000 years	Cervicornis group – Eocene: Lutenian (49-41.3my– Bartonian 41.4 – 37.5my)	Wallace, 1999; Wallace 2008
A. paplillare*	None	Aspera group– Eocene: Bartonian 41.4-37.5 my	Wallace 2008
A. speciosa*	None	Loripes group - none	
A. jacquelineae*	None	Loripes group - none	
A. tortuosa	None	Horrida group - none	
A. caroliniana*	None	Loripes group - none	
A. granulosa	none	Loripes group - none	
I. cuneata	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	Genus Isopora - Eocene	Budd & Wallace 2008
A. vaughani	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	Horrida group - Pliocene - 5.32 – 1.81my to Pleistocene - 1.81my – 10,000 years	Wallace, 1999
A. pulchra	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	Aspera group– Eocene: Bartonian (41.4 – 37.5 my)	Wallace, 1999 Yabe & Sugiyama, 1935
A. aspera	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	Aspera group– Eocene: Bartonian (41.4 – 37.5 my)	Wallace, 1999 Pickett et al, 1985
A. longicyathus	Pleistocene - 1.81 – 10, 000 years	Echinata group - Pleistocene - 1.81 – 10, 000 years	Pickett et al, 1985
A. loripes	None	Loripes group - none	

A. gemmifera	Pleistocene - 1.81 – 10, 000 years	Humilis group II – Eocene; Priabonian (36-34.2 my)	Pandolfi, 1996; Wallace 2008
A. microphthalma	Miocene - 25 – 5.32 my; Pleistocene - 1.81 – 10, 000 years	Horrida group - none	Wells, 1964 Pickett et al, 1985
A. millepora	Pliocene - 5.32 – 1.81my Pleistocene - 1.81my – 10,000 years	Aspera group – Eocene: Bartonian (41.4-37.5 my)	Wallace, 1999 Pickett et al, 1985
A. digitifera	Holocene – 10,000 years to present	Humilis group I - Eocene	Camoin et al.,1997; Wallace 2008
A. humilis	Miocene - 25 – 5.32 my; Pleistocene - 1.81 – 10, 000 years; Holocene – 10,000 years to present	Humilis group II – Eocene – Priabonian (36-34.2 my)	Wallace, 1999; Wells, 1964; Wallace 2008
A. austera	Pleistocene - 1.81 – 10, 000 years	Rudis group - none	Wallace, 1999
A. cerealis	Pliocene - 5.32 – 1.81my	Nasuta group - none	Wallace, 1999
A. nasuta	none	Nasuta group - none	
A. valida	Pleistocene - 1.81 – 10, 000 years	Nasuta group - none	Pickett et al, 1985; Wallace, 1999

Table 10. Summary of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) topologies highlighting allele/haplotype sharing and abundance/distribution patterns.

Species	Abundance/ Distribution	nDNA (n= number of sequences)	nDNA allele sharing	mtDNA haplotype sharing	mtDNA (n=number of sequences)
A. walindii	Rare/Restricted	2 alleles in Clade III (n= 4)	*A. kirstyae & A. batunai*	*A. kirstyae*	Polyphyletic. 1 haplotype Clade IV, one Clade III (n= 2).
A. rongelapensis	Rare/Restricted	5 alleles in clade III (n= 5)			3 haplotypes clade III (n= 3).
A. loisetteae	Rare/Restricted	Polyphyletic. 5 alleles, 2 in Clade V, 3 in Clade III (n= 5)	*A.spathulata, A. valida (separately)*		2 haplotypes clade III (n= 2)
A. lokani	Rare/Restricted	4 alleles in Clade III (n= 4)			3 haplotypes clade III (n= 3)
A, pichoni	Rare/Restricted	4 alleles clade III (n= 4)	*A. tenella*		Monophyletic group in clade V. 2 haplotypes 100% prosterior probability (n= 3)
A. derawanensis	Rare/Restricted	Polyphyletic. 4 alleles. 2 in clade III, 2 in clade V (n= 4)	*A. jacquelineae*		2 haplotype with extremely long branch length Clade IV (n= 2)
A. tenella	Rare/Restricted	4 alleles in clade III (n= 4)	*A. chesterfieldensis & A. pichoni (separately)*		3 haplotypes Clade III (n= 3)
A. batunai	Rare/Restricted	Polyphyletic. 1 allele in clade V and 4 alleles in Clade III (n= 6)	*A, walindii, A. kirstyae*		2 haplotypes clade IV (n= 2). One haplotype has extremely long branch length.
A. chesterfieldensis	Rare/Restricted	4 alleles in Clade III (n= 4)	*A. tenella*		3 haplotypes Clade III (n= 3)
A. kimbeensis	Rare/Restricted	4 alleles in clade III (n= 4)			Monophyletic group clade III, 2 haplotypes (n= 3)
A. prolifera	Rare/Restricted	Clade III (n1)	*A. palmata*		not included in Mt study
*A. spathulata**	Rare/Restricted	Polyphyletic. 7 alleles. 2 om Clades V, 5 in clade III (n= 7)	*A. loisetteae*		Monophyletic group in Clade III, 1 haplotype (n= 3)
A. palmata	Common/Restricted	Clade III (n1)	*A. prolifera*		not included in Mt study
A. kirstyae	Rare/Widespread	6 alleles in terminal part of Clade III (n= 6)	*A. walindii* & *A. batunai*	*A. walindii*	3 haplotypes cluster together in Clade IV (n= 3).
A. cervicornis	Common/Restricted	Clade III (n= 2)			2 haplotypes, monophyletic clade III (n= 2)
A. papillare	Rare/Restricted	Polyphyletic. 7 alleles. 2 alleles in Clades V and 5 alleles in Clade III (n= 7)	*A. gemmifera*		Monophyletic group in Clade III. 1 haplotype. 100% prosterior probability (n= 3)
A. speciosa	Common/Restricted	6 alleles in Clade III (n= 6)	*A caroliniana & A. vaughnai (separately)*		3 haplotypes cluster together Clade III (n= 3)
A. jacquelineae	Rare/Restricted	5 alleles cluster in Clade V (n= 5)	*A. derawarensis*		Polyphyletic. 1 haplotype clade IV, 2 haplotypes cluster together clade III (n= 3).
A. tortuosa	Common/Widespread	Polyphyletic. 10 alleles. 3 alleles in clade V and 7 in clade III (n12)	*A. loripes, A. granulosa, A. valida (separately)*		Monophyletic group, single haplotype Clade III (n= 3).
A. caroliniana	Rare/Restricted	3 alleles in Clade III (n= 5)	*A. speciosa*		3 haplotypes cluster together Clade III (n= 3)
A. granulosa	Common/Widespread	4 alleles in clade III (n= 4)	*A. tortuosa*		3 haplotypes cluster together Clade III (n= 3)
A. carduus	Common/Widespread	1 allele Clade III (n1)		*A. longicyathus*	1 haplotype in Clade III (n1).

A. vaughani	Rare/Widespread	4 alleles in Clade III (n= 6)	*A. micropthalma A. valida, A. speciosa*		Polyphyletic. I divergent haplotype Clade IV, 2 clade II (n= 3)
A. pulchra	Common/Widespread	2 alleles Clade III (n= 2)		*A. millepora*	1 haplotype present in Clade III (n1).
A. aspera	Common/Widespread	4 alleles Clade IV (n= 4)			3 haplotypes Clade IV (n= 3)
A. longicyathus	Common/Widespread	Polyphyletic. 2 alleles clade I and 1 in clade III (n= 3)		*A. carduus*	Polyphyletic. 1 haplotype in Clade I, 1 haplotype in Clade III (n= 3).
A. horrida	Common/Widespread	3 alleles in monophyletic Clade II (n= 3)			3 haplotypes in monophyletic group in Clade II (n= 3)
A. loripes	Common/Widespread	4 alleles in Clade III (n= 4)	*A. tortousa*		3 haplotypes Clade III (n= 3).
A. gemmifera	Common/Widespread	2 alleles in Clade III	*A. palillare*		2 haplotypes Clade III (n= 2)
A. elseyi	Common/Widespread	I allele Clade III (n1)			1 haplotype in Clade III (n1)
A. microphthalma	Common/Widespread	3 alleles in Clade III (n= 3)	*A. vaughnai & A. valida*		Polyphyletic. 2 haploypes Clade IV, 1 Clade III (n= 3)
A. millepora	Common/Widespread	5 alleles Clade III (n= 5)		*A. pulchra*	3 haplotypes cluster together in Clade III (n= 3).
A. austera	Common/Widespread	2 alleles Clade I (n= 2)			1 haplotype clade I (n1)
A. cerealis	Common/Widespread	4 alleles in Clade III (n= 4)		*A. nasuta*	3 haplotypes Clade III (n= 3).
A. nasuta	Common/Widespread	6 alleles in Clade III (n= 6)		*A. cerealis*	1 haplotype clade III (n1).
A. valida	Common/Widespread	Polyphyletic. 4 alleles, 1 in clade V and 3 in Clade III (n= 4)	*A. vaughani and A.microphthalma, A. loisetteae, A. tortuosa (separately)*		3 haplotypes clade III (n= 3)
A. digitifera	Common/Widespread	1 allele in clade IV (n1)			2 haplotypes clade III (n= 2)
A. humilis	Common/Widespread	not included in nuclear study			3 haplotypes clade IV (n= 3)
A. tenuis	Common/Widespread	not included in nuclear study			3 haplotypes in monophyletic group clade I (n= 3)

The results indicate many cases of polyphyly that occurs most often in nDNA but also in mtDNA. Polyphyly was not restricted to rare species however it was more frequent in rare species. For example, the rare species *A. derawanensis**, *A. loisetteae* A. batunai*, A. papillare*, A. spathulata** and *A. tortuosa* are polyphyletic in nDNA whereas the only common species displaying polyphyletic nDNA patterns were *A. valida* and *A. longicyathus*. The occupation of a single species in different positions in a phylogenetic analysis has been demonstrated previously in the genus *Acropora* (e.g., *A. longicyathus* (van Oppen *et al.,* 2001), and *A. hyacinthus* and *A. cytherea* (Márquez *et al.,* 2002) and this conflicts with the expectation of the biological species concept that alleles/haplotypes from a species will fall within a single clade.

It is possible that the observed intraspecific heterogeneity may reflect alternating phases of population expansion and contraction across a species range (Ricklefs and Bermingham, 2002). In this case, multiple colonization or expansion/contraction events could have resulted in the evolution and co-existence of moderately differentiated lineages within a single species. Ecological factors such as environmental change and competition drive pulses of range expansion (Erwin, 1981). Between pulses, a species range is maintained or contracts and local adaptation could lead to a degree of differentiation. While intraspecific polymorphism could possibly be explained by multiple expansion/contraction events, intra-individual polymorphisms are significantly more difficult to explain. In the case of *A. tortuosa* and *A. loisetteae**, different alleles sequenced from a single PCR product from a single individual occurred in divergent clades. Such patterns are not without precedent as they have been observed in other scleractinian coral genera including *Madracis* (Diekmann *et al.,* 2001); *Montipora* (Willis *et al.,* 1997; van Oppen et al. 2004); *Platygyra* (Miller and Babcock, 1997; Miller and Benzie 1997; Willis *et al.,* 1997) and *Montastrea* (Szmant *et al.,* 1997) and they provide significant challenges to coral systematics. In these studies, complex evolutionary patterns are interpreted as resulting from either incomplete lineage sorting (i.e. shared ancestral polymorphisms), or hybridisation (Arnold, 1992; van Oppen *et al.,* 2001; Wolstenholme *et al.,* 2004; Vollmer and Palumbi, 2002). In Chapter Four I further explore the hypotheses that may explain the observed patterns of allele sharing and polyphyly.

One of the most interesting species included is this study is *A. valida* because it has the most widespread distribution of all *Acropora* species (Wallace, 1999). It known to be morphologically plastic (E. Turak, JEN Veron pers comm.) and karotyping suggests it is tetraploid (Kenyon, 1997). From the complex patterns of allele sharing and polyphyly

shown here, I hypothesize that *A. valida* acts as a reservoir and conduit for genetic variation (examined further in Chapter 5).   The dispersal of genetic diversity between species via conduits may be an overlooked but functionally important process in *Acropora*.   This process does not however appear to be restricted to species that maintain a widespread global distribution, moreover local abundance is likely to drive the pattern.   An example is *A. tortuosa* in the Marshall Islands.   This species is geographically restricted to the Pacific Ocean however in some locations, it is abundant and dominant in (marginal) inter-reefal lagoonal habitat (e.g. at Rongelap Atoll). Results presented here (e.g. allele sharing and intraspecific and intra-individual polyphyly) suggest *A. tortuosa** may also act as a conduit for genetic diversity in a similar fashion to *A. valida*.

The addition of rare species to the existing *Acropora* phylogeny has revealed novel evolutionary information about modes of speciation. I suggest that in a similar fashion to other members of the animal kingdom (Lynch 1989, Turelli *et al.,* 2001), allopatric speciation may be the primary mode of speciation in *Acropora*. Coral species currently restricted to the central Indo-Pacific at depths relatively unusual for *Acropora* species (i.e., below 30 m, *A. tenella**) are prime examples of taxa that have speciated via allopatry driven by vicariant events pertaining to the last glacial sea level falls (Wallace, 1999). Other species in this study exhibit complex evolutionary histories that cannot be explained by allopatric speciation alone (see Chapter 4). In the case of *A. tortuosa* I hypothesize that polyploidy, perhaps also driven by introgression may have led to the sympatric speciation of *A. tortuosa** in the Pacific Ocean.

One result that remains to be explained is the finding that almost one third of the species included in this study have higher mtDNA than nDNA intraspecific diversity. This pattern is unexpected because mtDNA is known to evolve slowly in Anthozoa (Shearer *et al.,* 2002). Most of the species exhibiting this pattern of higher mtDNA diversity are rare (*A. walindii*, A.lokani*, A. derawanensis*, A. chesterfieldesis*, A. speciosa*, A. jacquelineae**). High mean nuclear diversity was found in the geographically restricted coral *A. derawanensis** largely because one of its nuclear alleles was highly divergent. A recent study shows in animals, the mitochondrial diversity does not reflect its population size (Bazlin *et al.,* 2001) and variations in the mutation rates among species could be invoked to explain the discrepancy between mtDNA and nDNA.   On the other hand, I detected limited mtDNA among three rare species (*A. kirstyae*, A. papillare** and *A. tortuosa*)* and high nuclear diversity. In these

cases, recurrent fixation of advantageous mutations may have lead to a loss of diversity i.e. "genetic draft" (Gillespie 2001). However data relating to variation in genetic diversity should be interpreted with caution because of the small sample sizes examined here (3 individuals per species). This topic is revisited in Chapter 5.

This study shows that the rare species examined here are among the most recent additions to five different *Acropora* lineages. However, phylogeny alone does not explain the rarity of these species. I demonstrate that morphology can conceal evolutionary relationships within the genus *Acropora* and examples of where species group classifications do not imply taxonomic affinity. I provide evidence that at least two species may be functionally important for the maintenance and potential exchange of genetic diversity between species. My analysis of rare versus common species has provided insight into the complex evolutionary processes operational in *Acropora* and provides novel information useful for conservation purposes (see Chapter 7).

CHAPTER 4: Hybridisation as a mode of coral speciation

4.1 Introduction

It was suggested over a decade ago that regional Indo-Pacific endemic corals and putative subspecies' could be morphogically unique hybrid species (Veron, 1995). Hybridization is thought to have been an important factor in the evolutionary success of the genus *Acropora* (Willis *et al.,* 2006). However there are few unambiguous examples of hybrids or hybrid coral species. The only accepted hybrid is *A. prolifera* a product of crosses two *Acropora* species that are restricted to the Atlantic Ocean - *A. palmata* and *A. cervicornis* (Van open *et al.,* 2000; Vollmer and Palumbi 2002). In the Indo-Pacific, up to 60 *Acropora* species can occur in sympatry (WW *Acropora* database unpublished), and this greatly complicates unravelling interspecific relationships. Previously, complex patterns of allele sharing and polyphyly have been proposed as evidence for introgressive hybridisation however alternative explanations have not been ruled out (Ordorico and Miller, 1991; Hatta *et al.,* 1999; van Oppen *et al.,* 2001; Wolstenholme *et al.,* 2003).

It is evident form the common *Acropora* species that have been studied to date, that *Acropora* have a reticulate evolutionary history because extensive genetic overlap is present among some species (Ordorico and Miller, 1991; Hatta *et al.,* 1999; Veron, 1995; van Oppen *et al.,* 2001, 2002; Marquez, 2003). However, the unspecified age of many extant Indo-Pacific species makes it difficult to distinguish between introgressive hybridisation, incomplete lineage sorting (i.e. shared ancestral polymorphism - van Oppen *et al.,* 2001; Vollmer and Palumbi, 2002; Wolstenholme *et al.,* 2003) or morphological convergence (van Oppen *et al.,* 2001; Marquez *et al.,* 2002; Wolstenholme *et al.,* 2003). For the common species examined to date (e.g. *A hyacinthus; A. aspera, A. pulchra, A. humilis, A. gemmifera, A. digitifera*, *A. florida, A. sarmentosa, A. intermedia*), census population sizes *(N)*, effective population sizes (N_e) and coalescence times were not measured. It is most likely however that in these common species, coalescence times are long meaning that if alleles are shared between species this is indicative that the species have not fully diverged.

If divergence times could be estimated it would be possible to distinguish between some of the causes of genetic overlap between species. For example, if two species with large populations were recently evolved, the most likely explanation for their identical sequence data would be a recent common ancestry. However in *Acropora*,

the fossil record limits molecular clock calibration to the earliest record for the genus {Palaeocene (approx 57 mya) (Carbone *et al.,* 1993) and to species group records (Wallace, 2008). I consider rare species can provide new insights into the evolution of reef corals due to their intrinsically limited population sizes and therefore very short coalescence times. If reticulate patterns are present in rare species, this may provide for the first time in the Pacific Ocean, unambiguous evidence in support of interspecific hybridisation.

Further, because some rare *Acropora* species occur outside typical reef flat, reef crest and upper reef slope habitats (i.e. 2-30 m) in marginal interreefal, shallow exposed or deep locations there is a strong possibility that some rare corals may be hybrids that occupy atypical or non-parental niches. Such is the case for the Caribbean hybrid species *A. prolifera* (Vollmer and Palumbi, 2002). To address to address the question of whether rare Indo-pacific *Acropora* species might also be hybrids, I analysed DNA sequence data from nuclear and mitochondrial loci in a range of rare and common *Acropora* species from the Indo-Pacific and Caribbean.

4.2 Methods

4.2.1 Sample collection

New samples (n = 1 – 3 individuals per species) of 14 rare and 8 common Indo-Pacific species of *Acropora* (Table 11) were collected from the Great Barrier Reef (Palm Island Group), the Marshall Islands (Rongelap Atoll) and Papua New Guinea (Kimbe Bay) for molecular analyses (Same samples examined in chapter 3 analysis). These were combined with existing sequences of 10 common corals from (van Oppen *et al.,* 2001) and existing sequences from the three Caribbean *Acropora* species (van Oppen *et al.,* 2000) and *Isopora cuneata* (Marquez *et al.,* 2002). Corals sampled were separated by at least 10 meters to avoid the possibility of sampling of clonemates. Skeletal and matching tissue samples were collected from all corals sampled (n=102 corals). Richards and Wallace identified material with reference to the World Wide *Acropora* Collection at the Museum of Tropical Queensland. (www.mtq.qld.gov.au).

Table 11: 36 target species, abundance/distribution, range, ecological niche, number of global site records in the Worldwide *Acropora* database, collection locations and outgroups.

Species	Abundance	Range	Ecological niche	Collection location
A. walindii	Rare	PNG	deep sandy reef slopes 8-40m	Kimbe Bay, PNG
A. rongelapensis	Rare	Marshall Is. Irian Jaya	protected sandy slopes 15-40m	Rongelap Atoll, RMI
A. loisetteae	Rare	Malaysia, W. Aust, Micronesia	protected sandy lagoons 5-25m	Rongelap Atoll, RMI
A. pichoni	Rare	PNG, Micronesia	submerged shelf reefs, shipwrecks 25-70m	Kimbe Bay, PNG
A. lokani	Rare	SE Asia	shallow reef flat 2-8m	Kimbe Bay, PNG
A. derawanensis	Rare	SE Asia	protected deep sandy slopes8-40m	Kimbe Bay, PNG
A. tenella	Rare	SE Asia	subtidal protected slopes, shelfs 25-70m	Kimbe Bay, PNG
A. batunai	Rare	Indonesia, PNG	submerged reefs, slopes 10-40m	Kimbe Bay, PNG
A. chesterfieldensis	Rare	Chesterfield Is., Micronesia	submerged shallow reefs 5-12m	Rongelap Atoll, RMI
A. kimbeensis	Rare	PNG, Micronesia	submerged reef flat 3-12m	Kimbe Bay, PNG
A. spathulata	Rare	GBR, PNG	reef flat and slope to 5m	Orpheus Island, GBR
A. kirstyae	Rare	Indo., GBR, PNG, New Caledonia	protected interrefal locations 10-20m	Orpheus Island, GBR
A. papillare	Rare	W. Australia, GBR, Japan	ultra shallow and exposed reef 0-5m	Orpheus Island, GBR
A. speciosa	Rare	SE Asia, GBR, Central Pacific	subtidal, protected slopes and walls 15-40m	Rongelap Atoll, RMI
A. jacquelineae	Rare	Indonesia, PNG	reef slopes and submerged reefs, 10-35m	Kimbe Bay, PNG
A. caroliniana	Rare	SE Asia-Pacific	submerged habitats to 12m	Kimbe Bay, PNG
A. tortuosa	Common	Central Pacific	subtidal, protected sandy lagoons	Rongelap Atoll, RMI
A. granulosa	Common	Indo-Pacific	reef slopes and walls 15-50m	Rongelap Atoll, RMI
A. vaughani	Common	Indo-Pacific	protected subtidal habitats 5-35m	Orpheus Island, GBR
A. pulchra	Common	Indo-Pacific	intertidal or shallow subtidal	Van Oppen et al. 2001
A. aspera	Common	Indo-Pacific	intertidal or shallow subtidal	Van Oppen et al. 2001
A. longicyathus	Common	SE Asia-Pacific	subtidal habitats	Van Oppen et al. 2001
A. loripes	Common	Indo-Pacific	subtidal shallow reef habitats 5-25m	Rongelap Atoll, RMI
A. gemmifera	Common	Indo-Pacific	intertidal or shallow subtidal	Van Oppen et al. 2001
A. microphthalma	Common	Indo-Pacific	subtidal habitats 5-30m	Orpheus Island, GBR
A. millepora	Common	Indo-Pacific	intertidal or shallow subtidal	Van Oppen et al. 2001
A. digitifera	Common	Indo-Pacific	intertidal or shallow subtidal	Van Oppen et al. 2001
A. humilis	Common	Indo-Pacific	intertidal or shallow subtidal	Van Oppen et al. 2001
A. austera	Common	Indo-Pacific	shallow subtidal habitats	Van Oppen et al. 2001
A. cerealis	Common	Indo-Pacific	shallow subtidal habitats	Van Oppen et al. 2001
A. nasuta	Common	Indo-Pacific	shallow subtidal habitats	Van Oppen et al. 2001
A. valida	Common	Indo-Pacific	shallow subtidal habitats 5-15m	Magnetic Island, GBR
A. palmata	Outgroup	Atlantic Ocean	subtidal habitats	Van Oppen et al. 2000
A. prolifera	Outgroup	Atlantic Ocean	subtial habitats	Van Oppen et al. 2000
A. cervicornis	Outgroup	Atlantic Ocean	subtidal habitats	Van Oppen et al. 2000
I. cuneata	Outgroup	Indo-Pacific	subtidal habitats	Van Oppen et al. 2001

4.2.2 DNA Extraction, PCR, Cloning and Sequencing

Conditions of Pax-C (nDNA) and mitochondrial Control Region (mtDNA) sequencing and cloning are described in 3.2.2, 3.2.3, and 3.2.4.

4.2.3 Phylogenetic Analysis

Sequences were manually aligned in Sequencher 4.5 against a subset of the existing *Acropora Pax-C* intron and mitochondrial control region sequences (van Oppen *et al.,* 2001; Marquez *et al.,* 2002) before phylogenetic analysis in a Bayesian statistical framework in Mr Bayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Genetic distances were calculated as Kimura 2-parameter distances (Kimura, 1980). The optimal model of sequence evolution was identified using hierarchical likelihood ratio tests in MrModeltest 2.2 (Nylander, 2004). The (MCMC) analyses were run for 5 million generations, with burn-in times of 20,000-50,000 ($p<0.05$). Trees generated from the *Pax-C* data were rooted using sequences from *Isopora cuneata,* whereas the mtDNA tree was rooted with *A. cervicornis* as in this case the degree of divergence of the *I. cuneata* sequence effectively precluded unambiguous alignment. Analyses were conducted on the full alignments without the exclusion of indels or repeat regions because I found the overall topology remained the same when large indels or repeat regions are included, excluded or weighed down (see also van Oppen 2001). Results of the phylogenetic analysis are compared with previous published molecular phylogenetic results sequences (van Oppen 2001; Marquez *et al.,* 2002). Alignments for nuclear and mitochondrial data are submitted to Genbank accession numbers – EU918202-EU918288 and EU918771-EU918925.

4.2.4 Census Estimation

Global census size estimates (N) are calculated in order to get an estimate of effective population size (Ne). Mean global census size of rare species (+SE) was estimated using global estimates of reef area (km^2) within regions (e.g. SE Asia, Micronesia) (Wilkinson 2004). Mean global reef area was calculated as the sum of the mean regional reef habitat available for all regions in which each species is known to occur (see Table 12). It was estimated a certain percentage of the overall reef area available was destroyed prior to 2004 (Wilkinson, 2004). Hence reef remaining after 2004 was also calculated for each region using the percentages presented in Table 12. I assume that rare species occupy a small proportion of the reef available within the regions that it is present, hence I calculate that 10-30% of regional reef habitat is available to rare species by calculating the mean (+SE). Mean (±SE) global census sizes were

estimated by multiplying the mean global reef area available to each species by its mean local abundance per unit area (Table 13).

Region	Total reef area (km2)	% of reefs destroyed >2004	Reef remaining after 2004 (km2)	10-30% region occupied by rare species	Mean available regional reef habitat	SE
S Asia	19210	45	10565.5	3169.65	1056.55	1056.55
SE Asia	91700	38	56854	17086.2	5695.4	5695.4
E & N Asia	5400	14	4644	1393.2	464.4	464.4
Australia & PNG	62800	2	61544	18463.2	6154.4	6154.4
SW Pacific	27060	3	26248.2	7874.86	2624.82	2624.82
Polynesia	6733	2	6598.34	1979.49	659.83	659.83
Micronesia	12700	8	11684	3505.2	1168.4	1168.4

Table 12. Regional estimate of reef habitat > 2004.

The combined global reef area available to each species was calculated from the mean available regional reef habitat (+SE) summed for all regions each species is known to occur within (Wallace, 1999; Veron, 2000). Habitat preferences were assigned to each species; this determined the percentage of a site available to target species according to their habitat preference. For example 10% of a site is available to intertidal species, 40% is available to reef flat/crest species, 40% is available to shallow slope species <30m, and 10% of a site is available to deep slope species >30m. The result is an estimate of the reef area available to target rare species. The mean local abundance was determined for each species from relative estimates of local population size (Richards unpublished). To determine the mean global census size from the reef available to target species, I estimated that one RAP biodiversity swim (Rapid Visual Assessment) covers approximately 3000m^2, thus to estimate mean global census size (n^{global}) I standardize the distance measure by multiplying reef area available to target species (+SE) by 1000 (km^2) and divide by 3000 m^2 (average area covered on a single rapid visual biodiversity assessment), and multiply by the mean local site abundance.

$$N^{global} = \underline{\text{Reef area available to rare species x 1000}} \quad \text{x mean local abundance}$$
$$3000$$

Effective population sizes were assumed to be approximately 11% of the calculated mean global census sizes; this relationship is based on a comprehensive meta-analysis of data for 102 species of animals (Frankham, 1995). Effective population

Table 13. Estimate of mean global census size +SE for target rare species.

Species	Region	Combined Global Reef Area (km²)	Habitat preference	% site available as per habitat	Reef area available to rare sp (km²)	Mean local abundance	Mean Global Census size	SE
A. papillare*	SE Asia-Australia-Sth Asia	25812.7 +/- 12906.35	intertidal	10%	2581.27 +/- 1290.64	3	2581.27	1290.64
A. spathulata*	Australia	12308.8 +/- 6154.4	reef flat	40%	4923.52 +/- 2461.76	20	32823.47	16411.73
A. batunai*	SE Asia-PNG	23699.6 +/- 11849.8	deep slope	10%	2369.96 +/- 1184.98	2	1579.97	789.98
A. pichoni*	SE Asia-PNG	23699.6 +/- 11849.8	deep slope	10%	2369.96 +/- 1184.98	6	4739.92	1139.08
A. tenella*	SE Asia-Micronesia-PNG	26036.4 +/- 8032.2	deep slope	10%	2603.64 +/- 803.22	6	5207.28	1606.44
A. walindii*	PNG	12308.8 +/- 6154.4	deep slope	10%	1230.88 +/- 615.44	3	1230.88	615.44
A. tortuosa	Micronesia-Polynesia-SW Pacific	20297.1 +/- 4453.05	inter-reefal sand	100%	20297.1 +/- 4453.05	10	67657	14843.5
A. derawanensis*	SE Asia	11390.8 +/- 5695.4	shallow slope	40%	4556.32 +/- 2278.16	3	4556.32	2278.16
A. kirstyae*	SE Asia-Australia	23699.6 +/- 11849.8	inter-reefal sand	100%	23699.6 +/- 11849.8	3	23699.6	11849.8
A. speciosa*	SE Asia-MicronesiaPolynesia-PNG	27356.06 +/- 13678.03	shallow slope	40%	10942,44 +/- 5471.2	3	10942.44	5471.21
A. caroliniana*	SE Asia-Australia	23699.6 +/- 13018.2	shallow slope	40%	9479.84 +/- 5207.28	6	18959.68	10414.56
A. chesterfieldensis*	MicronesiaPolynesia-SW Pacific	20297.1 +/- 4453.05	reef flat	40%	8118.8 +/- 1781.22	3	8118.8	1781.22
A. rongelapensis*	Micronesia	2336.8 +/- 1168.4	deep slope	10%	233.68 +/- 116.84	3	223.68	116.84
A. jacquelineae*	SE Asia-PNG	23699.6 +/- 11849.8	shallow slope	40%	9479.84 +/- 5207.28	10	31 599.47	17 357.6
A. lokani*	SE Asia-PNG	23699.6 +/- 11849.8	reef flat-shallow slope	40%	9479.84 +/- 5207.28	6	18 959.68	9479.84
A. kimbeensis*	SE Asia-Micronesia	13727.6 +/- 6863.8	reef flat	40%	5491.04 +/- 2745.52	6	10 982.08	6425.76
A. loisetteae *	SE Asia-Australia	23699.6 +/- 11849.8	inter-reefal sand	100%	23699.6 +/- 11849.8	10	78998.66	39499.33

sizes in reef corals are expected to be significantly smaller than census sizes for a number of reasons (Hughes *et al.,* 1992). First, variation in census population sizes is sometimes extreme due to perturbations such as storms and cyclones, bleaching, crown-of-thorns starfish outbreaks, etc., and this substantially reduces effective sizes because it diminishes the proportion of the population involved in reproduction (Frankham, 1995). Second, high variance in fecundity occurs in corals (Wallace, 1985), which reduces N_e because neither juveniles nor senescent adults take part in reproduction (Caballero, 1994). Third, some *Acropora* species reproduce asexually by fragmentation or fission (Ayre and Hughes, 2000), which again reduces N_e.

4.3 Results

Allele/haplotype data from nuclear and mitochondrial loci were determined for 17 rare and 15 common Indo-Pacific *Acropora* species as well as all 3 Caribbean species of *Acropora* (Table 11) and *Isopora cuneata*. Only samples from taxonomically unambiguous individuals were included in this study; the morphology of the corals sampled was absolutely consistent with their formal description. To avoid the possibility of sampling clonemates, corals sampled were separated by at least 10 meters. The extreme rarity of several of the species examined limited the number of samples that it was possible to examine.

4.3.1 *Pax-C* intron data

Results of phylogenetic analyses of *Pax-C* intron data (Figure 23) are broadly consistent with previous results, but some details differ due to the selection of taxa. To facilitate comparison with previous analyses, clades are labelled according to published trees (van Oppen *et al.,* 2001; Marquez *et al.,* 2002). As in previous analyses, the basal clade contains *A. longicyathus*, and, in the present case, *A. austera*. In the present tree, a polytomy then gives rise to strongly supported clades corresponding to IIIA, IVB, IIID of previous studies; a major difference is the novel clade V which is composed exclusively of rare species with the exception of a single allele of *A. valida*. The nuclear tree distinguishes the Caribbean species in the highly supported clade IIID. Within the large terminal clade, two novel subclades (III F + G) were identified, containing predominantly sequences from rare species.

4.3.2 Mitochondrial control region data

Phylogenetic analyses of the mtDNA Control Region (Figure 24) were also broadly consistent with previous results and clades were labelled as in previous publications (van Oppen *et al.,* 2001; Marquez *et al.,* 2002). The basal clade (IA/IB) again contains *A. longicyathus* and *A. austera*, with *A. tenuis* added. In the present case, clade III is expanded and clade IV contracted relative to published analyses, due to differences in composition of the datasets. Clade IV includes *A. aspera, A. humilis* and several rare species (e.g. *A. kirstyae, A. derawanensis*).

4.3.3 Census Sizes

Mean (±SE) global census population sizes for rare species in this study varied from 32823 (±16412) for *A. spathulata* to 224 (±117) for *A. rongelapensis*. Based on the N_e estimate of 11% of the census population size, *A. spathulata* has a mean effective global population size of 3611 (±1805) and A*. rongelapensis*, 25 (±13) (Figure 22). Local population census and effective population sizes are likely to be substantially smaller than these conservative global estimates.

Figure 24. Mean (±SE) global census sizes (black histograms) and predicted effective population sizes (red histograms) for rare species included in this study.

Figure 25. Bayesian majority rule (>50%) consensus tree of nuclear sequence data for the thirty-five *Acropora* species studied here, with *Isopora cuneata* defined as outgroup. Bayesian analyses used likelihood settings from best-fit model (HKY+G) selected by hLRT in MrModeltest 2.2 (Nylander, 2004): 5 million generations; burn in = 50, 000.

Figure 26. Bayesian majority rule (>50%) consensus tree of mitochondrial sequence data for thirty-five Indo-Pacific *Acropora* species with the Caribbean species *Acropora cervicornis* defined as outgroup. Bayesian analysis used likelihood settings from best-fit model (HKY+I+G) selected by hLRT in MrModeltest 2.2 (Nylander, 2004): 5 million generations; burn in = 20,000.

4.4 Discussion

In both the *Pax-C* and mitochondrial phylogenies many *Acropora* species are polyphyletic. Previous work (van Oppen *et al.,* 2001; Marquez *et al.,* 2002) provides precedents for this pattern, which has been interpreted as evidence for interspecific hybridization. However, the Indo-Pacific species examined in these previous studies are widespread and locally common, and in these cases lineage sorting will occur slowly. As the fossil record of *Acropora* is extremely limited, for common and widespread species incomplete lineage sorting cannot be rigorously excluded as an alternative explanation for the observed polyphyletic patterns. However, for the rare species included in the present study, effective population sizes are so small that lineage sorting will occur on very short time scales, so in contrast to the position with common species, polyphyletic patterns observed for rare species provide unequivocal evidence for hybridization.

Comparison of the trees generated from nuclear and mitochondrial data shows that three of the rare species studied here - *A. pichoni*, *A. kimbeensis* and *A. papillare* - are monophyletic for the mtDNA marker but are polyphyletic and contain highly divergent alleles at the nuclear marker, even within individual corals (Figure 26). The presence of species-specific mitochondrial haplotypes is unusual in *Acropora* (van Oppen *et al.,* 2001; Marquez *et al.,* 2002). Of the 49 species studied to date, the only other *Acropora* species that is monophyletic in mtDNA is *A. tenuis* (however, see also below), which is known to be reproductively isolated through a difference in spawning time (van Oppen *et al.,* 2001).

The mitochondrial phylogeny implies that the three monophyletic rare species have evolved relatively recently, because they fall within derived positions of the large terminal clade that reflects the post-Miocene Indo-Pacific speciation of *Acropora* (i.e. <5.32 my) (van Oppen *et al.,* 2001, Wallace and Rosen, 2006). In contrast, sequences from these three species are widely distributed throughout the nuclear tree; for example, alleles from *A. papillare* occur in both Clades III and V. This pattern in nuclear versus mtDNA loci can be explained by the known faster lineage sorting of mitochondrial haplotypes than alleles at single copy nuclear loci (Tavare, 1984). Unlike their more common relatives, the small effective global population sizes of these three rare species (*A. pichoni* = 521 ±125; *A. kimbeensis* = 1208 ±707; *A. papillare* = 284 ±142) effectively rules out the possibility of incomplete lineage sorting, because of their small population sizes, these rare species have very short coalescence times.

Figure 26. Comparison of nuclear and mitochondrial phylogenies. Asterisks indicate posterior probability values of 100% (black) or >70% (red); for clarity, asterisks are shown only at nodes affecting the positions of sequences from *A. papillare*, *A. pichoni*, *A. kimbeensis*, *A. spathulata* and *A. tortuosa.*

There is no evidence that these rare species were historically more common. Moreover, these observed patterns – monophyly with respect to mitochondrial haplotypes accompanied by polyphyly at nuclear loci - couldn't be explained as consequences of either recent population crashes or population bottlenecks. Under a population crash scenario one would expect to find divergent mitochondrial haplotypes as well as divergent nuclear alleles, whereas under a population bottleneck scenario (i.e. a crash occurring less recently) low diversity at both nuclear and mitochondrial loci is expected. These alternate possibilities can therefore be ruled out, and the most parsimonious explanation for the observed patterns of allele/haplotype distribution is that *A. pichoni*, *A. kimbeensis* and *A. papillare* are unidirectional hybrids.

In the Caribbean, the hybrid species *A. prolifera* colonizes habitats that are distinct from those of the parental species (van Oppen *et al.,* 2000; Vollmer and Palumbi, 2002). Similarly, two of the three rare putative hybrid species from the Indo-Pacific, *A. pichoni* and *A. papillare,* occur in atypical habitats. Whereas the vast majority of *Acropora* spp. occurs in relatively shallow reef flat, crest and slope habitats (2-30m), *A. pichoni* occurs below 40m and *A papillare*, is found in extremely shallow intertidal habitats (<2m). Specialization in extremely shallow or deep habitats is atypical for *Acropora* species hence my data provides support for the hypothesis that hybrid species may exploit atypical (or non-parental) niches.

Other rare species occurring in small and isolated populations (e.g. *A. walindii, A. loisetteae, A. derawanensis* and *A. jacquelineae*) are polyphyletic with respect to both nuclear alleles and mitochondrial haplotypes. Whilst these patterns are again consistent with hybridization, in these cases alternative explanations, such as recent population crashes, cannot be rigorously excluded. Two species that are restricted to the Pacific Ocean but are locally common (*A. spathulata* and *A. tortuosa)* are also monophyletic at the mitochondrial marker but polyphyletic at the nuclear marker. However, in these latter cases, incomplete lineage sorting cannot be ruled out because of the longer coalescence times for these species resulting from their larger census and predicted effective population sizes.

The transfer of genetic variation among lineages is an important creative force in many plants (Grant, 1973; Reisberg and Wendel, 1993) and animals (Dowling and Secor, 1997; Arnold, 1997; Lewontin and Birch 1966). It has been proposed that hybridization may be an evolutionary strategy utilized by corals that may increase genetic diversity either through new combinations of parental alleles or the generation of novel alleles, the origin of new species, and the breakdown of reproductive barriers (Hatta *et al.,* 1999; Willis *et al*. 1997; van Oppen *et al.,* 2002; Wolstenholme *et al.,* 2004; Willis et al 2006; van Oppen & Gates 2006). Overall, the introgression of new alleles into a population is a process that may promote speciation (Willis 1997), facilitate ecological partitioning (van Herwerden *et al*. 2006) and adapting to/or colonizing new habitat after disturbance (Rieseberg *et al.,* 2003) or at the periphery of a species range (Seehausen, 2004). Hybridization also allows for adaptation to changes in environmental conditions (Lewontin and Birch, 1966; Willis *et al.,* 2006; van Oppen & Gates 2006).

In plants, it has been empirically demonstrated that hybrids do not necessarily express intermediate phenotypes (Riesberg and Ellstrand, 1993). This could be explained by the possibility that only subsets of genes are exchanged following hybrid events and that morphological genes have not introgressed. If corals act in a similar manner to what has

been described in plants, hybrids are difficult to recognize without genetic data. Furthermore, while these results suggest that hybridization occurs more often than currently understood in *Acropora* communities, it is likely that different patterns of hybridization occur in different biogeographical regions at different rates depending on the local species composition and the condition of the coral community. In some cases, hybridization may have led to polyploidy and quantifying this is a critical future area of research because duplicated alleles can evolve freely with little selection pressure which may help organisms adapt to a new environments or survive different stress conditions (Comai, 2005).

The results presented here imply that a number of rare Indo-Pacific *Acropora* species are the products of recent hybridization events, and highlight the significance of hybridization in coral diversification. Whether these species have hybrid origins or have evolved and then hybridised in the absence of conspecific gametes remains to be elucidated. In summary, although it has often been assumed that small populations have a decreased potential for adaptation (Willi *et al.,* 2006), my analyses implies that some rare Acroporid corals may actually have increased adaptive potential as a consequence of introgressive hybridisation (Seehausen, 2004), and therefore may be less vulnerable to extinction than has been assumed.

CHAPTER 5 - Genetic diversity, inbreeding and population structure of rare and common *Acropora* coral species

5.1 Introduction

Theoretically, the expected relationship between genetic diversity and population size is linear (Wright 1931) meaning populations of rare species are expected to be genetically depleted.   In terrestrial systems, rare species become genetically depleted because genetic drift, founder effects, directional selection and high levels of inbreeding cause genetic erosion under low population size (Kimura and Ohta, 1971; Avise, 1994, Frankham *et al.,* 2002; Willi *et al.,* 2006).   Hence rare species are of particular conservation interest because they may not have the genetic resilience needed to adapt or survive in changing environments.

Genetic resilience, which encompasses the amount of genetic diversity in a population or species, is critical for long-term survival and continued evolution of populations or species (Lande and Barrowclough, 1987). Without it, disturbance events, outbreaks of pathogens (Coltman, *et al.,* 1999), variable recruitment (Jones *et al.,* 2002) and other stochastic events can force populations to extinction (Goodman, 1987; Elstrand and Elam, 1993; Frankham et al, 2002; Fagen *et al.,* 2002). For these reasons, population genetic analyses of rare species are critical for the assessment of present and future population viability and aid conservation decision-making (Palumbi, 2003).

Unravelling the factors accounting for existing levels of rarity requires comparisons be made between common and rare congeneric species (for examples see: Karron *et al.,* 1988, Soltis and Soltis, 1991, Ge *et al.,* 1999, Dodd and Helenurm, 2002).   Even though the causes of rarity are many and varied, the species in question is generally confined to a narrow range, often resulting in high genetic structure among and low genetic diversity within populations (Gaston 2003). The theory that rare species are genetically depleted and less able to respond to changing circumstances has largely been developed and tested in terrestrial systems (Frank, 1968; Maruyama and Kimura, 1980; Baskauf *et al.,* 1994; Endean and Cameron, 1990).

In marine ecosystems, the initial view that oceans are open and interconnected has evolved into one recognizing that complex patterns of genetic structuring occur on local and regional scales in most marine organisms (Williams and Benzie, 1998; Chenoweth *et al.,* 1998; Gopurenko *et al.,* 1999; Barber 2002; Benzie *et al.,* 2002; Lourie *et al.,*

2005; Rohfritsch and Borsa 2005; Kochizius and Nuryanto 2008; Timm *et al.,* 2008). Likewise, in corals, complex patterns of genetic discontinuity have been described and sometimes on surprisingly small scales (Knittweis *et al.,* 2008; van Oppen *et al.,* 2008; Underwood *et al.,* 2007).

The genetic resilience of rare corals has not been examined. Of the 845 species of extant hermatypic zooxanthellate scleractinian corals, published estimates of genetic diversity and structure exist for 1.4% of species (n=12) (Table 14). All of these species are common and/or widespread with the exception of *A. palmata,* which has been the focus of population genetic studies because it is critically endangered (Baums, 2005).

Table 14. Summary of population genetic data available for zooxanthellate scleractinian corals.

Family	Species	Reference
Pocilloporidae	*Seriatopora hystrix*	Ayre and Duffy, 1994; Ayre and Hughes, 2000; Ayre and Hughes, 2004; Maier, 2005; Underwood *et al.,*2007; van Oppen 2008; Noreen *et al.,* 2009
Pocilloporidae	*Stylophora pistillata*	Ayre and Hughes, 2000; Takabayashi *et al.,*2003; Ayre and Hughes, 2004; Nishikawa, 2008
Pocilloporidae	*Pocillopora damicornis*	Stoddart, 1984, 1988; Benzie *et al.,*1995; Ayre, Hughes and Standish, 1997; Adjeroud and Tsuchiya, 1999; Ayre and Hughes, 2000; Miller and Ayre, 2004; Ayre and Hughes, 2004; Whitaker, 2006
Pocilloporidae	*Pocillopora meandrina*	Magalon, *et al.,* 2005
Pocilloporidae	*Pocillopora verrucosa*	Ridgway *et al.,* 2001
Acroporidae	*Isopora cuneata*	Ayre and Hughes, 2000; Ayre and Hughes, 2004
Acroporidae	*Isopora palifera*	Benzie *et al.,* 1995; Ayre and Hughes, 2004
Acroporidae	*Acropora aspera*	Whitaker 2006
Acroporidae	*Acropora cervicornis*	Vollmer and Palumbi 2006
Acroporidae	*Acropora cytherea*	Ayre and Hughes, 2004; Marquez *et al.,* 2002
Acroporidae	*Acropora digitifera*	Whitaker 2004; Nishikawa, 2008; Nakajima *et al.,* 2009
Acroporidae	*Acropora hyacinthus*	Ayre and Hughes, 2004; Marquez *et al.,* 2002
Acroporidae	*Acropora millepora*	Ayre and Hughes, 2004; Smith-Keune and Van Oppen, 2006
Acroporidae	*Acropora nasuta*	Mackenzie *et al.,* 2004
Acroporidae	*Acropora palmata*	Baums, 2005
Acroporidae	*Acropora tenuis*	Underwood *et al.,*2007, Marquez *et al.,*2002; Nishikawa, 2008; Underwood 2009

Acroporidae	Acropora valida	Ayre and Hughes, 2000; Ayre and Hughes, 2004
Faviidae	Plesiastrea versipora	Rodriguez-Lanetty and Hoegh-Guldberg, 2002
Faviidae	Goniastrea aspera	Nishikawa and Sakai, 2003, Nishikawa, 2008
Faviidae	Platygyra sinensis	Ng and Morton, 2003
Pectiniidae	Mycedium elephantotus	Yu et al., 1999; Dai et al., 2000
Fungiidae	Fungia fungites	Gilmour 2002
Fungiidae	Heliofungia actiniiformis	Knittweis et al., 2008
Dendrophylliidae	Balanophyllia europaea	Goffredo et al., 2004

Given the predictions about extinction risk for rare corals and that very few rare corals have been examined in a population genetic context, obtaining these data is imperative for biodiversity conservation. In this study, I examine how rarity affects the genetic diversity of 9 rare and 5 common *Acropora* corals (NOTE = rare species are marked with asterisk throughout text). I examine marker transferability and size homoplasy of 9 microsatellite loci in *Acropora* species collected from 11 geographic locations. More specifically, this study is concerned with the level of genetic diversity and level of inbreeding in rare and closely related common species and the amount of broad-scale genetic structure between isolated populations of 2 rare and 4 common species.

5.2 Methods

5.2.1 Sampling locations

Samples of 14 species (Table 15) were collected from 11 locations across the Indo-Pacific (Figure 27). Dr. Paul Muir collected samples of *A. microphthalma* and *A. austera* from the Seychelles and Madives. Dr Akira Iguchi collected samples of *A. papillare** from Nth Okinawa. Natalie Rosser collected samples of *A. papillare** and *A. millepora*. Dr Maria Beger collected samples of *A. pichoni** from Chuuk, Micronesia. Dr David Abrego collected samples of *A. valida* from Heron Island. All molecular samples donated for this project have matching skeletal voucher specimens that were identified by the author and verified by Dr Carden Wallace. All samples from Orpheus Island, Kimbe Bay, Majuro Atoll, Arno Atoll and Rongelap Atoll collected by the author.

Figure 27. Sampling locations for population genetic analysis

5.2.2 Coral Sampling, DNA Extraction & PCR

Small branches (2-5 cm) were collected from individual colonies and stored in absolute ethanol. To avoid sampling across multiple recruitment cohorts and asexually derived clone mates, colony sizes and spacing were standardized (20-50 cm colony size, >20 m between colonies).

DNA was extracted from approx. 20 mg of coral branch. Branches were air dried briefly on paper towel to remove ethanol. The coral skeleton was crushed in a sterile mortar and pestle with 750 µl of grinding buffer (100 mM Tris pH 9.0, 100 mM EDTA, 1% SDS, 100 mM NaCl, double distilled H_2O) to enable maximum disruption the cell membrane and release of DNA. The ground material was transferred to an eppendorf tube and its contents were vortexed for 30 sec and incubated for 3 hours at 65ºC. After incubation, tubes were vortexed and cooled on ice. To precipitate proteins, 187.5 µl of ice-cold 5 M KOA (Potassium Acetate) was added to the tube, mixed, and incubated on ice for 10 min. The samples were spun for 10 min in a benchtop eppendorf centrifuge at a maximum speed of (12000 rpm) to separate proteins and cell debris. The supernatant was then transferred to a new eppendorf tube. Six hundred µl isopropanol was added to each tube to precipitate the DNA. After mixing, samples stood at room temperature for 5 min. Samples were then spun in a benchtop eppendorf centrifuge at a maximum speed of for 15 min (12000 rpm), and the supernatant was removed. One-hundred-and-fifty µl of 70% ethanol was added to the

DNA pellets, mixed and centrifuged for 5 min. The supernatant was removed and the pellets were air-dried for 5 min. The purified DNA pellet was then resuspended in 100 µl 0.1 M Tris pH=9 and stored at -20°C.

Table 15. Summary of species, population sample sizes, and number of loci included in the final analysis. Species marked with asterisk are rare.

SPECIES	POPULATION	Geographic Region	Sample Size	Number of loci
A. microphthalma	Orpheus Island	Central GBR	25	7
" "	Maldives	North Indian Ocean	12	7
" "	Seychelles	South Indian Ocean	22	7
" "	Kimbe Bay	Papua New Guinea	25	7
A. valida	Orpheus Island	Central GBR	29	7
" "	Heron Island	Southern GBR	26	7
" "	Kimbe Bay	Papua New Guinea	20	7
A. austera	Maldives	Indian Ocean	29	6
" "	Arno Atoll	North Central Pacific	18	5
" "	Majuro Atoll	North Central Pacific	24	5
" "	Majuro - 20 branches from single colony	Central Pacific	20	5
A. millepora	Ningaloo Reef	Indian Ocean	34	8
" "	Orpheus Island	Central GBR	27	8
A. horrida	Orpheus Island	Central GBR	27	8
A. papillare *	Ningaloo Reef	East Indian Ocean	31	7
" "	Orpheus Island	Central GBR	20	8
" "	Okinawa - Japan	North Pacific	14	8
A. pichoni *	Kimbe Bay	Papua New Guinea	6	7
" "	Chuuk Lagoon	Central West Pacific	6	7
A. spathulata *	Orpheus Island	Central GBR	28	7
A. kirstyae *	Orpheus Island	Central GBR	27	8
A. tortuosa	Rongelap Atoll	North Central Pacific	12	7
A. jacquelineae *	Kimbe Bay	Papua New Guinea	20	7
A. kimbeensis *	Kimbe Bay	Papua New Guinea	14	7
A. rongelapensis *	Rongelap Atoll	North Central Pacific	12	7
A. walindii *	Kimbe Bay	Papua New Guinea	14	8

5.2.3 Microsatellite Cross-amplification

Variation at eight variable tandem repeats (microsatellite markers) was documented. Seven of the microsatellites were developed for *Acropora millepora* at the Australian Institute of Marine Science (van Oppen *et al.,* 2007) and the last was developed for *Acropora palmata* from the Atlantic Ocean (Baums *et al.,* 2005) (Table 16). Microsatellite PCR products were initially examined using denaturing gel

electrophoresis on the Corbett GelScan2000 to determine if they would cross-amplify. Microsatellite PCR products were visualized using fluorescently-labelled forward primers and unlabelled reverse primers.   The microsatellite denaturing gel was prepared using a 100 ml stock solution composed of 42 g UREA, 6 ml 10x TBE, 1.5 ml Acrylamide 40% and MiliQ water (Corbett Research).   Urea was dissolved at 50° Celcius in a hot water bath and the mix was filtered through Whatman paper #1. Fifteen ml of the gel mixture was placed in a beaker and 100 µl APS (0.05G/500µl MQH$_2$0) and 10 µl Temed added before pouring approximately 6 ml in between the two glass plates and inserting a gel front comb. The gel was left to set for approximately one hour.   Before loading on the gel, samples were denatured at 94ºC for three minutes in a thermocycler and put on ice. One µl of sample/LB mix (using formamide loading buffer (LB) at a ratio of 1 µl sample to 3 µl loading buffer) was loaded into wells and the samples were pulse loaded for 15 seconds. After pulsing, excess product was flushed out and the gel was pre-run for 10 minutes before the actual run. Once it was confirmed via initial GelScan screening that the microsatellites would cross-amplify genotyping was undertaken following the procedure described in 5.2.4.

5.2.4 Genotyping

Microsatellites were pooled into three multiplex reactions (Table 17). Each PCR primer was labelled with a different fluorescent dye (TET, HEX or FAM) and alleles were scored as PCR product size in base pairs.   Where high levels of heterozygosity occurred, PCR products were cloned for subsequent sequencing to ensure peaks were real and not amplification of ambiguous data (see 5.2.5). Conditions for the PCR reaction included using 150-200 ng of DNA template and 5 µl 2x Qiagen Multiplex PCR kit master mix in a 10 µl reaction in the presence of 1 µl of each primer and 3.25 µl of H$_2$0. PCR profile consisted of the initial denaturation step of 15 mins followed by 35 cycles of 94º for 30 seconds, 50º for 90 seconds and 72º for 60 seconds. The mix was incubated at 60ºC for 30 min. Three µl of the PCR product was electrophoresed in a 2% TAE-agarose gel in 1xTAE buffer to assess the yield. Successful products were then cleaned using the following Sephadex clean-up protocol.

Five µl of each reaction was cleaned through Sephadex in Whatman Unifilter 800 96-well plates. Sephadex resin was added to a Millipore multiscreen loader. Three hundred µl of Mili Q water was added and left for 3 hours to swell the resin. The plate was spun in a benchtop centrifuge at 1500 rpm for 3 mins to pack the Sephadex into mini-columns using an empty 96 well plate to collect the water. One-hundred-and-fifty

μl of Mili Q water was added to each well and spun in a benchtop centrifuge at 1500 rpm for 3 mins.   The unifilter plate was placed on a new 96 well plate and 5 μl PCR product added to the well and spun in a benchtop centrifuge at 1500 rpm for 5 min. One μl of the purified PCR product was transferred to a skirted 96-well plate and sent for genotyping at the JCU Advanced Analytical Centre.   Fragment analysis was conducted on the Amersham MegaBase.

Table 16. Primer sequences

Locus Name	Primer Sequence (5"-3")
Amil2_002	F – ACAAAATAACCCCTTCTACCT
	R - CTTCATCTCTACAGCCGATT
Ami2_006	F – CTTGACCTAAAAAACTGTCGTACAA
	R – GTTATTACTAAAAAGGACGAGAGAATAACTTT
Amil5_028	F – GGTCGAAAAATTGAAAAGTG
	R – ATCACGAGTCCTTTTGACTG
Amil2_022	F – CTGTGGCCTTGTTAGATAGC
	R – AGATTTGTGTTGTCCTGCTT
Amil2_23	F – GCAAGTGTTACTGCATCAAA
	R – TCATGATGCTTTACAGGTGA
Amil2_007	F – TAATGAGCAAACTCATTCATGG
	R - CTTTT CCAAGAGAAGTCAAGAA
Amil2_010	F – CAGCGATTAATATTTTAGAACAGTTTT
	R - CGTATAAACAAATTCCATGGTCTG
Amil2_012	F – TTTTAAAATGTGAAATGCATATGACA
	R – TCACCTGGGTCCCATTTCT

Table 17. Multiplex reactions

	Locus Name	Repeat Type	Label
Multiplex 1	Amil2_002	(TG)10	HEX
	Ami2_006	(CA)4TA(CA)4	FAM
	Amil5_028	(TCACA)7TCAC(TCACA)4TCACTCACTCACA	TET
Multiplex 2	Amil2_022	(AC)10	TET
	Amil2_23	(AG)7	HEX
Multiplex 3	Amil2_007	(TG)7AG	TET
	Amil2_010	TA(TG)11	FAM
	Amil2_012	GA(CA)6GA(CA)2	HEX

5.2.5 Cloning

Microsatellite PCR products were cloned using the ligation kit, pGEM T easy (Promega) (5 μl ligation buffer, 1 μl pGEM-T Easy Vector, 3 μl PCR product, 1 μl DNA ligase) and incubated for 1-4 hrs at room temperature or overnight at 4° C. The bacterial cells were transformed with a ligated vector and transformed using 60 μl of NM522 competent cells. Following 20 min on ice, transformations were heat shocked at 42°C for 90 seconds. One ml LB broth was added and the transformations were incubated at 37° C for 1 hour. Transformations were transferred to 1.5 ml tubes and centrifuged for 30 seconds. Supernatant (800 μl) was removed and the remaining pellet was resuspended in the last drop of LB by pipetting up and down. The transformation was added to warm LB Agar Plates (Tryptone, 10 g; Yeast, 5 g; NaCl 5 g, H_2O 950 ml/litre) composed of X-Gal (4 ml/litre), IPG (5ml/litre) and Ampicillin (1 ml/litre). The plates were placed overnight in a 37°C incubator.

Up to 15 colonies that appeared to have taken up the insert (white colonies) were picked and put into a 1.5 ml tube with 3 μl of PCR grade H_2O. Sixteen sectors were drawn on a separate AIX plate and swiped by each positive clone. Cultures grew on plates overnight in a 37° C incubator. PCR tubes were heated at 95° C for 5 mins to denature the DNA and transferred to ice.   The PCR reaction was performed as previously described and the product was electrophoresed to see which clones have an insert. Fifteen positive clones were transferred from the overnight cultures to 50 ml falcon tubes with 5 ml of LB media and 5 μl Ampicillin. Clones were incubated overnight in a shaker at 37° C. The remaining cultures were spun in a benchtop centrifuge for 5 mins at 4000 rpm. The supernatant was removed and DNA isolated using the plasmid isolation protocol in the RBC Hyfield Plasmid Mini Kit.   The concentration of DNA was determined using a spectrometer and a sufficient quantity of purified DNA was dried down to reach a final concentration of approximately 100 ng/μl when resuspended in 10 μl of H_2O.   Dried DNA was sent to Macrogen Inc. (www.macrogen.com.au) for sequencing using SP6 and M13F vector primers.

5.2.6 Analysis

Fragment length analysis was used to score microsatellite alleles as a simple function of PCR product size. Genotypes for all loci were manually scored from electrophoretic data. Data was organized in excel and exported to GenAlex (Peakall and Smouse, 2005). Using a GenAlex template file, conformity to the expectations of Hardy Weinberg equilibrium (HWE) were established using a chi-square test (Miller and

Benzie 1997) and significance values were adjusted with Benjamini-Hochberg (BY) correction for multiple comparisons (Benjaini and Yekutieli, 2001; Narum 2006). Genepop on the web (Raymond and Rousset, 1995) was used to test for independence between loci under the following Markov Chain parameters: 1000 dememorization, 100 batches, 10 000 iterations per batch. Descriptive statistics, including proportion of polymorphic loci (P), number of alleles per locus (A), and observed and expected heterozygosity were calculated to illustrate the distribution of genetic diversity within and between populations (Lewis and Zaykin 2001). Allele richness was calculated in Fstat v 2.9.3 (Goudet 2001) with correction for variable sample size. Allelic diversity and standard genetic distance were computed according to Nei (1987) and significance was corrected for multiple pairwise comparisons (Benjaini, 1995).

The extent of asexual reproduction was estimated from the genetic diversity of each population using Ng/N whereby Ng equals the number of unique multilocus genotypes and N equals the number of genoytped individuals. Ng/N = 1 where no clone mates are present and approaches zero when the population is entirely clonal (Coffroth and Lasker, 1998). The extent of inbreeding was summarized by the inbreeding coefficient, Fis, in Fstat on the Web. This inbreeding coefficient assesses the effects of non-random mating within subpopulations, as a measure of reductions in the heterozygosity of individuals. The amount of population subdivision was estimated from pairwise population Fst estimates. Fst estimates and p-values were calculated in Fstat v2.9.3 (Goudet 2001) and corrected for multiple comparisons. The hierarchical population genetic structure within each species data subset was explored using an analysis of molecular variance in GenAlex. I use the infinite allele model (Kimura and Crow, 1964) which assumes that every mutation that occurs within a population creates a unique allele rather than step-wise mutation model (which assumes mutations are additions or subtractions of repeat units - Kimura and Ohta, 1978) because insertions and deletions were not restricted to the repeat region and also found in 3' and 5' flanking regions.

The presence of null alleles (inconsistent amplification of alleles due to mutations in the primer binding region) was assessed in Microchecker v 2.2.3 (van Oosterhout 2004). If a population was determined to have null alleles at a locus, pairwise Fst were re-analyzed in Fstat (Goudet 2001) with corrected data. The corrected dataset was re-run in Microchecker v. 2.2.3 (van Oosterhout 2004) to determine if the correction resolved the heterozygote deficit. Statistical significance of pairwise Fst values was based on 10,000 permutations and p-values were corrected for multiple comparisons. Statistical

differences in genetic diversity and level of inbreeding among rare and common species were determined using Kruskal Wallace implemented in SPSS 16.   This non-parametric Krustal Wallace test was chosen because it caters for ordinal data that is independent of each other and makes no assumptions about the shape of the underlying distributions.

5.3 Results

5.3.1 Marker transferability

A total of 531 individuals in 14 species of *Acropora* were genotyped. To minimize genotyping errors, all automated scorings of alleles were checked manually, and uncertainties were cleared by running the clean PCR product again. Thirty-eight percent of samples were genotyped twice to resolve peaks and 4% were genotyped three times. Genotyping results for 1 in 42 individuals were considered erroneous and disregarded from the analysis. Overall the markers that were developed for *A. millepora* amplified well in other *Acropora* species (Table 18), however, locus Apam3_166 (which was developed on *A. palmata* from the Atlantic Ocean) did not amplify or amplified poorly in all samples. In most instances the TET labelled Amil2_022 peaks pulled the FAM labelled Apam3_166 peaks up giving a false Apam3_166 score. Subsequently, Apam3_166 data was removed from the analysis. Amil2_007 and Amil2_012 also provided mixed results and did not amplify some populations of some species. For example, Amil2_007 did not amplify *A. papillare** from Ningaloo Reef but did amplify in *A. papillare** from the Orpheus Island and from Japan; Amil2_012 did not amplify in any *A. austera* populations but worked for all other species examined. Amil5_028 showed the largest size range and Amil2_012 showed the smallest range of size difference (Figure 28).

Figure 28. Microsatellite size range difference in all species and populations examined showing Amil5_028 has the largest size range. Note: Populations showing polyploidy at Amil2_022 are excluded (see section 5.3.3).

Table 18. Allele size ranges and marker transferability

Species	Locus2	Locus6	Locus28	Locus 22	Locus 23	Locus7	Locus 10	Locus 12
A. pichoni*	91-107	99-121	132-186	x	113-147	x	144-162	85-105
A. millepora	95-107	95-101	135-200	147-187	117-137	135-143	154-188	99-107
A. austera	87-101	97-107	96-142	x	131-157	x	144-156	x
A. papillare*	87-105	89-103	128-186	131-168	129-141	121-135	140-170	85-101
A. microphthalma	81-111	89-105	102-190	x	113-141	101-135	106-174	91-109
A. valida	91-105	89-105	132-192	x	113-131	148-160	x	93-99
A. kirstyae*	89-93	89-101	116-174	141-203	113-141	109-131	142-158	85-101
A. kimbeensis*	87-101	93-101	134-186	X	131-157	127-131	148-162	99-107
A. horrida	91-95	85-105	102-178	161-189	129-133	95-103	98-106	83-93
A. walindii*	93-95	99-101	152-182	143-165	131	133	142-148	97
A. jacquelineae*	97-103	97-99	124-164	x	113-155	127-135	148-162	95-99
A. spathulata*	95-97	97-101	156-180	165-179	133-135	x	146-168	89-103
A. rongelapensis*	95-105	87-101	156-180	x	111-131	103-133	146-162	93-107
A. tortuosa	89-105	93-101	99-160	157-167	131-133	x	152-158	95-107
OVERALL	81-105	89-121	96-200	131-203	111-157	95-160	98-188	83-109

5.3.2 Clonality

Individuals sampled were predominately sexually produced. All individuals of *A. microphthalma*, *A. horrida, A. tortuosa, A. pichoni*, *A. kimbeensis*,* and *A. rongelapensis** were sexually produced*. The Heron Island *A. valida* population had the highest proportion of clonality (31%) however the Orpheus Island and Kimbe Bay *A. valida* populations were entirely sexually produced. Of the individuals sampled from the Majuro Atoll *A. austera* population, 17% were clonal whilst all individuals sampled in the Arno Atoll and Maldive populations were 100% sexually produced. Sixteen percent of the *A. jacquelineae* individuals sampled in Kimbe Bay were clonal. Eleven percent of the *A. kirstyae** individuals genotyped were clonal. Six and 7% of the Ningaloo Reef *A. millepora* and *A. papillare** (respectively) populations had clonal origins. Lastly, 4% of the *A. spathulata** population was clonal. A single genotype was shared between *A. papillare* and A. millepora* from Ningaloo Reef. In situations where multi-locus matches were identified within species, one individual from each pair was removed from subsequent analyses so that each unique genotype was represented only once. After clonemates were removed, 487 individuals were included in the analysis.

5.3.3   Potential polyploidy or multi-copy loci

Genotyping showed more than two peaks (three to five) in 15% of the individuals sampled (73/531) (Figure 29), suggesting either some of the loci are not single-copy in some species or that some species or populations are polyploid. Species showing such

patterns included *A. microphthalma, A. valida, A. austera, A. kirstyae*, A. kimbeensis* Acropora pichoni**.   The presence of multiple alleles necessitated further testing to confirm if the unexpected peaks were real.   As summarized in Table 18, cloning verified that the ≥2 allele scores obtained were real for locus Amil2_022 in *A. valida* and *A. kimbeensis**, but not for any of the other loci or species. This suggests this locus has undergone duplication in these species, rather than these species being polyploid. The existence of multiple peaks prevented the inclusion of locus Amil2_022 data, however data for this locus was included for populations that showed ≤2 alleles. In some cases, blast searches of unusual PCR products matched ambiguous data from repeat regions (McMillan and Miller, 1989).

Figure 29.   Chromatogram showing multiple peaks in locus Amil2_022 in *Acropora valida* from the Orpheus Island.

Table 18. Comparison of genotyping and cloning scores for some of the individuals showing more than two peaks. Genotyping and cloning matches are in bold. Some of the 1-2 base pair differences between genotyping and cloning product scores can be explained by scoring biases or migration biases due to the dye-labels.

Population	Species	Sample No.	Locus	Genotyping Product Scores	Cloning Product Scores
Orpheus Is.	*A. microphthalma*	2	Amil2_010	153, 159	151, 157
Kimbe Bay	*A. kimbeensis*	12	Amil2_007	96, 98, **130**	**130**
Maldives	*A. microphthalma*	5	Amil2_002	91, 100, **106**	95, **106**
Rongelap	*A. rongelapensis*	2	Amil2_007	102, 106, 124,**132**	**124, 132**
Rongelap	*A. rongelapensis*	12	Amil2_007	102, **124, 132**	**124, 132**
Orpheus Is.	*A. valida*	3	Amil2_022	141, 155, **162, 166**	160,**162, 166**
Orpheus Is.	*A. valida*	5	Amil2_022	141, 149, **162**	152, **162**
Kimbe Bay	*A. valida*	12	Amil2_022	142, **162, 164**	158, **162, 164**
Kimbe Bay	*A. valida*	14	Amil2_022	**170, 172, 182, 184**	**170,172**,180,**182, 184**
Kimbe Bay	*A. kimbeensis*	4	Amil2_022	**158**, 164	156, **158**, 160
Kimbe Bay	*A. kimbeensis*	5	Amil2_022	152, **160,** 165	141, **160**
Kimbe Bay	*A. kimbeensis*	6	Amil2_022	154, **158**, 164	156, **158**, 160
Kimbe Bay	*A. kimbeensis*	12	Amil2_022	**160,** 164	158, **160**

5.3.4 Size homoplasy

I found in the markers used here, mutations are not restricted to the repeat region (Table 19). For example, in *A.valida* from Kimbe Bay across the five alleles that were identified, sequence differences included: two single A/G nucleotide changes in the 5' flanking region; the loss of one, two, five or seven repeat motifs; a single A/G nucleotide change in 3' flanking region and a single C/T nucleotide change in 3' flanking region. Given mutations occur in the flanking regions it is worthwhile noting that size-scoring procedures can be inaccurate because of electromorph size homoplasy. Electromorph size homoplasy refers to the situation when genetic diversity is concealed because two alleles have the same length but contain different numbers of repeats with compensatory differences in the 3' and 5' flanking regions (Roberts *et al.,* 2004).

Table 19. Description of cloning results and sequence differences.

Species Sample #	Collection Location	Locus	# Clones	# Alleles	Description of differences
A. valida 14	Kimbe Bay	Amil2_022	11	5	Two single A/G nucleotide changes in the 5' flanking region. Single repeat loss. Loss of 2 repeats. Loss of 5 repeats. Loss of 7 repeats. Single A/G nucleotide change in 3' flanking region. Single C/T nucleotide change in 3' flanking region.
A. valida 12	Kimbe Bay	Amil2_022	11	3	Single A/G nucleotide change in 5' flanking region. Loss of single A from repeat region. Loss of AATAAG segment from 3' flanking region. Single G/C nucleotide change in 3' flanking region.
A. valida 5	Orpheus Island	Amil2_022	13	2	Loss of CTTCCAAATC segment from 5' flanking region. Single A/G nucleotide change in 5' Flanking region. Single T/G nucleotide change in 5' flanking.
A. kimbeensis 12	Kimbe Bay	Amil2_022	14	2	Single repeat missing. Single G/ A nucleotide change in 3' flanking region.
A. kimbeensis 5	Kimbe Bay	Amil2_022	11	2	Single G/A nucleotide change in 5' flanking region. CTAATTCTTTGCCAAGAACCCTAAA segment loss from 5' flanking region. Loss of AATAAG segment from 3' flanking region.
A. kimbeensis 4	Kimbe Bay	Amil2_022	14	3	Single A/G nucleotide change in 5' flanking region. Loss of single repeat. Loss of 2 repeats. Single nucleotide A/G change in repeat region.
A. kimbeensis 6	Kimbe Bay	Amil2_022	8	3	Single A/C nucleotide change in 5' flanking region. Loss of single repeat. Loss of 2 repeats. Single T/C nucleotide change in 3' flanking region.
A. microphthalma 2	Orpheus Island	Amil2_010	8	2	Single A/G nucleotide change in 5' flanking region. Loss of single repeat. Loss of 4 repeats. Single A/T nucleotide change in 3' flanking region.
A. kimbeensis 12	Kimbe Bay	Amil2_007	6	1	Single A/G nucleotide change in 5' flanking region.

A. microphthalma 5	Maldives	Amil2_002	3	2	Loss of four repeats. Loss of TT from 3' flanking region. Single A/G nucleotide change in repeat region.
A. rongelapensis 2	Rongelap Atoll	Amil2_007	3	2	Loss of 4 repeats.
A. rongelapensis 12	Rongelap Atoll	Amil2_007	4	2	Loss of 4 repeats. Single C/T nucleotide change in 3' flanking region.

5.3.5 Heterozygosity

Seventy-one percent of samples have a lower observed heterozygosity than expected under Hardy Weinberg equilibrium (Table 20), however, only 29% are statistically significant at p<0.05.   Two common species (*A. millepora* and *A. valida*) have the greatest proportion of loci with significant heterozygote deficits (number of loci in deficit = 62% and 70% respectively).   Nevertheless, significant heterozygote deficits were also detected in rare species (e.g. *A. papillare*, *A. pichoni*, *A, kimbensis** and *A. spathulata**). Forty-five cases of null alleles were encountered. Heterozygote deficits due to null alleles were corrected in 73% of cases. For the remaining 27% of cases, significant deficits remained after correction for null alleles suggesting there are additional reasons for the deficits or there was not enough data to correct the null alleles.   Correction reduced Fis scores and increased the number of populations with heterozygote excess.   For example all three populations of *A. papillare** changed to heterozygote excess after correction for null alleles while the Majuro *A. austera* population remained in deficit after null alleles were corrected.   No null alleles were detected in *A. rongelapensis** or *A. jacquelineae**. Significant heterozygote excess was detected in *A. rongelapensis** at 3 loci and *A. papillare** at 3 loci.

Eighteen locus pairs were affected by linkage disequilibrium (Table 21). Two loci pairs (2 & 28; 28 & 10) were affected in all *A. microphthalma* populations. In *A. tortuosa** and *A. kirstaye** loci with significant LD also showed significant heterozygote deficits however deficits were in most cases found to be related to null alleles. In one case, heterozygote deficit was due to large allele dropout whereby shorter alleles are preferentially amplified resulting in the less efficient amplification of large alleles. These data could be corrected.

Ten species had 100% polymorphic loci (Table 21), including *A. millepora,* the species upon which the microsatellites were developed. Species showing <100% polymorphic loci were: *A. papillare*, A. valida, A. austera and A. walindii**. In *A. valida*, two populations were 100% polymorphic but the third (Heron Island) was not (57%

polymorphic). In the Ningaloo reef *A. millepora* population 52% of alleles were private and in *A. austera* 55% of the Maldive population alleles were private. As opposed to this, 18% of alleles in the *A. valida* Heron population were private and 14% of the alleles in the Orpheus Island *A. microphthalma* population were private.

Table 20. Species, population, locus and number of samples (N), alleles (A), expected (H$_e$) and observed (H$_O$) heterozygosity. Inbreeding coefficient (F$_{IS}$), Null Alleles (Null), Corrected inbreeding coefficient (F$_{IS}$C). Large allele drop-out (LAD), not enough data (ned). Asterisks indicate significant deviations from Hardy-Weinberg equilibrium (P<0.05)

		locus	N	A	He	Ho	Fis	Null	FisC
A. microphthalma	Kimbe Bay	2	23	12	0.849	0.826	0.105	no	0.105
		6	23	5	0.691	0.783	-0.147	no	-0.147
		28	23	6	0.509	0.435	0.096	no	0.096
		22	na	na	na	na	na	no	na
		23	23	6	0.778	0.652	0.136*	no	0.136
		7	22	6	0.478	0.045	0.92*	yes	*0.119*
		10	25	10	0.728	0.72	0.031	no	0.031
		12	25	6	0.406	0.32	0.232*	no	0.232
	Seychelles	2	22	8	0.747	0.611	0.141	no	0.141
		6	22	7	0.638	0.529	0.191	no	0.191
		28	22	20	0.787	0.389	0.515*	yes	0.169
		22	na	na	na	Na	na	na	na
		23	22	3	0.575	0.714	-0.171	no	-0.171
		7	22	7	0.702	0.591	0.181*	no	0.181
		10	22	12	0.778	0.545	0.32*	yes	0.267
		12	22	6	0.635	0.318	0.516*	yes	0.115
	Maldives	2	19	3	0.661	0.579	0.15	no	0.15
		6	19	6	0.726	0.789	-0.061	no	-0.061
		28	19	7	0.74	0.684	0.102	no	0.068
		22	na	na	na	Na	na	na	na
		23	19	4	0.35	0.421	-0.176	no	-0.176
		7	19	5	0.56	0.333	0.566	yes	0.186
		10	19	5	0.738	0.533	0.176*	yes	0.16
		12	19	5	0.616	0.867	-0.397	no	-0.397
	Orpheus Is.	2	22	6	0.624	0.773	-0.216	no	-0.216
		6	22	5	0.636	0.818	-0.264	no	-0.264
		28	22	4	0.17	0.091	0.485	yes	-0.05
		22	na	na	na	Na	na	na	na
		23	22	4	0.458	0.273	0.423*	yes	0.133
		7	22	5	0.522	0.409	0.238	no	0.238
		10	22	6	0.667	0.864	-0.273	no	-0.273
		12	22	6	0.674	0.909	-0.329*	no	-0.329
A. valida	Orpheus Is.	2	28	6	0.596	0.357	0.281*	yes	0.028
		6	25	6	0.728	0.52	0.238*	yes	0.105
		28	26	13	0.877	0.654	0.231*	yes	0.225
		22	na	na	na	Na	na	na	na
		23	27	4	0.141	0.074	0.378	yes	0.043
		7	28	7	0.694	0.179	0*	no	0
		10	28	3	0.364	0.321	0.143	no	0.143
		12	28	2	0.069	0	1*	no	1
	Heron Is.	2	17	7	0.78	0.588	0.223	ned	0.223
		6	13	3	0.506	0.077	0.727*	ned	0.727
		28	14	3	0.612	0	0.87*	ned	0.87
		22	na	na	na	Na	na	na	na
		23	16	1	0	0	0	ned	0
		7	na	na	na	Na	na	ned	na

Species	Location	Locus							
		10	17	2	0.111	0.118	-0.045	ned	-0.045
		12	17	1	0	0	0	ned	0
	Kimbe Bay	**2**	19	6	0.735	0.737	0	no	0
		6	19	6	0.719	0.368	0.535*	yes	0
		28	19	15	0.904	0.421	0.571*	yes	0.085
		22	na	na	na	Na	na	no	na
		23	19	4	0.389	0.368	0.089	no	0.089
		7	19	4	0.609	0.105	0*	no	0
		10	19	5	0.503	0.368	0.3*	no	0.3
		12	19	2	0.188	0	1*	yes	0.3
A. austera	Majuro	**2**	12	4	0.462	0.417	0.141	no	0.141
		6	12	4	0.66	0.417	0.405	no	0.405
		28	12	4	0.601	0.25	0.612*	yes	0.12
		22	na	na	na	Na	na	na	na
		23	11	1	0	0	0	no	0
		7	na	na	na	Na	na	no	na
		10	12	1	0	0	0	no	0
		12	na	na	na	Na	na	na	na
	Arno	**2**	14	5	0.666	0.857	-0.253	no	-0.253
		6	14	4	0.666	0.857	-0.253	no	-0.253
		28	10	4	0.27	0.3	-0.04	no	-0.04
		22	na	na	na	Na	na	na	na
		23	14	2	0.191	0.214	-0.083	no	-0.083
		7	na	na	na	Na	na	na	na
		10	14	1	0	0	0	no	na
		12	na	na	na	Na	na	na	na
	Maldives	**2**	28	7	0.733	0.786	-0.054	no	-0.054
		6	28	6	0.625	0.536	0.161	no	0.161
		28	28	3	0.307	0.286	0.087	no	0.087
		22	na	na	na	Na	na	na	na
		23	29	3	0.511	0.586	-0.131	no	-0.131
		7	na	na	na	Na	na	na	na
		10	29	6	0.728	0.655	0.118*	no	0.118
		12	29	6	0.702	0.448	0*	no	na
A. papillare	Ningaloo	**2**	28	3	0.07	0.036	0.5*	yes	-0.038
		6	28	4	0.563	0.179	0.692*	yes	-0.041
		28	28	6	0.735	0.357	0.528*	yes	0.028
		22	25	9	0.788	**1**	-0.25*	no	-0.25
		23	25	7	0.626	0.6	0.061	no	0.061
		7	na	na	na	Na	na	na	na
		10	28	7	0.721	0.571	0.225*	yes	0.015
		12	26	5	0.57	0.462	0.208*	no	0.208
	Orpheus Is.	**2**	20	7	0.639	0.75	0.164	no	0.164
		6	20	3	0.411	0.2	0.532*	yes	-0.282
		28	20	8	0.8	0.8	0.026	no	0.026
		22	20	14	0.814	**1**	-0.204	no	-0.204
		23	20	5	0.585	0.15	0.755*	yes	-0.078
		7	20	3	0.421	0.15	0*	no	0
		10	20	7	0.706	0.75	-0.036	no	-0.036
		12	20	4	0.554	0.8	-0.424	no	-0.424
	Japan	**2**	14	6	0.755	0.643	0.185*	no	0.185
		6	14	4	0.543	0.571	-0.015	no	-0.015
		28	14	4	0.707	0.071	0.906*	ned	0.906
		22	14	4	0.612	0.857	-0.368	no	-0.368
		23	14	4	0.199	0.143	0.316	no	0.316
		7	14	4	0.487	0.643	0	no	0
		10	14	5	0.691	0.714	0.004	no	0.004
		12	14	1	0	0	0	no	0
A. millepora	Orpheus Is.	**2**	26	3	0.556	0.385	0.326	no	0.326
		6	26	4	0.499	0.385	0.248*	no	0.248
		28	26	4	0.362	0.192	0.485*	yes	0.162
		22	26	16	0.873	0.846	0.05	no	0.05
		23	26	3	0.473	0.308	0.367*	yes	0.006

Species	Location	Locus							
		7	18	3	0.642	0	1*	yes	1
		10	26	13	0.749	0.731	0.044	no	0.044
		12	26	2	0.074	0.077	-0.02	no	-0.02
	Ningaloo	**2**	32	5	0.409	0.469	-0.131	no	-0.131
		6	32	5	0.445	0.156	0.658*	yes	0.168
		28	33	6	0.786	0.394	0.51*	yes	0.155
		22	33	12	0.805	0.727	0.112	no	0.112
		23	33	7	0.597	0.515	0.152	no	0.152
		7	32	7	0.754	0.219	0.718*	yes	-0.084
		10	33	13	0.865	0.576	0.348*	yes	0.102
		12	33	7	0.648	0.333	0.498*	yes	0.022
A. pichoni	Kimbe Bay	**2**	6	6	0.694	0.5	0.362	ned	0.362
		6	6	3	0.611	0	1*	ned	1
		28	6	8	0.847	0.833	0.107	ned	0.107
		22	na	na	na	Na	na	ned	na
		23	6	4	0.514	0.5	0.118	ned	0.118
		7	2	2	0.375	0.5	0	ned	0
		10	6	3	0.542	0.167	0.737	ned	0.737
		12	6	6	0.806	0.667	0.259	ned	0.259
	Truk	**2**	6	6	0.806	**1**	-0.154	ned	-0.154
		6	6	5	0.667	0.5	0.333	ned	0.333
		28	6	7	0.806	0.833	0.057	ned	0.057
		22	na	na	na	Na	na	ned	na
		23	6	2	0.375	0.5	-0.25	ned	-0.25
		7	2	3	0.625	0.5	0.5	ned	0.5
		10	5	5	0.78	0.6	0.333	ned	0.333
		12	6	6	0.806	0.333	0.643*	ned	0.643
A. horrida	Orpheus Is.	**2**	26	3	0.144	0.038	0.742*	yes	0.059
		6	26	8	0.803	0.346	0.582*	yes	0.019
		28	26	10	0.686	0.346	0.51*	yes	0.067
		22	22	8	0.834	0.364	0.579*	yes	0.012
		23	23	4	0.427	0.565	-0.303	no	-0.303
		7	17	2	0.457	0	1*	yes	-0.333
		10	18	4	0.335	0.389	-0.133	no	-0.133
		12	18	5	0.466	0.611	-0.285	no	-0.285
A. jacquelineae	Orpheus Is.	**2**	17	3	0.631	0.529	0.191	no	0.191
		6	17	3	0.258	0.176	0.342	no	0.342
		28	17	4	0.306	0.176	0.448	no	0.448
		22	na	na	na	Na	na	no	na
		23	19	7	0.578	0.789	-0.343	no	-0.343
		7	11	3	0.657	0.545	0.216*	no	0.216
		10	11	4	0.442	0.455	0.02	no	0.02
		12	11	2	0.236	0.091	0.643	no	0.643
A. kimbeensis	Kimbe Bay	**2**	13	6	0.719	0.385	0.496*	yes	0.163
		6	13	4	0.666	0.538	0.229	no	0.229
		28	13	10	0.858	0.615	0.319	yes	0.319
		22	na	na	na	Na	na	no	na
		23	14	4	0.459	0.143	0.708*	yes	0.071
		7	13	2	0.497	0.462	0.111	no	0.111
		10	14	7	0.694	0.857	-0.2	no	-0.2
		12	14	4	0.656	0.429	0.378	no	0.378
A. tortuosa	Rongelap Atoll	**2**	12	4	0.462	0.25	0.492*	yes	0.135
		6	12	3	0.559	0.25	0.582*	yes	-0.021
		28	12	4	0.608	0.25	0.616*	LAD	0.616
		22	12	4	0.563	0.917	-0.603	no	-0.603
		23	12	2	0.153	0.167	-0.048	no	-0.048
		7	na	na	na	Na	na	na	na
		10	12	2	0.278	0.333	-0.158	no	-0.158
		12	12	5	0.778	0.833	-0.028	no	-0.028
A. kirstyae	Orpheus Is.	**2**	24	2	0.499	0.125	0.759*	yes	-0.004
		6	24	4	0.582	0.167	0.724*	yes	-0.129
		28	24	7	0.777	0.583	0.269	LAD	0.269

					Ho	He	F		
		22	23	14	0.765	0.913	-0.173	no	-0.173
		23	23	7	0.433	0.348	0.218	no	0.218
		7	22	5	0.249	0.136	0.471*	yes	-0.122
		10	23	5	0.631	0.826	-0.288	no	-0.288
		12	23	5	0.632	0.609	0.06*	no	0.06
A. spathulata	Orpheus Is.	**2**	26	2	0.038	0.038	0*	no	0
		6	26	3	0.443	0.077	0.832*	yes	-0.098
		28	24	4	0.666	0.5	0.269*	no	0.269
		22	13	4	0.388	0.154	0.628*	yes	0.13
		23	12	2	0.153	0.167	-0.048	no	-0.048
		7	na	na	na	Na	na	no	na
		10	20	8	0.8	0.9	-0.1	no	-0.1
		12	22	5	0.546	0.636	-0.142	no	-0.142
A. walindii	Kimbe Bay	**2**	14	2	0.408	0.571	-0.368	ned	-0.368
		6	13	2	0.426	0.154	0.662	ned	0.662
		28	12	5	0.681	0.5	0.305	ned	0.305
		22	14	3	0.518	0.5	0.071	ned	0.071
		23	14	1	0	0	0	ned	0
		7	2	1	0	0	0	ned	0
		10	14	3	0.135	0.143	-0.02	ned	-0.02
		12	14	1	0	0	0	ned	0
A. rongelapensis	Rongelap Atoll	**2**	12	6	0.722	1	-0.347*	no	-0.347
		6	12	5	0.726	0.75	0.01	no	0.01
		28	12	8	0.83	0.833	0.039*	no	0.039
		22	na	na	na	Na	na	no	na
		23	12	3	0.538	1	-0.846*	no	-0.846
		7	12	4	0.726	0.917	-0.222*	no	-0.222
		10	12	5	0.694	0.667	0.083	no	0.083
		12	12	5	0.674	1	-0.451	no	-0.451

Table 21. Total number of alleles screened across all loci (N), number and percentage of private alleles, number of locus pairs in linkage disequilibrium (LD) and percentage polymorphic loci.

Species	Population	N	Private alleles (%)	LD	% Polymorphic loci
A. microphthalma	Kimbe Bay	51	11 (22%)	2	100
	Seychelles	53	16 (30%)	2	100
	Maldives	35	11 (31%)	2	100
	Orpheus Is.	36	5 (14%)	2	100
A. valida	Orpheus Is.	41	14 (34%)	0	100
	Heron Is.	17	3 (18%)	0	57.14
	Kimbe Bay	42	15 (36%)	0	100
A. austera	Majuro	12	3 (25%)	0	50
	Arno	14	3 (21%)	0	66.7
	Maldives	29	16 (55%)	0	100
A. papillare*	Ningaloo	41	16 (39%)	0	87.5
	Orpheus Is.	51	21 (41%)	0	100
	Japan	32	10 (31%)	0	87.5
A. millepora	Orpheus Is.	48	18 (38%)	0	100
	Ningaloo	62	32 (52%)	0	100
A. pichoni*	Kimbe Bay	32	12 (38%)	0	100
	Truk	34	14 (41%)	0	100
A. horrida	Orpheus Is.	43	-	2	100
A. jacquelineae*	Orpheus Is.	26	-	0	100
A. kimbeensis*	Kimbe Bay	37	-	1	100
A. tortuosa	Rongelap Atoll	24	-	2	100
A. kirstyae*	Orpheus Is.	49	-	3	100
A. spathulata*	Orpheus Is.	28	-	0	100
A. walindii*	Kimbe Bay	18	-	0	62.4
A. rongelapensis*	Rongelap Atoll	36	-	2	100

5.3.6 Intraspecific comparisons of genetic diversity

The overall mean number of alleles per locus ranged from 3.76 ($\pm$0.38 SE) at locus Amil_023 to 8.8 ($\pm$1.56 SE) at locus Amil_022. The Seychelles *A. microphthalma* population was particularly diverse with 20 different alleles at locus Amil5_028 (Figure 30) and the Kimbe Bay and Orpheus Island *A. valida* populations also had a high number of alleles at this locus. Patterns of allele richness were extremely variable between species and do not covary between loci (Figure 31). For example, in case of *A. kirstyae** allelic richness was low for locus Amil2_002 but high for loci Amil2_022 and Amil2_023. In the Orpheus Island *A. valida* population, allelic richness is high at locus Amil2_028 and low at locus Amil2_002. *A. walindii** exhibited low allelic richness across all eight loci suggesting it is genetically eroded. Across the entire dataset, seven cases of allele fixation were detected. Allele fixation occurred in the Heron Island *A. valida*, Japan *A. papillare**, Majuro *A. austera* and Kimbe Bay *A. walindii*.*. In three cases, locus Amil2_023 was fixed. Mean allelic richness was greatest in *A. microphthalma*, *A. papillare**, *A. millepora* and *A. kirstyae**. Mean allelic richness was lowest in *A. walindii**, *A. pichoni**, *A. jacquelineae**, *A. tortuosa** and *A. spathulata** (Figure 32). Mean expected heterozygosity was greatest in *A. rongelapensis**, *A. pichoni**, *A. kimbeensis** and *A. microphthalma*. Mean expected heterozygosity was lowest in *A. walindii**, *A. austera*, *A. spathulata**.

Figure 30. Number of alleles per locus/population showing locus Amil5_028 to be the most polymorphic followed by locus Amil2_022 (note: multi-copy data removed for locus Amil2_022). Note evidence of allele fixation in *A. walindii, A. papillare* from Japan, *A. austera* from Majuro and Arno and *A. valida* from Heron Island. KB = Kimbe Bay, SY = Seychelles, ML = Maldives, PI = Orpheus Island, HI = Heron Islands, MA = Majuro, NG = Ningaloo, JP = Japan, TL = Truk Lagoon, RA = Rongelap Atoll, AA = Arno Atoll.

Figure 31. Genetic diversity of rare and common *Acropora* species. Data was pooled for species sampled across multiple populations. Denoted is mean allelic richness (+/-SE) in grey and mean expected heterozygosity (+/-SE) in black. Species are listed from most widespread (*A. valida*) to most geographically restricted (*A. rongelapensis*). Asterisk indicates a rare species.

5.3.7 Biogeographic comparisons of genetic diversity

Allelic richness scores varied considerably within *A. microphthalma* with the Seychelles population generally having the highest allelic richness and the Orpheus Island population having the lowest allelic richness, however, all locations were similar at locus Amil2_012. Among the *A. valida* populations studied here, the Kimbe Bay population had the highest allelic richness followed by the Orpheus Island population and lastly the Heron Island population (Figure 32). Amil5_028 showed high allelic richness in both Orpheus Island and Kimbe Bay populations. The allelic richness of *A. millepora* was higher in the Ningaloo population than Orpheus Island population for six of the eight loci. In *A. austera* allelic richness was higher in the Maldive population than in the Arno and Majuro atoll populations. In *A. papillare**, allelic richness scores peak at Locus Amil2_022 in the Orpheus Island population but allelic richness is very similar at all locations at Amil2_006 and Amil2_010. In *A. pichoni*, allelic richness is consistent and low in both the Kimbe Bay and Chuuk population (Figure 33).

The Mann-Whitney test detected a significant difference (p<0.05) in the level of allelic richness between species that are geographically restricted versus widespread (U=9, p=0.016) (Table 22). This difference appears to be driven by *A. walindii** because when this species is excluded (because it was highly genetically depleted), the difference in the level of allelic richness becomes weakly insignificant between rare and common species (U=14, p=0.059). No significant difference was detected in expected heterozygosity between widespread and restricted species. When data was regrouped according to local abundance, there was no significant difference in expected heterozygosity or allelic richness between rare or common species. Correction for null alleles failed to pick up any significant differences between the expected heterozygosity, allelic richness or level of inbreeding in geographically restricted and widespread species or between locally common and rare species.

Figure 32. Allelic richness of *Acropora* species from different geographic localities.

Table 22. Mann-Whitney test of the significance of differences in level of allelic richness between rare and common species.

Non-corrected data	He	No significant difference in expected heterozygosity between geographically restricted and widespread species (U=20, p=0.739) No significant difference in expected heterozygosity between geographically restricted and widespread species when *A. walindii** is excluded (U=15, p=0.464) No significant difference in expected heterozygosity between rare and common species (U=19, p= 0.641)
Non-corrected data	AR	**Significant difference in allelic richness between geographically restricted and widespread species (U= 9, p = 0.016*)**

		Marginally non-significant difference in allelic richness between geographically restricted and widespread species when *A. walindii** is excluded (U = 14, p = 0.059). No significant difference in expected heterozygosity between rare and common species (U = 19, p = 0.641).
Non-corrected data	F_{IS}	No significant difference in level of inbreeding between geographically restricted and widespread species (U=16, p = 0.386) No significant difference in the level of inbreeding between geographically restricted and widespread species when *A. rongelapensis** is excluded (U=16, p = 0.558)
Corrected data	He	No significant difference in expected heterozygosity between geographically restricted and widespread species (U=19, p = 0.386) No significant difference in expected heterozygosity between rare and common species (U = 16, p = 0.524).
Corrected data	AR	No significant difference in allelic richness between geographically restricted and widespread species (U = 10.5, p = 0.109) No significant difference in allelic richness between geographically restricted and widespread species when *A. walindii** is excluded (U = 10.5, p=0.164) No significant difference in allelic richness between rare and common species (U= 13.5, p = 0.230)
Corrected data	F_{IS}	No significant difference in level of inbreeding between geographically restricted and widespread species (U=19, p= 0.641). No significant difference in level of inbreeding between geographically restricted and widespread species when *A. walindii** is excluded (U = 12, p=0.705) No significant difference in expected heterozygosity between rare and common species (U = 21, p = 0.841)

5.3.8 Inbreeding

When F_{IS} estimates were calculated from null allele corrected data, F_{IS} scores were reduced and the number of populations displaying heterozygote excess increased. All *A. papillare*, A. spathulata*, A. kirstaye** and *A. horrida* populations changed from having heterozygote deficits to heterozygote excesses. Fis values were variable ranging from 0.54 to -0.215 after correction for null alleles. The Mann-Whitney test showed no significant difference in the level of inbreeding in species that are restricted or widespread or rare or common before or after correction for null alleles (Table 23). There is also no clear trend between rarity and heterozygote deficiency or excess as both common and rare species (e.g. *A, valida* and *A. pichoni**) show deficits and excesses (e,g. *A. austera* and *A. rongelapensis**) (Figure 33).

Figure 33. Level of inbreeding in rare and common corals from different locations. Data corrected for null alleles (with the exception of Heron Is. *A. valida*; Kimbe Bay and Chuuk Atoll A. pichoni; and Kimbe Bay *A. walindii*). Species are listed from most widespread (*A. valida*) to most geographically restricted (*A. rongelapensis*). Rare species denoted by an asterisk. Positive F_{IS} values suggest heterozygote deficit, negative F_{IS} values suggest heterozygote excess.

5.3.9 Genetic structure

Analysis of Molecular Variance (AMOVA) results revealed that the variance within populations was larger than between populations (Figure 34). This parallels the results in allozymes (Yu et al 1999; Ayre and Hughes 2000; Ridgway *et al.,* 2001) and in ITS-1 (Takabayashi *et al.,* 2003). Pairwise F_{ST} values were statistically significantly different

from zero for all comparisons after BY correction except one (*A. pichoni* Chuuk/Kimbe Bay) In *A. microphthalma*, the only species for which we examined four populations, pairwise F_{ST} values revealed the largest genetic subdivision to be present between Orpheus Island and Maldive populations (Table 23).

Figure 34. Analysis of Molecular Variance (AMOVA) results showing variance is higher within populations than among populations.

Given the large spatial scales over which species have been sampled, the high levels of population structure observed are not surprising. In *A. valida* the large amount of population structure is driven largely by the divergent Heron Island and Orpheus Island populations (mean F_{ST} = 0.442) (p<0.0001; Figure 35a). In *A. microphthalma* the highest population structure was between the Maldive and Orpheus Island populations (mean F_{ST} = 0.226 – Figure 35b). No transoceanic gene flow was inferred for *A. papillare** (mean F_{ST} = *0.414 – Japan v's Ningaloo Reef)* (Figure 35c). In *A. millepora* a low level of population structure was detected between the Ningaloo Reef and Orpheus Island populations (mean F_{ST} = 0.074 Figure 35d) with low pairwise F_{ST} estimates (mean F_{ST} = 0.074 in *A. millepora*). In *A. austera*, Indian and Pacific Oceans are also clearly separated with 35% of the total molecular variance being partitioned among populations (mean F_{ST} = 0.349) (Maldives and Arno Atoll comparison – Fgure 35e) and the largest pairwise differentiation occurred between the Maldives in the Indian Ocean and Arno Atoll in the Central Pacific Ocean (mean F_{ST} = 0.439). *A. pichoni* populations were not significantly different from each other and the F_{ST} scores not significantly

different from zero indicate these populations have not diverged from each other (Figure 35f). However, only 6 individuals were surveyed from each population meaning the data for this species are not robust and there was not enough data to correct for null alleles.

Table 23. Pairwise population F_{ST} estimates probabilities based on 9999 permutations below diagonal using data uncorrected (bold) and corrected (plain type) for null alleles. P-values above diagonal uncorrected (bold) and corrected (plain type) # = significant at p≤0.05 after BY correction (there was not enough data to correct for null alleles in the Heron Is. *A. valida* and both *A. pichoni* populations).

A.micropthalma	Kimbe Bay	Seychelles	Maldives	Orpheus Is.
Kimbe Bay	-	**0.000#** (0.000#)	**0.000#** (0.000#)	**0.000#** (0.000#)
Seychelles	**0.157**	-	**0.000#** (0.000#)	**0.000#** (0.000#)
Maldives	**0.219**	**0.133**	-	**0.000#** (0.000#)
Orpheus Is.	**0.131**	**0.224**	**0.226**	-
A. austera	Majuro	Arno	Maldives	
Majuro	-	**0.000#** (0.000)	0.000# (0.000#)	
Arno	**0.119**	-	0.000# (0.000#)	
Maldives	**0.333**	**0.349**	-	
A. valida	Orpheus Is.	Heron Is.	Kimbe Bay	
Orpheus Is.	-	**0.000#**	**0.000#** (0.017 ns)	
Heron Is.	**0.442** (0.399)	-	**0.000#**	
Kimbe Bay	**0.045** (0.038)	**0.417**	-	
A. millepora	Orpheus Is.	Ningaloo		
Orpheus Island	-	**0.000#** (0.000#)		
Ningaloo	**0.074**	-		
*A. papillare**	Ningaloo	Orpheus Is.	Japan	
Ningaloo	-	**0.000#** (0.000#)	**0.000#** (0.000#)	
Orpheus Is.	**0.351**	-	**0.000#** (0.000#)	
Japan	**0.414**	**0.235**	-	
*A. pichoni**	Kimbe Bay	Chuuk Lagoon		
Kimbe Bay	-	**0.000#**		
Chuuk Lagoon	**0.062**	-		

Figure 35. Principal Coordinates analysis of pair wise population estimates of differentiation before correction for null alleles.

5.4 Discussion

Comparison of genetic diversity and levels of inbreeding at selectively neutral loci shows that in general, the rare *Acropora* species studied here do not have significantly lower genetic diversity or higher levels of inbreeding than common species. The amount of genetic diversity at neutral microsatellite loci may reflect wider genome diversity, hence, this finding has important implications for the conservation of rare species because it suggests that not all rare coral species conform to the predicted deleterious effects of small population size. Putatively neutral loci are not functionally constrained or under selection, hence genetic drift rather than selection determines their allele number and heterozygosity (Frankham *et al.,* 2002) therefore neutral markers provide a useful estimate of background population genetic diversity.

*Acropora walindii (*which is restricted to Kimbe Bay - Papua New Guinea) is genetically depleted. With an estimated global population size of only 1231 +/- 615 individuals and a mean global effective population size estimated to be only 135 +/- 68 individuals (see Chapter 4), the low allelic richness and expected heterozygosity of *A. walindii* suggests the long-term survival and continued evolution of this species is jeopardized. Without genetic resilience, small and isolated populations (such as that of *A. walindii* in Kimbe Bay) are subject to genetic erosion through drift, and deleterious alleles may become fixed which can force local extinction following a major disturbance. Other rare species such as *A. jacquelineae* and *A. pichoni* also had low allelic richness although for these species expected heterozygosity was comparatively higher.

Overall, the mean number of alleles per locus across the 25 *Acropora* populations surveyed (relating to 14 species) is 5.26 (+ 0.23 SE). When sample size is accounted for, the mean allelic richness becomes 4.64 (+ 0.2 SE). This level of genetic diversity is lower that that reported in a recent review that showed the mean number of alleles per locus in scleractinian corals is 8.27 (+0.75 SE) (Shearer *et al.,* 2009). Data presented in this review was pooled from members of the families Acroporidae, Faviidae, Pocilloporidae and Poritidae and it may be problematic to compare levels of genetic diversity between familes. The mean estimate of number of alleles per locus I present here is also smaller that a previous estimate from one population of *Acropora millepora* using the same microsatellite loci (mean 8.7). It is similar to the mean number of alleles presented in Mackenzie et al (2004) for *A. nasuta* (mean 3.9, single locus), but well short of the number presented for *A. palmata* (mean 14.4). The lower genetic diversity I detect here in Indo-Pacific Acropora species may be a result of total genetic

diversity being underestimated due to a small sample size. Rarefaction analysis conducted by Shearer et al (2009) suggests sample sizes of 50 were insufficient to capture all the genetic diversity within a population thus 100% of the total population diversity has not been quantified.

Not all rare corals were genetically depleted. The most extreme example of non-depletion in the rare corals examined here is *A. rongelapensis*. *A. rongelapensis* is restricted to the North West Pacific Ocean and known only from deep protected reef locations and is the rarest of all corals included in this study. Data presented here shows significant heterozygote excess at 6/7 loci and 100% observed heterozygosity at three loci. There are several explanations for this result. Firstly, if *A. rongelapensis* has a large effective local population size that could explain the high heterozygosity. In Chapter 4 I estimate the global effective population sizes of the rare species in this study. Gobal Ne was relatively small (i.e. <5000) for all rare species and *A. rongelapensis* had the smallest global census size of all (224+/-117 individuals). Local population census sizes would be even smaller than the effective global population sizes estimated here. I find no evidence that any of the rare species studied here have especially large local population sizes, nonetheless *A. rongelapensis*. I cannot unambiguously rule out that the rare species examined here formally had larger population sizes and that high initial levels of diversity may conceal the impact historical population crashes or bottlenecks have had on genetic diversity, however, there is no evidence of this in the fossil record.

Some populations display high genetic diversity if they are old, stable and persistent or if recruits are derived from various genetically divergent sources (van Herwerden *et al.,* 2009). However *A. rongelapensis* is relatively young with restricted source populations so the existence of diverse source populations is not likely to explain the high genetic diversity. The most likely explanation is that *A. rongelapensis* is a F1 hybrid. The finding of 100% observed heterozygosity at 3 loci in *A. rongelapensis* is a clear signature of hybridisation. The viability of hybrid species remains a controversial subject, however, hybridization can create individuals capable of sexual reproduction via backcrossing or possibly through hybrid matings. Therefore this unique small population of genetically diverse coral most likely of hybrid origin that occurs in protected lagoonal habitats throughout Micronesia is important because of the overall contribution it makes to genetic diversity. In other rare species that were found to be genetically diverse (e.g. *A. papillare** and *A. kirstyae**)* it is possible that the introgression of alleles has contributed to the genetic variation of local populations (as

demonstrated for *A. cervicornis* by Vollmer & Palumbi, 2006). Phylogenetic results from mtDNA and nDNA are consistent with this finding for *A. papillare* (see Chapter 4).

The general finding that rare species do not have lower diversity than closely related species that reach a widespread distribution is not unprecedented. This emerging view of 'non-depleted' rare species is reported in both marine and terrestrial ecosystems (see Young and Brown, 1996; Karron, 1987, 1991; Hamrick and Godt, 1990; Gitzendanner and Soltis, 2000; Bay, 2006; Read 2006). As I have discussed, this pattern could arise if the rare species in question was old, had a large local effective population size or if it had a hybrid ancestry. Variation in genetic diversity may also result from spatial-temporal variability in reproductive success, recruitment from various sources and disturbance history. The retention of high genetic diversity in species with a small population size suggests the predicted deleterious effects of small population size are not always present in corals and that only the most severe population depletion events have lasting effects on genetic resilience.

Conspicuous in this study are two populations of widespread species (*A. austera* at Majuro Atoll and *A. valida* at Heron Island) that were shown as genetically depleted. In the case of *A. valida* at Heron Island, genetic diversity was low, there were significant heterozygote deficits and some alleles appear to be fixed. These patterns are not explained by clonality because clone mates were removed from the analysis. Heterozygote deficits are commonly caused by null alleles, Wahlund effects (i.e. inadvertently sampling disparate populations, Wahlund 1928), and an excess of inbreeding (Wright 1922) brought about by variance in reproductive success. In most cases, the presence of null alleles was corrected during analysis so inbreeding and Wahlund effects have most likely contributed to the heterozygote deficits present in the common species examined here. The finding of genetically depleted populations of common species is important because the loss of populations of common species can have disproportional effects on ecosystems structure, function and services (Gaston and Fuller, 2007).

The examples of duplicated alleles that I describe could be further signatures of hybridisation events, however the duplication event was restricted to a single locus (Amil2_022) and does not appear to represent a genome-wide (polyploidization) pattern. This single microsatellite duplication could be explained by scoring errors or PCR artefacts however this is not likely because cloning and sequencing verified genotyping results. Single locus duplication events have been reported in another

microsatellite study involving *A. millepora* (Wang *et al.,* 2008) In this study chimerism (where juveniles settle together and fuse - Barki *et al.,* 2002; Puill-Stephan *et al.,* 2009) and the retention of a polar body during fertilization (Baums, Hughes and Hellberg, 2005) were suggested as possible explains for the occurrence of more than 2 alleles per locus. However these explanations are not likely as DNA was only examined from a small portion of a branch.   It is possible that Amil2_022 is not single copy marker, however the finding that duplication was restricted to a few individuals indicates that this may also reflect a recent region-specific duplication event caused by transposable element activity, replication slippage or aberrant crossing over (Bennetzen, 2002). The fate of duplicated elements is not known however they may eventually become fixed or lost (Kimura, 1983).   Gene and genome duplications are important mechanisms for the evolution of genetic diversity (Ohno, 1970; Stebbins, 1950; Lynch and Conery, 2000; Zhang 2003).

This study is the first to use microsatellites to estimate large-scale (transoceanic) population genetic structure in *Acropora* species.   The populations sampled derive from a very broad geographic range thus the extent to which population connectivity can be interpreted is somewhat limited. Nevertheless, the data presented confirms that for *A. papillare* and *A. austera* gene flow is restricted between the Indian and Pacific Oceans whereas for *A. microphthalma*, it is not. Restricted transoceanic gene flow has been shown in other marine taxa including crustaceans, echinoderms and fish (see: McMillan and Palumbi, 1995; Williams and Benzie 1998, 1999; Duda and Palumbi 1999; Nelson *et al.,* 2000; Kochzius *et al.,* 2003; Barber et al 2006; Froukh and Kochzius 2008, Timm et al, 2008). In *Acropora*, a transoceanic divide has also been hypothesized to exist based on biogeographic evidence (Wallace, 1999). The level of transoceanic genetic connectivity has only been tested on one other coral species (*Heliofungia actiniforms* throughout the Indo-Malay Archipelago, Knittweis *et al.,* 2008) and individuals were found to show significant genetic structuring with separate Indian Ocean and Pacific Ocean clades apparent.   The lack of gene flow over large spatial scales is also reflected in the high numbers of private alleles observed (e.g. *A. millepora* Ningaloo Reef v's Orpheus Island population comparison).   The situations where large-scale gene flow was predicted by F_{ST} estimates (e.g. *A. microphthalma*), this may reflect historical gene flow and chance long distance dispersal events (Hellberg, 1996).

My results show that when multiple populations are sampled across a species range, there are strong differences in the level of genetic diversity, in allele frequencies, in the

extent of clonality, and the mean proportion of private alleles across that range. Evidently varying physical, chemical and biological factors interact and influence a corals population history and this tends to make the genetic composition of each population unique. It is possible that some of the unusual patterns of gene flow I observe (e.g. between Heron Is. and Kimbe Bay in *A. valida*) are artifacts of size homoplasy because the assumption that all changes in PCR product length are due to length differences in the repeat region was violated. Homoplasy may occur if the rate of mutation at microsatellite loci is too high relative to the length of time separating populations (Orti *et al.,* 1997). Hence because data here show flanking region mutations, a cautionary approach should be taken when interpreting F_{ST} estimates because the magnitude and effect of homoplasy is difficult to estimate and may have reduced the ability to detect population subdivision.

The genetic effects of rarity are well established in theory but empirical support remains equivocal in *Acropora*. In general, this study found only weak evidence to support the predictions from rarity theory of lower genetic diversity and no evidence of higher levels of inbreeding in rare corals. This may generally be because unless the reduction in effective population size has been very severe, no major changes in genetic variability are detectable (Spencer *et al.,* 2000; Frankel and Soule, 1981). Nevertheless, genetic diversity is particularly important in the face of disturbance (van Oppen and Gates, 2006) because it increases a population's resilience, or ability to resist and recover from disturbances and to adapt to change. Genetic resilience is increased through gene flow, which enables new alleles to be integrated into a population through reproduction and by creating new gene combinations upon which selection can act. I hypothesize that introgressive hybridisation has led to the observed heterozygote excesses and higher genetic diversity than expected in some rare species at the neutral markers examined here. I assume this diversity reflects genome wide diversity however to confirm genetic diversity, population structure and the genetic consequences of small population size should be examined using both neutral and functional loci.

CHAPTER 6: New coral challenges conventional conservation practice

6.1 Introduction

The unique "elkhorn" growth form characteristic of *Acropora palmata* (Lamarck, 1816) (resembling a diagonal phylogram in three dimensions) makes it one of the most distinctive of all coral species at the level of whole colony morphology (Veron, 2000; Figure 36A). Moreover, this elkhorn morphology represents one extreme of the broad range of colony forms exhibited by members of the coral genus *Acropora*. Until recently, *A. palmata* was a dominant species on Caribbean reefs, but it is now critically endangered (Gardner *et al.,* 2003; Carpenter *et al.,* 2008). No reef-building coral species occurs in both Atlantic and Pacific Oceans, however I recently discovered a population of coral with archetypal elkhorn colony morphology growing in exposed spur and groove habitats on the western side of Arno Atoll in the Marshall Islands, central Pacific Ocean (Figure 36D). The Pacific elkhorn population consists of <100 mature colonies, growing at 3-5m depth along a 2 km stretch of exposed reef front. While the exact population size remains to be quantified, this growth form is so far, unique to this locale; no additional colonies were found during recent extensive surveys of nine other Marshall Island atolls, and there are no records from elsewhere in the Pacific Ocean to date.

Many *Acropora* species show considerable morphological variation according to their position on the reef (Wallace 1999), and some species can occasionally have coiled tabulate or irregular arborescent growth forms (particularly in the Maldives, West Indian Ocean – Wallace an Zahir, 2007; Pillai and Scheer, 1976). However, no currently recognised Indo-Pacific species has the distinctive elkhorn colony growth form that is characteristic of *A. palmata*. The Pacific elkhorn coral has regular divergent blade-like branches that radiate out from single or multiple large central stalks. Some Pacific elkhorn colonies are very large (1.5m high x 5m greater diameter) and are by far the largest of all the *Acropora* colonies observed at Arno Atoll, indicating that these colonies are relatively old. It is important for conservation purposes to resolve the identity of the Pacific elkhorn and its relationship to *A. palmata*.

6.2 Methods

Morphological features were described from 10 individual colonies of the Pacific elkhorn; these skeletal samples are stored at the Museum of Tropical Queensland, Australia.

DNA was extracted from a subset of samples in parallel with those used for the morphological analysis. To investigate the relationship between Atlantic and Pacific elkhorn corals, we used molecular phylogenetics based on existing data for a nuclear single copy marker (*Pax-C* 46/47 intron) and the putative mitochondrial control region (rns-cox3; Richards *et al.,* 2008). The *Pax-C* alignment contained 30 reference sequences from 9 *Acropora* species, and the mitochondrial alignment contained 28 reference sequences from 11 *Acropora* species (see the legend of Figure 2 for details). For PCR conditions see section 3.3.2 - 3.3.2. *A. robusta* was included in the molecular analysis because it is a close relative of *A. abrotanoides* and other species were included to represent the major clades recognised in previous analyses (van Oppen *et al.,* 2001). For the Pacific elkhorn corals, nuclear data were obtained from 4 individuals {2-5 clones per individual (GenBank accession #'s: FJ899043, 044, 045, 046, 051, 052, 053, 059, 060, 063)} and mitochondrial data obtained from 7 individuals (GenBank accession #'s: FJ899069-FJ8999075).

Phylogenetic analyses were conducted using Bayesian methods (Huelsenbeck and Ronquist, 2001), with *Isopora cuneata* defined as outgroup. Analyses of mitochondrial data were run for 5 million generations (p<0.05) with 100,000 trees disregarded as burn-in. Likelihood settings HKY+G were selected by hLRT (Posada and Crandall, 1998) {Lset Base=(0.24160, 0.16990, 0.26880, 0.31970) Nst=2 TRatio=1.4398 Rates=gamma Shape=0.5543 Pinvar=0}. Bayesian settings HKY+G selected by hLRT (Nylander, 2004) {Prset statefreqpr=dirchilet (1,1,1,1) Lset nst2 rates=gamma}. Phylogenetic analyses of nuclear data were run for 5 million generations (p<0.05) and 150,000 trees discarded as the burn-in. Likelihood settings HKY+G selected by hLRT (Posada and Crandall, 1998) {Lset Base=(0.2643 0.2173 0.2162) Nst=2 TRatio = 1.3616 Rates=gamma, Shape=0.5892 Pinvar=0}. Bayesian settings HKY+G selected by hLRT (Nylander, 2004) {Prset statefreqpr=dirchilet (1,1,1,1) Lset nst2 rates=gamma}.

6.3 Results

The Pacific elkhorn is clearly not *A. palmata*, as these were clearly resolved in phylogenetic analyses (Figures 37 and 38); *A. palmata* clustered with *A. cervicornis* (Lamarck, 1816) (another Atlantic species), whereas the Pacific elkhorn clustered with other Indo-Pacific species. Moreover, whilst overall colony morphology is shared, the Pacific elkhorn species differs from *A. palmata* in some detailed skeletal characters including in the former case the proliferation of branchlets at the branch tip, possession of radial corallites with dimidiate openings, limited septal development in axial corallites

and the presence of a mixture of spinule types (including large flaky spinules) in the coenosteum between radial corallites (compare Fig 36B, C with 36E, F).

Despite differences at the whole colony level, the Pacific elkhorn coral shares some morphological characters with *A. abrotanoides,* including dimidiate radial corallites (a feature of the *Acropora robusta* species group) and proliferation of branchlets at the branch tip (Table 24). However, the molecular analyses are consistent with these being closely related sister species rather than conspecifics. It is also possible that the Pacific elkhorn may be a new but rare species. However, I favour the hypothesis that the Pacific elkhorn coral represents another species recently lost in synonymy with *A. abrotanoides* (Wallace, 1999), *A. rotumana* (Gardiner, 1898).

Gardiner's description of *A. rotumana* from Rotuma in the South Pacific, and the figure of the type material are consistent with this assignment, although the colonies in the Marshall Islands appear to be a more mature form, having far more than the "two to four" plate-like branches originally described for *A. rotumana*. Molecular analyses using material from *A. rotumana* (from Rotuma) may resolve relationships between this species and the Pacific elkhorn coral however no material appropriate for molecular analyses are available. Whilst it is important to establish the identity of this coral, the Marshall Island population of elkhorn coral is unique in the Pacific Ocean and of particular scientific interest in terms of the evolution of this colony morphology. The elkhorn morphology is one extreme of coral colony architecture and although most *Acropora* colony morphologies are repeated in numerous species, the archetypal elkhorn shape is known in only one other species, the critically endangered *A. palmata*.

Figure 36. Gross and fine morphology of the Atlantic and Pacific elkhorn corals. (A): Typical whole colony morphology of *A. palmata in situ* (University of Exeter). (B): Broad branch of *A. palmata* showing anastomosed branch tip. (C): Portion of *A. palmata* branch showing tubular radial corallites with round openings, sample collected by A. Szmant from Florida Keys in 1997. (D): Typical whole colony morphology of Pacific elkhorn coral *in situ* at Arno Atoll May 2007. (E). Broad branch of Pacific elkhorn showing proliferation of branchlets at the branch tip. (F): Portion of Pacific elkhorn branch showing tubular radial corallites with dimidiate openings. All specimens are stored at the Museum of Tropical Queensland, Australia.

Table 24. Comparison of morphological characters used to distinguish the Pacific elkhorn from potentially related species.

Species	Colony Shape	Axial Corallite	Axial corallite inner size range (mm)	Axial corallite outer size range (mm)	Axial Septa	Radials	Coenosteum on radials	Coenosteum between radials
Pacific Elkhorn	Arborescent table with central **stalk**. Branches form broad anatomizing prostate blades that can form several layers with the **elkhorn formation**. Branchlets proliferate at the tip of branches.	Axial corallites often indistinct or **infilled** with flaky spinules or consolidated skeleton.	**0.5 –1.1**	**1.1 -2.3**	Primary septa present to ¼ **R**, directives may be slightly longer. Secondary septa may be **absent** or corallite opening infilled.	Dimorphic – large tubular radials with round or dimidiate openings interspersed between small immersed subimmersed or exsert tightly packed corallites that **touch**.	Costate or **broken costate**	Reticulate with a **mixture of spinule types**. Sometimes intergrading with broken costae on the sides of radials. Some specimens have **flaky** spinules. Some branches have radial corallites so dense there is **no coenosteum** between corallite walls.
A. abrotanoides (Lamarck,1816)	Arborescent table or subarborescent table with broad branches. Branchlets proliferate at the tip of branches	Axial corallites distinct but may be indistinct on stunted branchlets	0.7 –1.2	2.0 - 2.5	Primary septa present to 2/3 R. Secondary septa present up to ¼ R.	Dimorphic – long tubular corallites with dimidiate openings interspersed between subimmersed forms. Mostly not touching.	Smooth costae	Reticulate with occasional spinules.
robusta (Dana,1846)	Low subarborescent table with no stalk.	Axial corallites distinct	0.5-1.5	2.1 -4.0	Primary septa present to ¾ R. Secondary septa to 1/3 R.	Dimorphic – long tubular corallites with dimidiate openings interspersed with subimmersed forms. Mostly not touching.	Smooth costae	Reticulate with occasional spinules.
palmata (Lamarck 1816)	Large arborescent tables sometimes with several layers with elkhorn	May be indistinguishable along the edge	0.7-1.6	1.5 –2.3	Primary septa to ¾ R. Secondary	Dimorphic - even tubular with round to slightly dimidiate	Costate	Reticulate with simple spinules.

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	formation.	of branches.				septa to ½ R.	openings. Radials tightly packed and touch.		
rotumana (Gardiner, 1898)	Colony pedicellate. Broad plates formed by the fusion of branches radiating from a short and stout pedicle. Two to four plates arise from the pedicle and radiate out at right angles. The ends of branches are incompletely fused.		1mm	2.3-3.0mm	Indistinct	Dimorphic – nariform or tubular-nariform corallites interspersed with small immersed or subimmersed corallites.		Reticulate with low granular spines that may form striations.

Figure 37: Nuclear phylogeny resolves the Atlantic and Pacific elkhorn corals. The *Pax-C* topology shown are those with the highest log likelihood scores as constructed in Bayesian analysis. Details of the sequences used in the analyses: AF344420; AY083897; EU918816; EU918815; EU918893; EU918802; EU918252 FJ899043-899063).

Figure 38. Mitochondrial phylogeny resolves the Atlantic and Pacific elkhorn corals. The mitochondrial control region *rns-cox3* topology shown is the one with the highest log likelihood score as constructed in Bayesian analysis. Details of the sequences used in the analysis: AF507220; AF507257; AF507256; AF507255; AF507242; AF507217; EU918257; EU918217; EU918252; E918207; EU918233; EU918282l; FJ899064-899077.

6.4 Discussion

The Pacific elkhorn coral is restricted to a single atoll in the Marshall Islands, and the entire "species" may consist of fewer than 100 individuals. Despite its extreme rarity and importance to science, the Pacific elkhorn would not qualify for threatened species listing under current International Union for Conservation of Nature (IUCN) criteria on two counts. Firstly, the Pacific elkhorn is of uncertain taxonomic position and hence would be considered "data deficient". Secondly, some Pacific elkhorn colonies individuals appear to be hybrids; for example, colony #59 yielded four distinct alleles at the nuclear *Pax-C* locus, consistent with this individual being a polyploid hybrid. Although most coral species are diploid with 2n = 28, there are precedents for odd ploidy states in some *Acropora* species (Kenyon 1997). A number of other rare *Acropora* species are also probable hybrids (Richards *et al.,* 2008). Hybrid species and the Pacific elkhorn pose major challenges to conventional thinking with respect to the conservation and management of endangered "species".

Hybridisation is often considered to be an evolutionary dead end by zoologists; this view can be traced back to Darwin (Darwin, 1859) and is supported by the fact that terrestrial animals as diverse as insects and mammals generally follow Haldane's law – that in hybrids, heterogametic sex is infertile (Haldane 1932). Hybridisation is generally viewed as threatening the long-term persistence of rare species through assimilation or outbreeding depression (Frankham *et al.,* 2002) and/or otherwise contributing to the extinction of species (Levin *et al.,* 1996; Rhymer & Simberloff, 1996). The view that hybridisation threatens the genetic purity of parental species and is a violation of species integrity (Mayr, 1963) is pervasive throughout animal conservation literature and has, in some cases, led to attempts to eradicate hybrids from natural populations (Craw *et al.,* 1999). This negative view of the significance of hybridisation has been extrapolated to corals. For example, the Caribbean species *Acropora prolifera,* which is an F1 hybrid (van Oppen *et al.,* 2000), has been described by Vollmer and Palumbi (2002) as an 'immortal mule' with little evolutionary potential.

Hybridization can, however, have positive effects; it may lead to the introgression of new and potentially beneficial alleles, and to the creation of new species (Anderson, 1954; Arnold, 1997; Grant 1981). Introgression may enable the colonisation of new habitats or increases in fitness to occur more rapidly than through mutation (Stebbins, 1940; Lewontin and Birch, 1966). The significance of hybrid vigour is well established in the plant literature (Fitzpatrick and Shaffer, 2007) and amongst animals there are

apparent examples of positive selection for hybridisation (e.g. Rosenfield and Kodric-Brown, 2003). In the case of at least some coral lineages, hybridisation appears to be an evolutionary strategy (Willis *et al.,* 2006), and a number of rare species are hybrids (Richards *et al.,* 2008). Making analogies with mules is misleading, because unlike mules, corals can reproduce asexually and may persist on evolutionary time scales. Hybrids contribute to community processes – in the case of corals, through reef building, and thus the provision of habitat to a wide range of organisms. Further, hybrid corals compete for resources and may occupy non-parental niches. Hybrids should not be ignored simply because they do not fit traditional assumptions on how species evolve. Further, Haldane's law may simply not apply to many marine invertebrates; even in the case of *A. prolifera,* backcrossing occurs between the hybrid and parental lineages (Miller and van Oppen, 2003), so they are clearly not evolutionary dead-ends.

The ecological, and likely evolutionary, significance of hybrid corals poses a major challenge to conservation legislation, and this is compounded by taxonomic uncertainties. Traditionally, conservation resources have been directed away from hybrids and towards the protection of distinct and well-defined evolutionary lineages (Stuart and Parham, 2007). Hybrids are currently not considered for threatened species listing and, on this basis, the hybrid coral species *A. prolifera* was explicitly excluded from a recent red list assessment (Carpenter *et al.,* 2008). Species with associated taxonomic uncertainty, and all recently described species (in practice, all coral species described since 2000) are listed as "data deficient" in the IUCN Red List of Threatened Species, meaning that "there is inadequate information to make a direct, or indirect, assessment of its risk of extinction" (IUCN 2004).

One hundred and forty one coral species are listed by the IUCN as data deficient and on these grounds; their conservation status has not been assessed. In the case of the family Acroporidae, 30% (81 of 271) of currently recognised species are considered data deficient. A high proportion of these 81 species were described only recently by Veron (Veron, 2000), and are likely to have very limited population sizes and ranges. It is these species, for which we have little information, that face the highest level of threat (see also Mace and Kunin 1994; Mace 2002). The IUCN will re-evaluate the status of these species in approximately ten years, but this may be too late for many rare species.

The case of the Pacific elkhorn highlights the mismatch between existing IUCN criteria and real world conservation needs. Moreover, these issues are not unique to corals, and most likely apply to many other marine invertebrates. I consider that, despite the

present uncertainty surrounding the taxonomic position of the Pacific elkhorn and its possible hybrid ancestry, this evolutionarily significant and spectacular rare coral must be protected, and conservation measures to this end should be implemented immediately. More generally an urgent re-evaluation of conservation policies for marine invertebrates is warranted.

CHAPTER 7: Conservation of *Acropora* biodiversity

7.1 Rarity and threatened status

A species is considered threatened on a local scale if intrinsic or extrinsic factors cause its population size to decline past some critical point. Where this critical point lies is not always clear, especially in the case of rare species where population declines are not often detected. A distinction is commonly made between 'rare' and 'threatened' species suggesting that not all rare species are (necessarily) threatened (Mace & Lande 1991), however, making this distinction is important for conservation purposes because few species in ecological communities are exceptionally abundant. In fact, most species are rare (Magurran and Henderson, 2003), thus it is important to understand the implications of different types of rarity in terms of extinction probability. In this study, I determined that the level of threat facing species of the coral genus, *Acropora,* varies from very high (species categorised as occupancy type 1 that are genetically depleted, such as *A. walindii*) to low (species categorised as occupancy type 8 that have large populations sizes, large ranges and high genetic diversity, such as *A. microphthalma*). Taxa that display the other 5 types of occupancy fall in a grey area where there is little consensus about their relative threatened status (see Reed 1992; Kattan 1992). For these species, little baseline information is available from which to detect if population declines or range contractions are occurring. There is some urgency to understand the implications of these occupancy types for the conservation of coral biodiversity.

Thirty-two species of *Acropora* examined in the NW Pacific region fall in this grey area because they display occupancy types that do not conform to classic rarity or commonness. Twenty-two of the 83 species of *Acropora* examined had a restricted local distribution and small local abundance despite having a widespread global distribution (Occupancy type 2). It could be argued these species (such *A. kirstyae)* are not threatened in the conventional sense by virtue of their widespread global distribution. However, species that have a wide range but occur in low numbers across their range are vulnerable to local extinction events and population fragmentation (see section 7.2). I found that one species has a restricted global distribution but is locally widespread and common in Kimbe Bay PNG (*A. plumosa* – Occupancy type 4). Despite its large local population size, *A. plumosa* is still threatened because local populations are largely self-sustaining, meaning that any local disturbance or predator or disease outbreaks could lead to a severe population

bottlenecks. Similarly *Acropora chesterfieldensis,* which has a restricted global distribution but is locally widespread, and locally rare (occupancy type 5) in the NW Pacific region, is also threatened because it is vulnerable to population fragmentation. Eight species were widely distributed on global and local scales but occurred in low numbers across the range (occupancy type 6), e.g. *A. horrida.* I categorized these species as following persistence pathways because they can persist 'under the radar' and typically escape both disease and predator outbreaks because of their low densities. These species are not highly threatened unless there are widespread extrinsic threats or evidence of range contractions.

It is important to note that an unexpected outcome of this research, namely the finding that some populations of common species are in fact genetically depleted and apparently threatened with local extinction (discussed further in sections 7.2 and 7.3). Common species are disproportionately influential in shaping many macro-ecological patterns (Gaston and Fuller, 2007), so the loss of populations of common species could have devastating effects on ecosystem functioning as well as cascading ecosystem effects (Vermeij, 1993). High coral cover reduces the distance between neighbouring coral colonies (Connell, 2004) and thus between infected and healthy hosts, increasing the potential for horizontal disease transmission. It has been shown in many animals that high-density communities bolster the prevalence and transmission of disease (Lafferty 2004; Altizer and Augistine, 1997; Rudolf and Antonics, 2005; Anderson and May 1979), especially under thermal stress (Bruno et al, 2007). In the coming decades it will be particularly important to monitor the status of dominant coral species to enable population declines or range contractions to be detected.

I was able to test all ecological, phylogenetic and population genetic hypotheses on a total of 14 species (results are summarized in Table 25), (for the other 69 species only ecological or ecological and phylogenetic hypothesis were tested). Synthesising the results of hypothesis tested in this thesis I conclude all species that display a type of rarity are threatened, but some are far more threatened than others. Further, even those species considered not threatened can have populations that are genetically depleted and vulnerable to local extinction. Most Indo-Pacific *Acropora* species are relatively recently evolved (<5 million years, van Oppen *et al.,* 2001) and it is these recently evolved species that appear to face the highest level of threat. However, not all recently evolved species are threatened (e.g. *A. microphthalma*). Overall I conclude that any species that displays a rarity characteristic in terms of either distribution or

abundance faces an elevated level of threat because genetic resilience may be jeopardized and/or because there are fewer source populations to stimulate the recovery of depleted populations. Species with Occupancy types 1 and 2 face the highest levels of threat.

Table 25. Summary results of all hypotheses tested on 14 *Acropora* species. *Indicates rare species.

Species	Occupancy type (Chapter 2)	Ancestral or recently evolved (Chapter 3)	Evidence of hybrid history (Chapters 3, 4)	Evidence of genetic depletion (Chapter 5)	Evidence of population connectivity (Chapter 5)	Level of Threat
A. microphthalma	Occupancy type 8	Recent	Yes – interspecific allele sharing	No	Transoceanic connectivity	Low
A. valida	Occupancy type 8	Recent	Yes – interspecific allele sharing	Heron Is. pop. depleted	Heron Is. – Orpheus Is. divide	Medium in isolated populations
A. austera	Occupancy type 8	Ancestral	No	Majuro pop. depleted	Indian O. /Pacific O. divide	Medium in isolated populations
A. millepora	Occupancy type 8	Intermediate	Yes – interspecific allele sharing	No	East & West Aust. connectivity	Low
A. horrida	Occupancy type 6	Ancestral	No	Some loci depleted	NA	Medium in isolated populations
A. pichoni*	Occupancy type 1	Recent	Yes – interspecific allele sharing, polyphyletic at nDNA	Depleted allelic richness	PNG & Micronesia connectivity	Very high
A. kirstyae	Occupancy type 2	Recent	Yes – interspecific allele sharing, polyphyletic at nDNA and mtDNA	No	NA	High
A. kimbeensis*	Occupancy type 1	Recent	Yes- polyphyletic at nDNA	No	NA	High
A. walindii*	Occupancy type 1	Recent	Yes – interspecific allele sharing	Depleted allelic richness and expected heterozygosity	NA	Very high
A. jacquelineae*	Occupancy type 1	Recent	Yes – interspecific allele sharing, polyphyletic at nDNA and mtDNA	Depleted allelic richness	NA	Very high
A. spathulata*	Occupancy type 1	Recent	Yes – interspecific allele sharing, polyphyletic at nDNA	Depleted allelic richness	NA	Medium in isolated populations
A. rongelapensis*	Occupancy type 1	Recent	Yes – 100% observed heterozygosity in 3 loci	No	NA	High
A. tortuosa	Occupancy type 8	Recent	Yes – interspecific allele sharing, polyphyletic at nDNA	Depleted allelic richness	NA	Medium
A. papillare*	NA	Recent	Yes – interspecific allele sharing, polyphyletic at NDNA	Japan pop. depleted	Indian O. /Pacific O. divide	Medium in isolated populations

7.2 Global, local and ecological extinctions

The results presented here suggest that many *Acropora* species in the NW Pacific are vulnerable to local or ecological extinction. Local extinction (the disappearance of a species from part of it range) and ecological extinction (when a species is reduced to such low abundance that, although still present, it no longer plays its typical ecological role) are precursors to global extinction. Isolated populations have been shown to be vulnerable to local extinction because stochastic and genetic forces can push populations to local extinction (Ayre and Hughes, 2000). Data here suggests that the populations of *A. papillare* sampled in Japan, *A. valida* sampled at Heron Island and *A. austera* sampled at Majuro Atoll are vulnerable to local or ecological extinction.

The likelihood of a species re-establishing a locally extinct population depends upon the presence of source larvae, the continuity of non-degraded coral reef habitat and the presence of ocean currents to transport larvae. It also depends upon atmospheric, oceanographic and hydrodynamic stability. Environmental variation leads to a decrease in the probability of establishment because where there is a high level of variability in environmental conditions; the proportion of offspring that survive and reproduce is more variable. The more extreme temperature variation and more intense and frequent storm events are predicted in the future are likely to further increase the likelihood of local extinctions and decrease the likelihood that locally extinct populations can re-establish.

Results from Chapter 2 suggest that in isolated coral communities with low species richness, the local or ecological extinction of a species may have exaggerated effects at the community level in comparison to the local or ecological extinction of a species in a community with high species richness. Local extinctions are difficult to detect without rigorous long-term species-level monitoring (see section 7.5). Data presented here in Chapter 5 suggests that the threat of local extinctions is not restricted to rare species. Isolation contributes to local population instability and extinction by decreasing individual reproductive success or by increasing reproductive variance (Stephens *et al.,* 1999; Robertson and Butler, 2009) and this is not conducive to the long-term sustainability of any species (Worm *et al.,* 2007).

7.3 Conservation Genetics

Results from my population genetic study (Chapter 5) suggest that 91.4% of Acropora species examined here have lower mean allelic diversity at individual loci than a

'conservative mean' published in a recent review of scleractinian coral genetic diversity (Shearer *et al.,* 2009). Only 8.6% of loci had mean allelic richness higher than 8.27 alleles per locus (n = 107 loci/populations) (Figure 39). It is important to note that *A. palmata* (a species restricted to the Atlantic Ocean that is critically endangered) has far higher mean levels of allelic diversity (14.4 alleles/locus) than any of the Indo-Pacific species examined here regardless of whether they are rare or common. These comparisons are alarming because *Acropora* species generally make up a large proportion of the diversity and cover in Indo-Pacific coral communities – if *Acropora* populations have low genetic diversity they may have low resilience and a low capacity to adapt (Reusch *et al.,* 2005; van Oppen and Gates, 2006) and perhaps this helps to explain why *Acropora* are so sensitive to environmental changes.

Although the allelic richness results presented were corrected for unequal sample size in Fstat v 2.9.3 (Goudet 2001) are likely to underestimates the actual population genetic diversity of these *Acropora* populations. Rarefaction analysis has shown that although population samples of 10 capture greater than 50% of the estimated allelic diversity, population samples of 35 are needed to capture 90% or more of the population's genetic diversity (Shearer *et al.,* 2009). It is plausible that the genetic diversity estimates I present underestimate actual genetic diversity by at least 10% because the individual population sample sizes are <30 colonies. Even when the possibility of an additional 20% diversity is factored in, rare species such as *A. walindii**, *A. pichoni** and *A. jacquelineae** clearly have very low mean allelic richness across all or most loci examined.

The low level of mean genetic diversity that I report must be further interpreted with caution because data pertaining to loci that had more than 2 alleles were excluded from the genetic diversity estimates. This is because FSTAT is designed for haploid/diploid populations only. This means that the mean allelic diversity estimates should be higher for species such as *A. valida* at Amil2_022. My results show that some rare marine species are invariably vulnerable to genetic erosion and for these species their long-term persistence is of critical conservation interest. Further robust quantitative estimates of genetic diversity are required to fully understand the relationship between genetic diversity, adaptive potential and population persistence. .

Figure 39. Mean allelic richness for all species/loci examined in this study showing that most A*cropora* species have lower mean allelic richness than what is considered a 'conservative mean' in a recent review of scleractinian coral genetic diversity (Shearer *et al.,* 2009).

It is apparent from the data presented here that the level of genetic diversity a species displays varies greatly across its distribution range and therefore this means that populations of common species can be much more threatened than expected. Conversely, small populations can be more diverse and less threatened than expected. My findings of non-depletion in some rare species and depletion in some populations of common species, in conjunction with the lack of significantly greater levels of inbreeding in common species, means general conservation genetic hypotheses about the genetic resilience of rare/common species are not always relevant for species in the coral genus *Acropora*.

I provide evidence to suggest that some of the genetic diversity I detect in rare species results from hybrid relationships. As discussed in Chapter 6, hybrid corals have previously been considered to have limited evolutionary potential (Vollmer and Palumbi 2002), however evidence of successful embryogenesis has been demonstrated for a variety of interspecific *Acropora* crosses (Willis, 1997) and hybrid larvae are known to be competent to settle, metamorphosize and survive for up to 3.5 yrs (Willis et al. 2006). Further, whether being unidirectional or multi-directional, hybridisation can create individuals capable of sexual reproduction via backcrossing with parental lineages and thus, hybrid corals can potentially maintain populations that contribute to reef-building and ecosystem functioning over long periods of time (Arnold and Hodges, 1995; Willis *et al.,* 2006). As Illustrated by the Pacific elkhorn case study, establishing new policies to deal with the conservation of marine invertebrates with hybrid ancestries is a challenge that must urgently be dealt with in order to truly protect coral biodiversity.

7.4 Rare species, biodiversity and functioning are interrelated

There is a large amount of uncertainty about the structure and function of coral reef communities and this provides a major challenge to marine conservation (Halpern *et al.,* 2006). Generally, coral reef managers seek to efficiently protect key processes that maintain the biodiversity and functioning of coral reefs (Hughes *et al.,* 2003; Folke *et al.,* 2004). However, coral reefs are ecologically heterogeneous in terms of species composition and configuration, and we have little understanding of the key functional roles most species play. This uncertainty means that we have limited understanding of how ecosystems respond to either management or changing environmental conditions. In the absence of specific data that would optimise management for an area or species, there is a trend in coral reef management to manage for uncertainty by utilizing whatever general information is available (McCook *et al.,* 2009). Such an approach seeks to maximize the likelihood that ecosystem functioning will be maintained and assumes biodiversity will be protected indirectly.

The role rare species and wider biodiversity play in ecosystem functioning is not clear, and subsequently rare species are often thought to be functionally redundant. Numerous ecological studies have investigated how many species can be lost from an ecosystem before functioning is affected (Naeem *et al.,* 1995; Purvis and Hector, 2000; Petchey and Gaston, 2002). These studies found that there is limited redundancy in complex ecosystems and there is a strong link between biodiversity persistence and ecosystem functioning (Hooper at al, 2005; Danovaro *et al.,* 2008). In particular when an ecosystem is exposed to intensive use or pressure, a large pool of species is required to sustain ecostystem functioning (Loreau, *et al.,* 2001). Thus, even small changes in biodiversity can have significant impacts on functional diversity and ecosystem functioning (Micheli and Halpern, 2005) especially in isolated locations.

Part of the challenge of managing coral reefs to preserve ecosystem function is that in heterogenous ecosystems functionality is often cryptic. Here I introduce a new term - *cryptic functionality* - to describe the situation where a species (or group) is suited or designed for a function that is currently unknown, unrecorded or latent. Cryptic functionality is essentially a type of uncertainty. Accounting for cryptic functionality requires the application of the precautionary principle to encompass the conservation of species that may play a key (but yet unknown) role over long timeframes. In this respect, species (including rare species) that appear to be functionally redundant or neutral to environmental conditions may be important under changing conditions. Because we are only just beginning to discover that species may play multiple roles or have varying roles across spatio-temporal gradients (Bremner *et al.,* 2003), making generalizations about ecosystem functionality is problematic.

7.5 Species level monitoring of biodiversity

On reefs today, all evidence points towards the general demise of habitat quality (Hughes 2003; Bruno and Selig, 2007). The impact chronic habitat decline has on coral biodiversity remains elusive. On the Great Barrier Reef, coral biodiversity is predicted to be lost in degraded habitats over the next decade (DeVantier *et al.,* 2006). To evaluate current and future trends in biodiversity, detect species extinctions (Thomas *et al.,* 2004), depletions (Gaston and Fuller, 2007) or range shifts (Parmesan & Yohe, 2003), species-level monitoring is required. I argue that given the poor knowledge of the responses of coral reef species to environmental change and management actions, further targeted species-specific studies are needed to monitor the success of widespread conservation planning action. Given that we will never be able to identify all biodiversity on coral reefs or identify all

functions (Hector *et al.,* 2001), there is an urgent need to identify taxonomic groups or species that show a strong sensitivity to environmental parameters (for example temperature, salinity and water quality stress, disease susceptibility etc). Once identified, these species would be included in more general monitoring programs (such as the Great Barrier Reef Long Term Monitoring Programme). The sustained collection of targeted species-level data will help detect environmental change and improve conservation planning decision-making. Species distribution modelling techniques can be used to identify species that respond to selected environmental parameters (Beger and Possingham, 2008). Relevant target species would change spatio-temporally so regional-specific species distribution models should be developed.

7.6 Overall Summary

The processes that determine why some species are rare and others common are of great ecological and evolutionary interest and of primary importance for resource managers working to protect coral reef biodiversity threatened by global climate change. Although a limited number of modern global extinctions of rare corals are recorded, there is reason to believe that the threat of extinction is increasing with the frequency of disturbance events. In this thesis I have examined rarity with ecological, phylogenetic and population genetic tools. Current theory predicts that characteristics of rare species, such as restricted dispersal and low local abundance, confine them to narrow habitats or environmental conditions, resulting in high genetic structure among populations and low genetic diversity within populations. These characteristics make rare species of greater conservation concern than widespread species, however up until now, there has been very little data available about the population ecology and genetics of rare coral species against which to test these hypotheses. In this thesis my results suggest that:

- *Acropora* species in the NW Pacific display multiple patterns of rarity. Over half of the species examined *(*54%, n= 45 species) display one of five different types of rarity. Two factors contributed to the identification of a larger number of rare species than previously represented in the coral literature: 1) recognition of multiple types of rarity, and 2) explicit targeting of rare species in rapid visual assessment methodology which greatly improved the representation of structural zeros in the numerical data.

- The inclusion of rare species in numerical data reveals differences in assemblage structure between habitats and among locations, highlighting the importance of maintaining rare species in datasets and using appropriate analytical techniques, like inverse transformation, to capture their signal.

- In coral communities with lower species richness, the loss of a dominant species could have a lasting impact at the community level, whereas in a community with higher evenness, the loss of a single species may have lesser significance unless that species plays an irreplaceable functional role.

- Most of the rare species included in this analysis are recently evolved. However, phylogeny alone does not explain rarity in *Acropora* corals because some comparatively recently evolved species have wide distributions and large population sizes.

- Members of the *horrida* and *echinata* groups of *Acropora* occur in basal positions in both mitochondrial and nuclear topologies suggesting that these lineages are far older than currently understood and are likely to have arisen in the Eocene.

- Phylogenetic and population genetic data presented here show that *Acropora* species follow complex evolutionary pathways. I show the first unequivocal evidence for hybridization in Indo-Pacific species and infer that the ecological, and likely evolutionary, significance of hybrid corals poses a major challenge to conservation legislation. There is an urgent need to re-evaluate conservation policies for hybrid marine invertebrates.

- Some rare species are genetically depleted and for these, a narrow window of opportunity exists for management and conservation. However, not all rare corals have lower genetic diversity at neutral DNA loci than common species. I infer 'non-depleted' rare species may have more resilience and adaptive potential than has been previously assumed.

- Some populations of common species are genetically eroded, reinforcing concerns that isolated populations of common species are also vulnerable to genetic erosion.

- Recently evolved Indo-Pacific *Acropora* corals may be less genetically diverse than expected and this may account for their sensitivity to change.

7.7. Future Directions

An important future direction for biodiversity conservation is to further explore genetic resilience. Inferring the genetic resilience of corals is challenging because: 1). corals show chaotic genetic patchiness, and this means that different cohorts can be genetically differentiated (Hedgecock, 1994), 2). larvae may come from different source populations at different times (Hellberg, 2002), and 3). only a few adults may contribute to the effective population size of even large populations of coral. Moreover, this means that very robust spatio-temporal sampling designs must be strictly employed to standardize sampling of population sizes and to avoid Wahlund effects and clonemates.

As a consequence of the results presented in this thesis, I consider that unless greater financial investment is directed towards species-level coral biodiversity monitoring and research, many local populations, and ultimately, species will go extinct before we learn about their functional roles. While species-level monitoring may not be economically valid in our current economic framework, I advocate that this shift should be a future funding priority. At a minimum, a suite of target indicator species representing different categories of threat and resilience, along with some of those predicted to respond to environmental parameters should be chosen within different regions and incorporated into long-term monitoring initiatives. Prioritising target indicator species will enable a better understanding of current conservation practice and help increase the probability that biodiversity is conserved.

Ultimately, the loss of biodiversity is irreversible and the ecosystem effects are unpredictable. To maximize the retention of ecosystem functioning, it is important to preserve not only patterns of biodiversity, but also the processes that generate and maintain it (Smith et al. 2001; Ennos *et al.,* 2005). My results underscore the importance of investigating the role that hybridization and gene/chromosome duplication events play in shaping coral biodiversity. I suggest further karyotyping research and investigating genetic diversity and population structure at functional loci should be a research priority. The application of high-throughput technology (e.g. next generation sequencing technologies such as Illumina) would be the most efficient way to conduct future genotyping research.

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