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The population genetic structure of coral reef fishes on the Great Barrier Reef.

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Statement of contribution of others

All data chapters of this thesis include collaborative work with my supervisors Prof. Ross H. Crozier and Dr. M. Julian Caley. Chapter 3 is a collaboration with Monica Gagliano and Karin Buechler. While undertaking these collaborations I was responsible for the project concept and design, the collection of the majority of samples, the laboratory work, analysis, synthesis and preparation of manuscripts for submission to peer reviewed journals. Karin Buechler provided the samples used in Chapter 3 and Karin Buechler, Monica Gagliano, Shilo Ludke and undertook the laboratory work for this chapter.

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General Abstract

The population genetic structure of species may be determined by complex interactions among many ecological, evolutionary and genetic processes. I investigated the population genetic structure of coral reef fishes on the Great Barrier Reef (GBR), Australia to better understand how these various processes may interact in a natural system. I firstly examined the spatial genetic structure of a low dispersal species to determine if its genetic structure varied among spatial scales and among regions located in the centre and on the periphery of its distributional range. I then examined the population genetic structure of species with different dispersal potentials and among species sampled at central and peripheral locations in their species range.

Using mtDNA control region sequences and three microsatellite loci, I examined the spatial genetic structure of a direct developing coral reef fish, *Acanthochromis polyacanthus,* with comparatively low dispersal rates. The spatial genetic structure of this species was scale-dependent with evidence of isolation-by-distance among regions, but not within regions. Very strong genetic structure was detected among reefs within regions consistent with a metapopulation model. Pairwise genetic distances increased from offshore and older populations, to inshore and younger ones, supporting a metapopulation propagule-pool model of colonisation. Genetic diversities, mismatch, and coalescence analyses all identified large variation in the demographic history of this species among populations and regions. Evidence of genetic bottlenecks was detected by mismatch analysis in the majority of populations sampled, but in most populations these bottlenecks appeared to be older since genetic diversities and coalescence based population growth estimates did not indicate recent genetic bottlenecks. In contrast, three populations displayed low genetic diversities and large population growth rates indicating a more recent genetic bottleneck. Reductions in genetic diversities of local populations resulted in overall lower genetic diversity and a higher regional expansion rate in the southern region located towards the distributional margin of this species. In all, these results suggest that *A. polyacanthus* exists as a metapopulation within regions on the GBR and that metapopulation dynamics may differ among regions located in the centre and on the periphery of this species.

The pelagic larval duration (PLD) can both affect and record the ecology and evolution of coral reef fishes and emerging evidence suggests that this trait displays considerable intraspecific variation. Here I present new estimates of PLD for ten species of Pomacentridae and two species of Gobiidae, and coupled with previously published estimates, examine spatial and temporal variation of PLDs within and among these species. In eight of the twelve species examined here, within-population mean PLDs differed between sampling times, locations within regions, and among regions. In contrast, the range of these same PLD estimates overlapped at all spatial and temporal scales examined in eleven of the twelve species, but not between regions in one species (*Amphiprion melanopus*). Therefore, despite tight error estimates typically associated with estimates of PLD taken from a particular population at a particular time in some taxa, the overlapping ranges in PLD reported here indicate that the length of the pelagic larval phase is a much more plastic trait than previously appreciated.

Pelagic larval duration (PLD) is a commonly used proxy for dispersal potential in coral reef fishes. Here I examine the relationship between PLD, genetic structure and genetic variability in coral reef fishes from one family (Pomacentridae) that differ in mean larval duration by more than a month. Genetic structure was estimated in eight species using a mitochondrial molecular marker (control region) and in a sub-set of five species using nuclear molecular markers (ISSRs). Estimates of genetic differentiation were similar among species with pelagic larvae, but differed between molecular markers. The mtDNA indicated no structure while the ISSR indicated some structure between the sampling locations. I detected a relationship between PLD and genetic structure using both markers. These relationships, however, were caused by a single species, *Acanthochromis polyacanthus*, which differs from all the other species examined here in lacking a larval phase. With this species excluded, there was no relationship between PLD and genetic structure using either marker. Genetic diversities were generally high in all species and did not differ significantly among species and locations. Nucleotide diversity and total heterozygosity were negatively related to maximum PLD, but again, these relationships were caused by *A. polyacanthus* and disappeared when this species was excluded from these analyses. These genetic patterns are consistent with moderate gene flow among well-connected locations and indicate that at this phylogenetic level (i.e., within family) the duration of the pelagic larval phase is not the primary factor affecting patterns of genetic differentiation.

Using mtDNA (control region) and nuclear (ISSR) markers, I investigated the population genetic structure of three congeneric species pairs of pomacentrid coral reef fishes (Pomacentridae) in the context of species' borders theory. This theory predicts that population located on the periphery of the species' range should be smaller and more fragmented and hence, display stronger genetic structure and lower genetic diversities compared to more centrally located populations. Each species pair consisted of one species sampled at two central locations within its geographic range, and another species sampled at the same locations but which constituted one location toward the centre of its range and another close to its edge. Contrary to expectations from theory, I did not find the predicted border effects in the population genetic structure of the species examined. Gene flow estimates did not differ among central and peripheral species. Genetic diversities were not lower in peripheral populations compared to central populations or in species sampled towards the periphery compared to those sampled in the centre of their ranges. Indeed, genetic diversities were much greater in the peripheral species compared to their central counterparts. The distribution of genetic variation indicated that secondary contact among differentiated lineages may, in part, be responsible for the high genetic diversity in these peripheral species. Elevated mutation rates mediated by environmental stress on the species' margin may have contributed further genetic variability in these species.

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Chapter 1: General Introduction

The genetic structure of populations is determined by complex interactions among many genetic, ecological and evolutionary processes (Hartl and Clark 1997; Avise 2000). Ecological and demographic factors including population size, generation time, reproductive behaviour and patterns of migration among suitable habitats may affect the distribution of genetic variation within and among populations. Natural selection may shape allele frequencies in response to local conditions and genetic drift may be a powerful evolutionary force in isolated populations (Slatkin 1985). Genetic factors such as variation in mutation rates and recombination may further affect the genetic composition of populations (Hartl and Clark 1997). An understanding of the processes responsible for the genetic structure of populations therefore requires a detailed appreciation of how these factors interact, and how they vary spatially and temporally.

The physical characteristics of the marine environment and the biological attributes of marine species present a number of evolutionary paradoxes to geneticists seeking to understand the processes determining population structure and speciation in the sea (Knowlton 1993; Palumbi 1994; Grosberg and Cunningham 2001). The marine environment is fluid and there is a general absence of physical barriers to dispersal (Vermeij 1987). Despite this, many marine communities such as those inhabiting coral reefs are very speciose (Sale 1991) and many widespread species comprise groups of cryptic species (Knowlton 1993). Fishes on coral reefs live in a naturally fragmented ecosystem where the isolation of suitable habitat patches may facilitate genetic isolation of populations. However, the pelagic larval stage exhibited by many of these species (Leis 1991; Leis and Carson-Ewart 2000) allows them to disperse widely and may homogenise population genetic structures (Palumbi 1994). Coral reef fishes are generally characterised by large, local populations and have high reproductive outputs, which may decrease the importance of population bottlenecks and genetic drift associated with founder effects in isolated populations (Birky et al. 1989; Hellberg et al. 2002; Kritzer and Sale 2004). However, coral reefs have a dynamic evolutionary history where Pleistocene sea level fluctuations greatly affected the distribution of habitats as well the size and connectivity of populations (Benzie 1999). Therefore, the population genetic structure of many species may be affected by historical isolation, genetic bottlenecks and founder events associated with the colonisation of new habitats. Present patterns of genetic variation within and among populations of many coral reef organisms is therefore likely to be the result of complex interactions among historical and present day factors (Grant and Bowen 1998; Benzie 1999).

Estimates of genetic variability within and among marine populations are rapidly accumulating in the literature, but generalisations about which species are likely to have genetically structured populations, and the processes driving such differentiation, are still hard to draw (Planes 2002). Many studies have used vast trans-oceanographic sampling strategies of species with long pelagic larval durations and presumably high dispersal potentials (e.g., Pacific: Planes and Fauvelot 2002; Bay et al. 2004; Caribbean: Taylor and Hellberg 2003; Geertje et al. 2004; Atlantic: Muss et al. 2001; Rocha et al. 2002). In general, these studies have detected significant genetic structuring among regions (but see Geertje et al. 2004) although some gene flow commonly occurs (but see Taylor and Hellberg 2003). Consequently, a positive relationship between genetic differentiation and geographical distance (isolation-by-distance) has often been found (e.g., Bay et al. 2004). Investigation of population genetic structure of marine species at smaller spatial scales have tended to investigate species with either a short or no pelagic larval duration and hence, low dispersal potentials (Doherty et al. 1994; Nelson et al. 2000; Planes et al. 2001; Bernardi and Vagelli 2004; Hoffman et al. 2005), or species with longer larval durations (Doherty et al. 1995; Planes et al. 1996; Planes et al. 1998; Dudgeon et al. 2000; Bernardi et al. 2001; Messmer et al. 2005). Investigations of species with a short or no pelagic larval stage have generally identified strong population genetic structure over quite short distances (e.g., 5 – 10 km) implying that spatial isolation and genetic drift are important ecological and evolutionary factors in such species. In contrast, species with longer larval durations may (Doherty et al. 1995; Planes et al. 1996; Planes et al. 1998; Messmer et al. 2005) or may not (Doherty et al. 1995; Dudgeon et al. 2000; Bernardi et al. 2001) display strong local structure suggesting that the factors that influence population genetic structure at local scales may also be complex.

The processes that determine the population genetic structure of coral reef fishes have also been investigated in a comparative framework (Doherty et al. 1995; Shulman and Bermingham 1995; Planes et al. 1998; Dudgeon et al. 2000; Riginos and Victor 2001; Fauvelot and Planes 2002; Fauvelot et al. 2003; Rocha et al. 2005). Such comparative studies may be particularly important in elucidating the mechanisms determining genetic structure in marine species because they allow for factors affecting dispersal to be isolated and controlled (Bohonak 1999). Indeed, some of the more general conclusions about the roles of ecological specialisation (Rocha et al. 2005), larval behaviour (Riginos and Victor 2001) and historical habitat stability (Fauvelot et al. 2003) on the population genetic structure of coral reef fishes have emerged from such studies.

While all these studies have made valuable contributions to our understanding of the population genetic structure of coral reef fishes, very few have allowed for genetic variation to be partitioned among local scales within regions of any species, regardless of their presumed dispersal potential (but see Doherty et al. 1995). Consequently, we do not currently have a good appreciation of local-scale genetic patterns and their potential effects on regional patterns. Studies that allow genetic variation to be partitioned among local and regional scales, as well as controlled comparative studies among closely related species, have the potential to greatly increase our understanding of the general mechanisms that determine the population genetic structure of coral reef fishes. The general aim of this thesis was, therefore, to understand how the spatial and temporal complexity of coral reefs can influence the genetic structure of species occupying such environments. To this end, I examined the spatial genetic structure of one species in detail to elucidate the potential for local and regional scale variation in its population genetic structure. I then used a comparative approach to examine the roles of dispersal potential on gene flow, and how the population genetic structure can differ within the species range. In order to achieve these aims it was necessary to consider the major biological, historical and environmental factors that may influence the interpretation of population genetic data of coral reef fishes.

The genetic structure of populations is interpreted using several spatial models that vary in their complexity and the assumptions they make about the biological characteristics of the system under investigation. The complexity of these models and the degree to which assumption may be violated can greatly affect the resolution and interpretation of population genetic data. The island model, originally proposed by Sewall Wright (1931), estimates genetic differentiation (F_{ST}) by assuming that all populations are of equal size and have an equal probability of exchanging migrants regardless of their relative positions. The spatial position and the size of populations are likely to affect both patterns of emigration (i.e., larger populations may produce more emigrants) and the local effects of immigration (i.e., the effects of immigration may be greater in a smaller compared to larger population). Consequently, this model does not describe the spatial structure of many real populations very well, except when the migration rate is low (Palumbi et al. 2003). This is because the migration rate is inversely related to the log of genetic structure (F_{ST}), so that even moderate migration rates will produce very small F_{ST} estimates associated with relatively high error (Waples 1998). Differences in F_{ST} estimates among populations or species are therefore difficult to distinguish statistically, even where the migration rates producing them are different (Neigel 1997; Waples 1998). Most genetic investigations on coral reef fishes to date have used this island model.

The stepping-stone, or isolation-by-distance model incorporates spatial variation by assuming that populations in closer proximity are more likely to exchange migrants than more distantly separated ones (Wright 1943; Kimura 1955; Kimura and Weiss 1964; Weiss and Kimura 1964). Migration rates can be estimated by correlating the genetic differentiation of populations with the geographical distance separating them, and therefore, allow migration rates to be estimated more precisely than under the island model, especially when migration rates are high (Palumbi et al. 2003). The isolation-by-distance model has increasingly been applied to the population genetic structure of coral reef fishes (e.g., Planes et al. 1996; Planes and Fauvelot 2002; Bay et al. 2004) and has indicated that patterns of gene flow may differ among species with high dispersal potential.

Metapopulation genetic models consider differences in effective population sizes, colonisation patterns and extinction rates in the interpretation of migration and concomitant genetic structure of populations (Slatkin 1977, 1985, 1987; Wade and McCauley 1988; Whitlock and McCauley 1990). A metapopulation is composed of a number of spatially structured ephemeral populations that interact and persist through time via migration (Hanski 1991; Hanski and Gilpin 1997). The patterns of migration, extinction and re-colonisation of these populations can have profound effects on the distribution of genetic variation within and among the populations of a metapopulation (Pannell and Charlesworth 1999, 2000; Pannell 2003). For example, extinctions may decrease genetic variation within local populations but increase genetic structure among populations depending on the pattern of colonisation (Wade and McCauley 1988; Whitlock and McCauley 1990). Migration may reduce genetic differentiation among populations over time so that younger populations display stronger genetic differentiation than older populations (Giles and Goudet 1997). The physical structure of coral reefs suggests that the application of metapopulation theory holds much promise for understanding the spatial genetic structure of many coral reef species (Swearer et al. 2002). Despite this, we currently have a poor appreciation of the presence, spatial extent and genetic consequences of metapopulation dynamics in marine systems.

The vast majority of coral reef fishes have a bipartite life history where dispersal occurs primarily during the pelagic larval phase (Sale et al. 1980; Leis 1991; Leis and Carson-Ewart 2000). As such, most species have a potential for large-distance dispersal and characteristics of the larval phase have commonly been used to predict the genetic structure of populations. The dispersal potential of coral reef fishes have been investigated with respect to a range of larval traits including egg type (Shulman and Bermingham 1995; Shulman 1998), pelagic larval environment (Riginos and Victor 2001) and most commonly the length of the pelagic larval phase (PLD: Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001). When examined previously, a relationship between mean larval duration and genetic differentiation has generally been found (Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001). This relationship may be greatly influenced by the inclusion of highly genetically structured, directly developing species (Bohonak 1999; Riginos and Victor 2001). Furthermore, behavioural (e.g., Taylor and Hellberg 2003), physiological (e.g., Shulman 1998) and ecological (e.g., Rocha et al. 2005) factors may also affect dispersal abilities, and these may vary among taxonomic groups (Bohonak 1999). Despite the potential importance of such characteristics in determining variation in dispersal rates, examinations of the relationship between PLD and gene flow in marine fishes to date have incorporated a range of distantly related species, displaying different spawning characteristics and adult ecologies (Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001). Consequently, we do not have a good understanding of how PLD relates to dispersal in species that display little variation in their biology and ecology.

Under the neutral theory of molecular evolution, genetic variation in unlinked markers is generated by mutation, subsequently modified by random genetic drift in isolated populations and homogenised among populations via migration (Hartl and Clark 1997). An explicit assumption of many population genetic analyses is, therefore, that populations are in migration-drift equilibrium; their current genetic structure is the result of the opposing effects of genetic drift and migration (Hartl and Clark 1997). The distribution of genetic variation within and among populations can be greatly affected by historical effects such as genetic bottlenecks and founder events (Avise 2000) and such signatures may be retained in populations over many generations depending on their effective population sizes and rates of migration (Crow and Aoki 1984). Consequently, many species may not be in migration-drift equilibrium and their genetic structure may reflect historical as well as current patterns of gene flow (Benzie 1999). Under this scenario, the use of F_{ST} as an indicator of genetic isolation becomes problematic (Neigel 1997, 2002); recent coalescence-based maximum likelihood methods (Kuhner et al. 1998; Beerli and Felsenstein 1999, 2001) may ameliorate some of the problems associated with estimating population genetic structure in non-equilibrium species (Neigel 2002).

Sea level fluctuations associated with Pleistocene glacial events greatly affected the presence and distribution of coral reefs and are likely to have had profound effects on resident faunas (Paulay 1990; Benzie 1999). The historical effects on the present day patterns of genetic structure and genetic variability in coral reef fishes have increasingly been considered (Doherty et al. 1994; Shulman and Bermingham 1995; Dudgeon, 2000; Nelson et al. 2000; Planes et al. 2001; Fauvelot et al. 2003). Strong genetic differentiation among closely spaced populations has been interpreted in the context of historical isolation of populations (Nelson et al. 2000), or founder events associated with the colonisation of new habitats (Doherty et al. 1994; Planes et al. 2001). In species with low genetic structure, reduced genetic diversities have been associated with historical habitat stability (Fauvelot et al. 2003). While many coral reef fishes display low genetic variability indicating shallow coalescent histories (Grant and Bowen 1998), other species display high levels of genetic diversity (Planes 1998). Consequently, the current genetic structure of many coral reef fishes may be strongly influenced by historical events, however, the importance of such historical factors may vary among locations and species.

The factors determining the extent of a species geographic range have long interested biologists (Darwin 1859; Mayr 1963). The distributional range of a species is determined by spatial and temporal variation in demographic parameters such as births, deaths and dispersal (Holt et al. 2005). Biological and environmental conditions are generally assumed to be optimal in the centre of a species range and to decline towards its periphery (Hoffmann and Parsons 1991). Populations should therefore become smaller, more fragmented and experience increased extinction rates towards the edge of the range (Levins 1970; Lennon et al. 1997). These effects should be evident in the genetic structure of such populations. For example, gene flow may be reduced towards the species margin because of increased fragmentation and smaller population sizes (Levins 1970). Genetic isolation, smaller population sizes and increased extinction rates should, therefore, reduce genetic diversities in peripheral populations (Holt 1987). Many coral reef fish species have borders that are not associated with any obvious habitat discontinuities or barriers to dispersal. They, therefore, constitute a good system to test predictions from species border theory, however, variation in the population genetic structure among central and peripheral populations have rarely been considered (but see Planes and Fauvelot 2002).

In this thesis I examine the processes that may determine the population genetic structure in one family of coral reef fishes, the Pomacentridae, on the Great Barrier Reef (GBR). I capitalise on the unique attributes of the coral reef fish assemblages and the physical structure of the coral reefs on the GBR. The GBR is unique among many coral reef systems of the world in being a largely linear band of highly interconnected, though spatially separated, reefs of relatively recent origin (Hopley and Thom 1983; Larcombe 2001). Environmental conditions change along latitudinal and longitudinal gradients and concomitant effects on fish species distribution, abundance and demographic patterns are evident (Russ 1984; Gust et al. 2001; Gust 2004). The large number of individual reefs facilitates a detailed examination of local-scale genetic structure of coral reef organisms and its latitudinal and longitudinal variation. There is an absence of obvious dispersal barriers on the GBR, and species currently occupying the GBR are likely to have been affected by recent sea level changes. It is, therefore likely that the population genetic structure of coral reef fishes on the GBR may be affected by this disturbance history (Doherty et al. 1994; Planes et al. 2001), but there is no *a priori* reason why this should have affected some species differently from others. The high species richness of coral reef fishes on the GBR also enables comparative investigations of closely related and co-occurring species to be undertaken. Such a design can allow potential confounding factors such as ecological specificity, spawning characteristics and distributional range effects to be controlled.

To examine the processes driving patterns of gene flow and genetic variability in coral reef fishes on the Great Barrier Reef, I addressed four specific issues:

1. The potential for local spatial genetic structure of a low dispersal species and the utility of metapopulation theory to describe the population genetic structure in this species

2. The role of local extinctions on metapopulation dynamics of a low dispersal species, and how this may vary spatially among regions located in the centre and on the peripheral of the distributional range.
3. The relationship between dispersal ability, gene flow and genetic diversity in ecologically generalised and widespread species.
4. How metapopulation processes affect patterns of gene flow and genetic diversities on the species margin in ecologically generalised species.

This thesis is constructed as a series of stand-alone, but conceptually interconnected publications. **Chapter 2** examines the genetic structure of a common direct developing coral reef fish, *Acanthochromis polyacanthus* within and among regions on the Great Barrier Reef using a mitochondrial sequence marker and three microsatellite loci. I examine patterns of gene flow and reciprocal migration rates (i.e., migration from a to b, and vice versa) within and among regions and evaluate the conformation to different spatial genetic models at local and regional scales. The role of genetic bottlenecks and founder effects in *A. polyacanthus* were examined among the same locations in **Chapter 3**. I used frequentist and Bayesian maximum likelihood analyses to evaluate the roles of local extinctions and founder events on genetic diversities at local and metapopulation levels. I further examined if there was a difference in extinction dynamics towards the distributional range edge of this species. In **Chapter 4,** I examine the potential for intraspecific variation in the pelagic larval duration (PLD) of twelve coral reef fish species. Point estimates of mean PLD from the literature are commonly employed in a variety of applications including the prediction of genetic differentiation among populations (e.g., Doherty et al. 1995; Shulman and Bermingham 1995). Because genetic structure is greatly affected by even low levels of migration (Wright 1943), it is possible that maximum rather than mean PLD may better predict gene flow and emerging evidence suggests that PLDs may vary considerably temporally and spatially within species (Leis 1991; Cowen and Sponaugle 1997). Consequently a characterisation of intraspecific variation in this trait was necessary before it could be used to predict gene flow here. In **Chapter 5,** I examine the relationship between dispersal potential (mean and maximum PLD) and gene flow in eight pomacentrid species. To control for potentially confounding factors, this examination was conducted using closely related species that display similar spawning behaviours and generalised ecologies. The potential role of demographic processes in determining species' borders is examined in **Chapter 6**. Patterns of gene flow and genetic diversities were examined in three congeneric species pairs that displayed very similar biological and ecological attributes. Each species pair consisted of one species sampled at two locations in the centre of its range (central species) and another species sampled at a central and marginal location in its range (peripheral species). This design allowed for the genetic consequences of range margins to be elucidated.

Chapter 2: Population genetic structure in a metapopulation of a coral reef fish: asymmetric migration rates and scale-dependency.

Publication: Bay LK, Caley MJ and Crozier RH (In Review) Population genetic structure in a metapopulation of a coral reef fish: asymmetric migration rates and scale-dependency. Molecular Ecology

Abstract

Using mtDNA control region sequences (n = 283) and three microsatellite loci (n = 316), I examined the the spatial genetic structure on the Great Barrier Reef, Australia, of a direct developing coral reef fish, *Acanthochromis polyacanthus,* with comparatively low dispersal rates. I employed a hierarchical sampling design to test three models of genetic structuring (i.e., the island, isolation-by-distance and metapopulation model) at multiple geographical scales (among regions (n = 3), among continental shelf positions within regions (n = 3), and among reefs within regions (n = 5 – 6)). I also tested for asymmetric migration rates among locations using multiple molecular markers. The spatial genetic structure of this species was scale-dependent. Significant genetic structure ($\Phi_{ST} = 0.81$, $R_{ST} = 0.2$, $F_{ST} = 0.07$, $P < 0.0001$) and evidence of isolation-by-distance (Φ_{ST} vs. km $r = 0.77$, $P = 0.001$, R_{ST} vs. km $r = 0.53$, $P = 0.002$, F_{ST} vs. km $r = 0.46$, $P = 0.001$) was found among regions. Within regions, significant structuring across the continental shelf was evident in some regions (North: $\Phi_{\mathbf{ST}} = 0.31$, $P < 0.001$; Central: $R_{\mathbf{ST}} = 0.11$, $P = 0.015$) but no evidence of isolation-by-distance was present at this spatial scale ($P > 0.05$ in all cases). Very strong genetic structure was detected among reefs within regions (mean fixation within region: $\Phi_{ST} = 0.28 – 0.41$, $R_{ST} = 0.09 – 0.13$, $F_{ST} = 0.06 – 0.1$) suggesting that *A. polyacanthus* displays metapopulation dynamics at this scale. Pairwise genetic distances increased from offshore and older populations, to inshore and younger ones, in all comparisons that included significant fixation indices. These patterns support a metapopulation propagule-pool model of colonisation. Based on mtDNA, reciprocal migration rates were low and asymmetric, but based on microsatellites high and symmetrical. These contrasting patterns suggest that the genetic structure observed here may be influenced by male-biased dispersal.

Introduction

The evolution of spatial genetic structure in animal and plant populations has been a central focus of evolutionary studies, and has been important in the development of metapopulation theory. This theory is intended to understand systems of ephemeral, genetically subdivided populations that persist through time via colonisation and migration from source populations (Hanski 1991; Hanski and Gilpin 1997; Pannell and Charlesworth 2000). Such populations are characterised by having a level of migration that is high enough to recolonise extinct populations, but low enough for drift to generate measurable genetic differences among populations (Hanski and Gilpin 1997). While earlier models assumed that migration was infrequent, occurring only to re-colonise patches that had gone extinct (Levins 1970; Slatkin 1977), it is becoming evident that in metapopulations, migration rates may be asymmetric (Stacey et al. 1997) and vary temporally (e.g., Harrison 1991; Stacey and Taper 1992) and spatially (e.g., Pulliam 1988; Hanski and Gyllenberg 1993; Valone and Brown 1995). Behavioural differences among individuals (e.g., Aars and Ims 2000; Blundell et al. 2002; Fraser et al. 2004) can further contribute to such variation. In turn, such variation in migration rates should generate a diversity of genetic signatures depending on the relative importance of each process contributing to its generation (Pannell and Charlesworth 2000). Analytical techniques that can separate overall genetic differentiation into reciprocal migration rates (Beerli and Felsenstein 1999, 2001) should, therefore, be able to illuminate the roles of various processes in establishing patterns of genetic differentiation among sub-populations.

Models of the genetic structure of populations have developed from Wright's original island model (Wright 1931) to the stepping-stone, or isolation-by-distance models, by incorporating spatial variation (Wright 1943; Kimura 1955; Kimura and Weiss 1964; Weiss and Kimura 1964), and later to metapopulation models which incorporate differences in effective population sizes, colonisation and extinction rates (e.g., Slatkin 1977, 1985, 1987; Wade and McCauley 1988; Whitlock and McCauley 1990). Theory suggests that the sources and rates of colonisation relative to subsequent migration are critical determinants of the evolution of genetic structure of a metapopulation (Wade and McCauley 1988; Whitlock and McCauley 1990; Pannell and Charlesworth 2000). In a metapopulation with low levels of migration, the metapopulation propagule-pool model predicts high genetic differentiation if empty patches are colonised by individuals from a single source (Wade and McCauley 1988;

Whitlock and McCauley 1990). In contrast, under the metapopulation migrant-pool model, low genetic differentiation may occur if individual patches are colonised by migrants from a range of sources (Wade and McCauley 1988; Whitlock and McCauley 1990). Under the propagule-pool model, genetic differentiation will always be greater than under an island model. Under a migrant-pool model, a metapopulation should have greater genetic differentiation among populations compared to an island model if colonisation and migration rates are similar (e.g., $4N_em = 2k$, $4N_em$ = effective number of migrants, k = number of colonisers) (Wade and McCauley 1988; Whitlock and McCauley 1990). Despite recent developments in the theory of structured populations, the genetic consequences of metapopulation dynamics remain unclear (Olivieri et al. 1990; Gilpin 1991; McCauley 1991; Harrison and Hastings 1996) due, to a considerable extent, to a lack of empirical tests.

Separating the effects of colonisation pattern and subsequent migration in metapopulations is often difficult because the relative effects of colonisation and migration cannot be estimated from a single estimate of genetic differentiation (Giles and Goudet 1997). However, if the conditions of the propagule-pool model hold, or if colonisation and migration is the same process, then younger populations should display greater genetic differentiation compared to older ones (Giles and Goudet 1997; Pannell and Charlesworth 2000). Consequently, it should be possible to distinguish different types of metapopulation dynamics by the amount of genetic structure among populations and by the distribution of genetic differentiation among older and younger populations.

Fishes on coral reefs occupy a naturally fragmented environment where patches of suitable reef habitat are surrounded by unsuitable habitat such as open sand and deep water. This physical structure makes coral reefs a good system for studying metapopulation dynamics. At present, however, we know little about the presence, spatial extent and genetic consequences of metapopulations dynamics in marine systems (but see Planes et al. 1996). The vast majority of coral reef fishes have a bipartite life history (Sale et al. 1980; Leis 1991; Leis and Carson-Ewart 2000), where pelagic larvae have the potential to disperse widely (Doherty et al. 1995; Chapter 5). Associated with this life-history pattern, little genetic structure across relatively large geographical distances is commonly observed (Planes and Fauvelot 2002; Bay et al. 2004; Chapter 5). While such species may be characterised by isolation-by-distance at large spatial scales (e.g., Planes and Fauvelot 2002; Bay et al. 2004) the lack of within-location sampling by many of these studies make detailed conclusions regarding migration patterns of such species hard to draw. In contrast, species with short, or non-existent larval durations generally display considerable genetic structure across small spatial scales (Bernardi 2000; Planes et al. 2001; Bernardi and Vagelli 2004; Hoffman et al. 2005). While these studies indicate that such species may display metapopulation dynamics, the sampling strategies used have not permitted detailed examinations of this issue.

Species such as *Acanthochromis polyacanthus,* a common fish on the Great Barrier Reef (GBR) which do not have a dispersive larval phase, coupled with the physical history of the GBR, provides an opportunity to examine metapopulation dynamics and the evolution of genetic structure on small spatial scales in a natural marine system. The reefs of the GBR are relatively young (approximately 6000 - 9000 yrs) (Hopley and Thom 1983; Larcombe 2001). Colonisation of these reefs by fishes is likely to have taken place from Pleistocene fringing reefs and offshore refugia (Davies 1989). Based on reef position and present current patterns (Church 1987; Andrews and Clegg 1989), colonisation is likely to have progressed from the outer continental shelf to inner shelf locations. Here I define the age of populations based on reef position and the population expansion times estimated in Chapter 3. Where the variation in the expansion times did not allow the age of populations to be distinguished, I assume for the purposes of this chapter that populations of this species on the outer shelf are older than those at inner shelf locations and hereafter are referred to as older and younger populations. Previous investigations of *A. polyacanthus*, as well as the presence of several colour morphs on the GBR, suggest that sufficient time has elapsed since colonisation began for this species to have evolved genetic differences among populations separated by small geographic distances (Doherty et al. 1994; Planes et al. 2001).

Here I examine the genetic structure of *A. polyacanthus* on the Great Barrier Reef using rapidly evolving mtDNA and microsatellite molecular markers. I examine if and how the genetic structure of this species varies at three spatial scales (i.e., among reefs within continental shelf position, continental shelf position within regions, and among regions) and evaluate spatial and behavioural differences in migration rates. I then examine the evidence for an island, stepping stone or metapopulation model of genetic structure. First, I evaluate if genetic structure follows a stepping stone model by examining the evidence for isolation-by-distance using conventional genetic estimates of fixation. Next, I evaluate whether the spatial structure of this species follows predictions from the metapopulation propagule-pool and migrant-pool models by evaluating estimates of fixation among older and younger populations. Finally, I examine differences in estimates of genetic differentiation among molecular markers and statistical approaches and discuss potential sources of such variation.

Methods and Materials

Study species and sampling locations

A total of 327 individual *A. polyacanthus* was collected from 15 back-reef locations from 3 regions on the Great Barrier Reef during 2000, 2003 and 2004 (Table 1, Fig. 1) by either spearing with hand-held spears or baited fence netting and hand nets. Baited netting involved aggregating *A. polyacanthus* by baiting the water immediately in front of a 3 x 1.5 meter monofilament net with processed bran, then chasing the fish into the net before catching them with hand held nets. When using this procedure the fence nets were moved regularly to avoid collections of family groups. Fish were transported either alive or on ice to the nearest shore where genetic samples (fin clips) were taken and preserved in 80% EtOH. Genetic structure within regions (shelf effects) were explored independently for two regions (i.e., north and central). Because the southern region contains no true inner and midshelf, the genetic structure in this region was explored using pairwise genetic distances.

DNA extraction and amplification

356 base pairs of the mitochondrial hyper variable control region I were amplified, sequenced in both forward and reverse directions, and aligned in fish from 15 reefs in three regions following methods outlined in Chapter 5. Representative sequences have been deposited in GenBank under accession numbers DQ199666 – DQ199947.

Four microsatellite loci (Miller-Sims et al. 2005) were screened (AC33, AC37, AC42, AC45,) but only 3 loci consistently amplified in both southern, mid and northern populations (AC33, AC37, AC42). Analysis was, therefore, restricted to these loci. Population genetic investigations commonly use a single mitochondrial marker, which introduces some uncertainty about whether results are gene specific or representative of population level processes (Avise 2000). Therefore, the analysis of microsatellites here was intended to provide an assessment of population structure independent of the mtDNA. Because of the relatively low number of microsatellite loci screened, interpretations based on these data should be regarded with some caution.

Table 1: Locations, shelf position and geographic coordinates of the 15 populations of *Acanthochromis polyacanthus* sampled in this study. Location abbreviations used throughout this paper are also indicated. Number of alleles sampled: mtDNA = N, microsatellites = 2N.

					Number of alleles sampled	
Region	Shelf	Location	Abbreviation	Latitude; Longitude	mtDNA	Microsatellites
North	Outer	Yonge Reef	YON	14°37S; 145°37E	20	48
		Day Reef	DAY	14°31S; 145°33E	22	44
	Mid	Lizard Island	LIZ	14°40S; 145°28E	20	36
		North Direction	NDR	14°44S; 145°30E	19	48
	Inner	Martin Reef	MAR	14°45S; 145°20E	21	46
		Linnet Reef	LIN	14°47S; 145°21E	20	48
Mid	Outer	Pith Reef	PIT	18°13S; 147°02E	21	42
		Myrmidon Reef	MYR	18°16S; 147°23E	17	46
	Mid	Britomart Reef	BRI	18°14S; 146°39E	19	48
		Trunk Reef	TRU	18°23S; 146°40E	14	30
	Inner	Orpheus Island	ORP	18°37S; 146°29E	21	46
South	Outer	One Tree Island	OTI	23°30S; 152°05E	21	36
	Outer	Sykes Reef	SYK	23°26S; 152°02E	16	62
	Mid	Polmaise Reef	POL	23°34S; 151°41E	13	18
	Outer	Broomefield Reef	BRO	23°16S; 151°57E	19	34

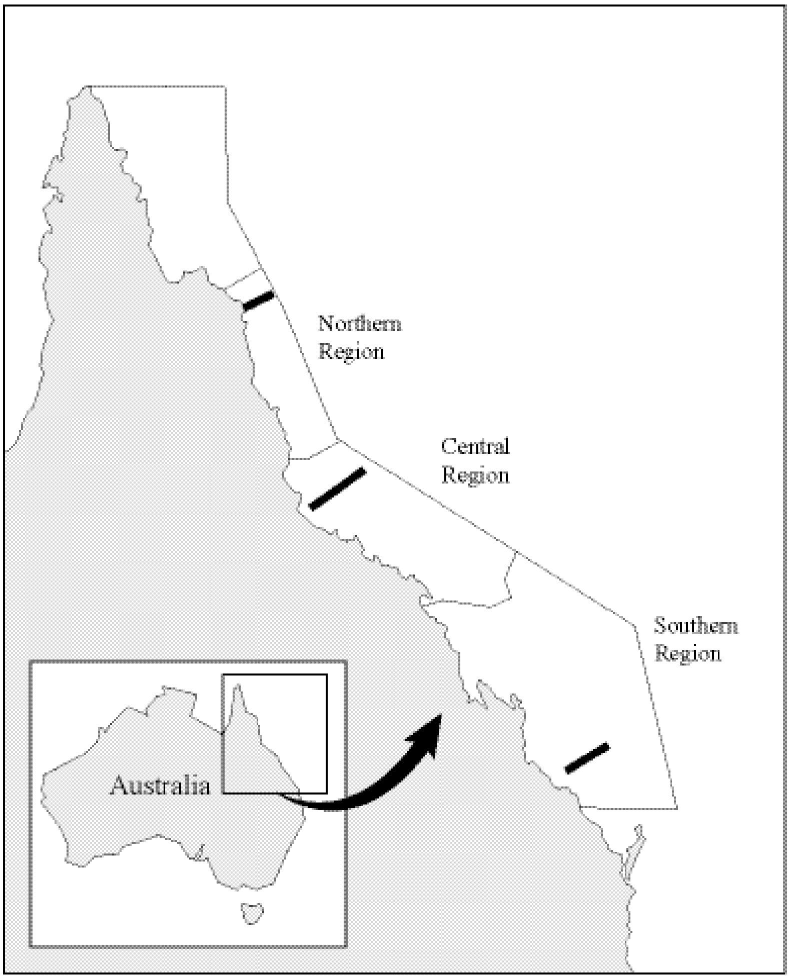

Fig. 1: The sampling locations of *Acanthochromis polyacanthus* on the Great Barrier Reef. Dark bars indicate locations of cross shelf sampling.

The three microsatellite loci were amplified in the same individuals in 15μl reactions containing 1x High Fidelity PCR Buffer, 2 mM $MgSO_4$, 200 μM each dNTP, 0.4 μM each primer, approx. 5 ng template DNA and 0.3 units of Hi Fidelity *Taq Polymerase* (Invitrogen Life Technologies). Microsatellites were amplified using a PCR cycling profile of 94^oC (5 min), 35 cycles of 94^oC (1 min), primer specific annealing temperature (1 min), 68^oC (1 min) followed by a final extension phase of 68^oC (10 min). Primer specific annealing temperatures were AC37 = 46^o, AC42 = 52^o and AC33 = 46^o. Flourolabelled PCR products were cleaned by centrifugation through 300 μl of sephadex G-50, multiplexed and 0.25 μl of ET400 standard (Amersham Biosciences) added before genotyping on a Megabase 1000 (Amersham Biosciences) at the Genetic

Analysis Facility in the Advanced Analytical Center, James Cook University. The microsatellite data set is available from the authors upon request.

Data analysis

The mtDNA control region sequences were aligned and edited using Sequencher 4.2 (GeneCodes Corp. Michigan USA) and ESEE (Cabot and Beckenbach 1989). The best model of nucleotide substitution was determined using Modeltest 3.5 (Posada and Crandall 1998) and PAUP* 4.0b10 (Swofford 1998). The hierarchical likelihood tests and Akaike Information Criteria agreed that the Tamura and Nei model (Tamura and Nei 1993) with $\gamma = 0.3012$ fitted the data best (-LogLikelihood = 1220.65; AIC = 2453.30). This model and rate heterogeneity estimate were used in all following analyses of population genetic structure. Base frequencies and the ts/tv ratio from all sampled fish combined were calculated using Modeltest. The role of saturation was explored by comparing the topology of neighbour joining trees (implemented in PAUP*) including and excluding transitions. All individuals retained membership in the same major clades and transitions were included in all further analyses. Linkage among the three microsatellite loci was investigated using Genepop on the Web (Raymond and Rousset 1995). The role of heterozygotic deficit and departure from Hardy-Weinberg equilibrium was investigated using Genalex 6 (Peakall and Smouse 2001) and significance levels were corrected for multiple tests by a sequential Bonferroni correction (Dunn-Sidak method, Sokal and Rohlf 1995). The fit of the Infinite Allele Model (IAM, Kimura and Crow 1963) and the Stepwise Mutation Model (SMM, Kimura and Otha 1978) was examined using Bottleneck 1.2.02 (Piry et al. 1999).

The mtDNA dataset did not contain any missing data but the microsatellite dataset contained 4% missing values and the majority of these missing values were associated with AC42. To avoid potential confounding effects due to these missing values being treated as a separate allele (Peakall and Smouse 2001), an average genetic identity was allocated to all missing data. This dataset was used in all subsequent analyses of microsatellites.

Population Genetic Structure

Hierarchical population genetic structure of *A. polyacanthus* among regions and reefs was explored using AMOVA using 1000 permutations (Weir and Cockerham 1984; Excoffier et al. 1992) implemented in ARLEQUIN 2.000 (Schneider et al. 2000).

Estimates of population differentiation using microsatellite data were based on both IAM and SMM mutational models. Pairwise genetic distances among populations were calculated from both markers and mutational models using ARLEQUIN and a sequential Bonferroni correction was applied to all pairwise comparisons (Dunn-Sidak method, Sokal and Rohlf 1995).

Migration

Differences in levels of gene flow among locations were investigated further using MIGRATE 1.7.6.1 (Beerli and Felsenstein 1999, 2001). This program calculates reciprocal migration rates (i.e., $4N_em$ from a to b, and vice versa) using a coalescence maximum likelihood approach (Markov Chain Monte Carlo with Hastings Metropolis importance sampling) and assumes constant mutation rates and equal effective population sizes. Because of the molecular divergences detected by phylogenetic and AMOVA analyses, MIGRATE was run on each geographical region separately and due to different effective population sizes the mtDNA and microsatellite data sets were analysed independently. Reciprocal migration rates were interpreted as significantly different when their 95% confidence intervals did not overlap. Extensive sampling regimes including 10 short chains sampled 10000 times each and 5 long chains sampled 100000 each were averaged over 5 replicate runs. MIGRATE was implemented on a SGI Origin 3800 computer in the James Cook University High Performance Computing Facility. A ts/tv ratio of 1.53 (estimated by Modeltest) was used for the sequence data and a stepwise mutational model was implemented for the microsatellite data. Repeated runs were highly consistent using this sampling strategy. To investigate the potential role of greater sample size of the nuclear marker on estimated patterns of migration, I reduced the microsatellite data sets by one third and one sixth using the same search parameters as above in two ways. First, I randomly removed one third and one sixth of individuals from each location. This resulted in highly inconsistent results among runs for the central and southern regions. Second, I removed two loci and half of the individuals of the remaining locus. This procedure was repeated on all three loci, but only resulted in consistent runs with one locus (AC33), most likely because this locus contained the most information. In the northern region, where the different data reduction strategies could be compared, the results were highly consistent. Therefore, only the reductions to one third and one sixth of individuals of AC33 for all regions are presented below.

Isolation-by-Distance

Isolation-by-distance was explored for both the mtDNA and the microsatellites. Geographical distances among locations were calculated using Vincenty's inverse method (http://www.ga.gov.au/nmd/geodesy/datums/distance.jsp). Genetic distances were estimated for mtDNA and microsatellites by conventional genetic distance estimators (Φ_{ST}, F_{ST}, R_{ST}) in ARLEQUIN. Reynold's distance D (Reynolds et al. 1983), Slatkin's linearised measure of similarity (Slatkin and Hudson 1991; Slatkin 1993; Rousset 1997) and Slatkin's measure of M (Slatkin 1995) were also calculated for mtDNA. Microsatellite migration parameters were calculated using IAM (N_em: Hartl and Clark 1997) and SMM ($M = N_em$: Slatkin 1995) models. Correlation between genetic and geographical distances were tested using a Mantel test (1000 permutations) of both log-transformed and non-transformed data following Smouse et al. (1986) and implemented in Genalex. A sequential Bonferroni correction was used to adjust the significance level of multiple tests (Sokal and Rohlf 1995). Transformations did not affect the overall results. Therefore, only non-transformed km versus Φ_{ST}/ F_{ST}/ R_{ST} are presented here.

Metapopulation Structure

Predictions from the metapopulation models were tested by comparing estimates of genetic differentiation among older and younger populations in the northern, central and southern regions. In the northern region, variation in the population expansion times (based on mismatch analysis presented in Chapter 3) did not allow older and younger populations to be identified and an age gradient from older outer shelf locations to younger inner shelf locations was examined. In the central region Orpheus Island and Trunk Reef and in the southern region One Tree Island and Sykes Reef were identified as younger (Chapter 3). These locations were compared to older locations (central regions: Myrmidon and Pith Reefs, southern region: Polmaise and Broomefield Reefs).

Results

356 bases of the mtDNA control region I was obtained from a total of 283 individuals collected from 15 reefs. The average base frequencies were AT biased (A= 0.41, T=0.40, C=0.07, G=0.12) as commonly observed in fish mtDNA (Wolstenholme 1992; McMillan and Palumbi 1997). The ts/tv ratio was 1.53:1 for all samples combined. The

three microsatellites were amplified in 316 individuals. Linkage equilibrium was not rejected for the three microsatellites (AC33 vs. AC37: $\lambda^2 = 37.735$ df = 30 P = 0.157, AC33 vs. AC42: $\lambda^2 = 28.253$ df = 30 P = 0.557, AC37 vs. AC42: $\lambda^2 = 25.871$ df = 30 P = 0.682). Heterozygotes were less abundant than expected according to Hardy-Weinberg expectations in 17 of 45 locus-by-population comparisons (29 of 45 comparisons before Bonferroni correction), however, these departures were not confined to any of the populations or loci in particular (Table 2). Both models of microsatellite evolution were supported: the IAM model was only rejected in 1 and SMM in 6 out of 45 locus-by-population comparisons (Table 2). Significant genetic structure was detected among regions ($P < 0.0001$) and the strength of fixation differed among molecular markers and mutation models (Table 3). Fixation indices ranged from very high 0.81 (Φ_{ST}), lower 0.2 (R_{ST}) and low 0.07 (F_{ST}) suggesting that the genetic structure among regions differed among the molecular markers used.

For mtDNA, most of the variation occurred among regions (81.2%), whereas, for microsatellites much less variation occurred at this spatial scale (IAM: 7.3 %, SMM: 19.5 %) and more variation was found within populations (IAM: 85.3 %, SMM: 70.3 %) (Table 3). Significant structure could be attributed to shelf position only in the northern region based on mtDNA ($\Phi_{ST} = 0.31$, $P < 0.001$, c.f. microsatellites: $F_{ST} = 0.04$, P = 0.06, $R_{ST} = 0.04$, P = 0.14). Significant shelf-position effects were evident in the central region based on microsatellites ($R_{ST} = 0.11$ P = 0.015) but not mtDNA ($\Phi_{ST} = 0.097$ P = 0.17) (Table 4).

Table 2: Observed (H_O) and expected (H_E) heterozygosity, probability test of heterozygote deficit (λ^2 (df)), P and Bonferroni corrected significance probability (α), probability of departure from the Infinite Allele Model (IAM) and Stepwise Mutation (SMM) models and their associated Bonferroni corrected significance probabilities (α) of the three microsatellite loci among 15 populations of *Acanthochromis polyacanthus* on the Great Barrier Reef.

				Heterozygote excess			IAM		SMM	
Location	Locus	H_O	H_E	λ^2 (df)	P	α	P	α	P	α
Day Reef	AC37	0.708	0.845	105.8 (78)	0.02	0.002	0.471	0.002	0.051	0.002
	AC42	0.833	0.944	385.7 (325)	0.012	0.002	0.429	0.004	0.250	0.004
	AC33	0.625	0.737	32.94 (21)	0.047	0.003	0.340	0.003	0.208	0.003
Yonge Reef	AC37	0.636	0.753	62.22 (28)	0.000	0.001	0.466	0.02	0.097	0.002
	AC42	0.636	0.777	134.6 (55)	0.000	0.001	0.255	0.03	0.004	0.001
	AC33	0.636	0.755	16.31 (15)	0.362	0.005	0.110	0.05	0.413	0.007
Lizard Island	AC37	0.778	0.764	47.25 (45)	0.381	0.006	0.204	0.002	0.012	0.001
	AC42	0.722	0.944	313.0 (253)	0.006	0.001	0.243	0.002	0.537	0.02
	AC33	0.778	0.776	38.72 (45)	0.734	0.01	0.271	0.002	0.007	0.001
North Direction	AC37	0.542	0.774	130.8 (55)	0.000	0.001	0.249	0.002	0.006	0.001
	AC42	0.625	0.928	263.3 (190)	0.000	0.002	0.115	0.002	0.628	0.05
	AC33	0.958	0.845	44.50 (66)	0.981	0.05	0.396	0.002	0.100	0.002
Linnet Reef	AC37	0.783	0.891	105.3 (105)	0.475	0.006	0.278	0.001	0.26	0.004
	AC42	0.522	0.940	381.7 (253)	0.000	0.001	0.128	0.001	0.567	0.025
	AC33	0.739	0.713	61.71 (21)	0.000	0.001	0.439	0.002	0.119	0.002
Martin Reef	AC37	0.792	0.833	48.66 (55)	0.714	0.01	0.325	0.002	0.139	0.002
	AC42	0.792	0.944	317.0 (276)	0.045	0.003	0.086	0.002	0.468	0.013
	AC33	0.458	0.703	38.12 (21)	0.012	0.002	0.488	0.002	0.070	0.002
Myrmidon Reef	AC37	0.826	0.870	109.5 (66)	0.001	0.002	0.139	0.002	0.375	0.006
	AC42	0.870	0.862	186.7 (136)	0.003	0.002	0.163	0.002	0.003	0.001
	AC33	0.435	0.580	54.24 (15)	0.000	0.001	0.296	0.002	0.030	0.002
Pith Reef	AC37	0.571	0.715	94.662 (55)	0.001	0.001	0.062	0.003	0.003	0.001
	AC42	0.905	0.934	249.760 (231)	0.189	0.003	0.33	0.004	0.31	0.004
	AC33	0.286	0.323	1.340 (6)	0.696	0.03	0.173	0.004	0.02	0.001

Table 2: Continued

				Heterozygote excess			IAM		SMM	
Location	Locus	H_O	H_E	λ^2 (df)	P	α	P	α	P	α
Trunk Reef	AC37	0.333	0.700	53.850 (21)	0.000	0.001	0.329	0.008	0.048	0.002
	AC42	1.000	0.920	177.000 (153)	0.090	0.003	0.466	0.01	0.211	0.003
	AC33	0.4	0.429	16.116 (6)	0.013	0.002	0.266	0.01	0.056	0.002
Britomart Reef	AC37	0.833	0.851	74.258 (55)	0.043	0.003	0.177	0.001	0.313	0.005
	AC42	0.917	0.943	253.667 (253)	0.476	0.007	0.056	0.001	0.34	0.005
	AC33	0.292	0.369	24.026 (3)	0.000	0.001	0.494	0.001	0.214	0.003
Orpheus Island	AC37	0.870	0.854	38.671 (55)	0.953	0.02	0.155	0.002	0.359	0.006
	AC42	0.826	0.911	205.949 (171)	0.035	0.003	0.454	0.003	0.102	0.002
	AC33	0.217	0.553	85.458 (36)	0.000	0.001	0.037	0.003	0.000	0.001
Polmaise Reef	AC37	0.769	0.698	23.111 (21)	0.338	0.004	0.294	0.004	0.049	0.002
	AC42	0.692	0.787	76.349 (45)	0.002	0.002	0.163	0.005	0.019	0.001
	AC33	0.154	0.500	9.030 (3)	0.029	0.002	0.366	0.005	0.419	0.008
Broomefield Reef	AC37	0.917	0.845	44.499 (45)	0.493	0.008	0.109	0.001	0.451	0.01
	AC42	0.708	0.799	56.154 (36)	0.017	0.002	0.311	0.001	0.185	0.003
	AC33	0.042	0.376	48.018 (6)	0.000	0.001	0.261	0.001	0.037	0.002
One Tree Island	AC37	0.722	0.832	50.604 (45)	0.262	0.004	0.314	0.003	0.199	0.003
	AC42	0.833	0.926	251.520 (190)	0.002	0.002	0.537	0.003	0.182	0.002
	AC33	0.500	0.637	22.926 (21)	0.348	0.005	0.208	0.003	0.01	0.001
Sykes Reef	AC37	0.774	0.770	48.331 (45)	0.341	0.004	0.464	0.006	0.023	0.002
	AC42	0.806	0.922	275.629 (210)	0.002	0.002	0.204	0.006	0.216	0.003
	AC33	0.290	0.674	134.038 (45)	0.000	0.001	0.127	0.007	0.003	0.001

Table 3: Analysis of Molecular Variance based on a) mtDNA control region, b) the Infinite Allele Model (IAM) and c) the Stepwise Mutation Model (SMM) of three microsatellite loci among three regions (North, Central and South) of *Acanthochromis polyacanthus* on the Great Barrier Reef. V = Variance component, % = percent variation explained, fixation = fixation index (mtDNA = Φ_{ST}, IAM = F_{ST} and SMM = R_{ST}) and P = significance.

	Among regions				Among populations within regions				Within populations			
	V	%	Fixation	P	V	%	Fixation	P	V	%	Fixation	P
a) mtDNA	17.99	81.21	0.812	<0.0001	1.93	8.71	0.463	<0.0001	2.23	10.0.8	0.90	<0.0001
b) IAM	0.101	7.24	0.072	<0.0001	0.104	7.51	0.081	<0.0001	1.183	85.26	0.147	<0.0001
c) SMM	189.7	19.45	0.195	<0.0001	99.95	10.24	0.127	<0.0001	685.9	70.31	0.297	<0.0001

Table 4: Analysis of Molecular Variance, fixation indices and significance based on a) mtDNA control region, b) the Infinite Allele Model (IAM) and c) the Stepwise Mutation Model (SMM) of three microsatellite loci among inner, mid and outer shelf locations in the northern and central regions of the Great Barrier Reef. V = Variance component, % = percent variation explained, fixation = fixation index (mtDNA = Φ_{ST}, IAM = F_{ST} and SMM = R_{ST}) and P = significance.

	Among shelves				Among populations within shelves				Within populations			
	V	%	Fixation	P	V	%	Fixation	P	V	%	Fixation	P
a) mtDNA:												
Northern	2.677	31.10	0.311	<0.001	1.625	18.87	0.274	<0.001	4.307	50.03	0.450	<0.001
Central	0.127	9.68	0.097	0.169	0.442	33.72	0.373	<0.001	0.742	56.60	0.434	<0.001
b) IAM:												
Northern	0.059	4.320	0.043	0.063	0.034	2.500	0.026	<0.001	1.268	93.18	0.068	<0.001
Central	0.113	8.860	0.089	0.064	0.049	3.820	0.042	<0.001	1.114	87.32	0.127	<0.001
c) SMM:												
Northern	36.36	3.630	0.036	0.138	107.3	10.73	0.111	<0.001	857.1	85.64	0.144	<0.001
Central	81.89	11.28	0.113	0.015	4.857	0.67	0.008	0.376	639.1	88.05	0.120	<0.001

Pairwise genetic distances among populations differed among markers and mutational models (Table 5) but were similar among regions (Table 6). Φ_{ST} values were significantly greater than 0 in more than 97% of all pairwise comparisons. Nuclear pairwise genetic distances were generally less than half those estimated by mtDNA and were statistically significant in 72% of comparisons (83.2% before Bonferroni correction) although this varied between mutational models (significant comparisons R_{ST} = 52% (71.4% before Bonferroni correction), F_{ST} = 91.5 % (97.1% before Bonferroni correction)). There was no consistent geographical pattern in the variation between models with F_{ST} indicating higher fixation in 37.3, 53.3 and 68% of northern, central and southern comparisons than comparable R_{ST} estimates. (Table 5).

Results of the isolation-by-distance analyses were largely congruent with those of the AMOVAs. Significant correlations between geographical and genetic distance were only evident at the largest spatial scale, i.e. among regions (Φ_{ST} vs. km: r = 0.77 P = 0.001; F_{ST} vs. km: r = 0.46 P = 0.001; R_{ST} vs. km: r = 0.53 P = 0.002 Fig. 2). Genetic and geographic distances did not correlate within regions using any of the genetic markers or distance measures ($P > 0.05$ in all cases, unpublished data).

The metapopulation propagule-pool model was supported in all three regions (Table 7). Fixation indices were higher among younger populations compared to older ones in all regions when based on mtDNA (Table 7). Fixation indices based on both microsatellite models were higher among younger populations in the central region, but not in the northern or southern regions. In both these regions, a large proportion of the pairwise genetic distances based on microsatellites were not significantly different from 0 and this lack of genetic structure may have affected this comparison.

There was substantial variation in migration rates among populations, regions and markers (Fig. 3). Migration rates based on mtDNA were generally low ($4N_eM$ mostly < 1) and reciprocal rates (i.e., $4N_eM$ (a to b) vs. $4N_eM$ (b to a) were significantly different in 26.7 % of northern, 40% of central and 66.7% of southern pairwise comparisons (Fig. 3). Migration rates based on microsatellites were generally higher (mostly ranging from 1 – 4) and significant reciprocal pairwise differences were less common (North = 6.7%, Central = 10% and South = 16.7%).

Table 5: Pairwise genetic distances among all sampling locations. Pairwise Φ_{ST} estimates are presented above the diagonal, and F_{ST} and R_{ST} estimates are presented below it. Location abbreviations follow Table 1.

		DAY	YON	LIZ	NDR	LIN	MAR	MYR	PIT	TRU	BRI	ORP	OTI	SYK	POL	BRO
		Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}
DAY		x	0.146	0.053	0.487	0.414	0.814	0.574	0.507	0.612	0.552	0.587	0.957	0.947	0.941	0.949
			*	ns	**	**	**	**	**	**	**	**	**	**	**	**
		x														
YON	F_{ST}	0.042	x	0.14	0.545	0.482	0.863	0.655	0.561	0.707	0.621	0.622	0.977	0.971	0.966	0.971
		ns		*	**	**	**	**	**	**	**	**	**	**	**	**
	R_{ST}	0.345	x													
		**														
LIZ	F_{ST}	0.062	0.096	x	0.263	0.201	0.65	0.407	0.344	0.427	0.373	0.403	0.913	0.895	0.886	0.901
		**	**		*	*	**	**	**	**	**	**	**	**	**	**
	R_{ST}	0.267	0.134	x												
		**	**ns**													
NDR	F_{ST}	0.067	0.098	0.011	x	0.034	0.214	0.592	0.559	0.594	0.579	0.579	0.882	0.858	0.847	0.867
		**	**	ns		ns	**	**	**	**	**	**	**	**	**	**
	R_{ST}	0.308	0.213	-0.014	x											
		**	**	ns												

Table 5: Continued

		DAY	YON	LIZ	NDR	LIN	MAR	MYR	PIT	TRU	BRI	ORP	OTI	SYK	POL	BRO
		Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}
LIN	F_{ST}	0.041 **	0.072 **	0.084 **	0.081 **	x	0.33 **	0.541 **	0.508 **	0.544 **	0.528 **	0.544 **	0.882 **	0.859 **	0.849 **	0.868 **
	R_{ST}	0.156 **ns**	0.027 ns	0.069 **ns**	0.127 **ns**	x										
MAR	F_{ST}	0.021 **ns**	0.053 *	0.074 **	0.078 **	0.024 **ns**	x	0.88 **	0.862 **	0.889 **	0.881 **	0.884 **	0.954 **	0.943 **	0.935 **	0.944 **
	R_{ST}	0.141 **ns**	0.053 ns	0.081 **ns**	0.134 **ns**	-0.018 ns	x									
MYR	F_{ST}	0.087 **	0.142 **	0.122 **	0.122 **	0.057 **	0.077 **	x	0.357 **	0.616 **	0.406 **	0.584 **	0.987 **	0.982 **	0.977 **	0.981 **
	R_{ST}	0.323 **	0.007 ns	0.08 **ns**	0.149 **	0.0 ns	0.015 ns	x								
PIT	F_{ST}	0.137 **	0.186 **	0.191 **	0.197 **	0.119 **	0.093 **	0.066 **	x	0.255 **	0.053 ns	0.384 **	0.98 **	0.975 **	0.97 **	0.975 **
	R_{ST}	0.429 **	0.009 ns	0.185 **	0.262 **	0.04 ns	0.056 ns	0.009 ns	x							
TRU	F_{ST}	0.118 **	0.168 **	0.18 **	0.173 **	0.101 **	0.079 **	0.079 **	0.019 ns	x	0.352 **	0.689 **	0.993 **	0.989 **	0.983 **	0.987 **

Table 5: Continued

		DAY	YON	LIZ	NDR	LIN	MAR	MYR	PIT	TRU	BRI	ORP	OTI	SYK	POL	BRO
		Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}
TRU	R_{ST}	0.321 **	-0.01 ns	0.115 **ns**	0.188 **	0.001 ns	0.014 ns	-0.017 ns	-0.023 ns	x						
BRI	F_{ST}	0.109 **	0.153 **	0.173 **	0.170 **	0.082 **	0.075 **	0.052 **	0.026 **ns**	0.014 ns	x	0.389 **	0.988 **	0.984 **	0.979 **	0.983 **
	R_{ST}	0.199 **	0.013 ns	0.096 ns	0.166 **	-0.016 ns	-0.004 ns	0.005 ns	0.046 **	0.002 ns	x					
ORP	F_{ST}	0.116 **	0.163 **	0.079 **	0.096 **	0.130 **	0.142 **	0.122 **	0.212 **	0.216 **	0.199 **	x	0.989 **	0.985 **	0.98 **	0.984 **
	R_{ST}	0.589 **	0.158 **	0.209 **	0.263 **	0.236 **	0.267 **	0.196 **	0.214 **	0.185 **ns**	0.251 **	x				
OTI	F_{ST}	0.109 **	0.148 **	0.06 **	0.069 **	0.126 **	0.133 **	0.135 **	0.22 **	0.211 **	0.198 **	0.062 **	x	0.459 **	0.085 *	0.59 **
	R_{ST}	0.595 **	0.235 **ns**	0.281 **	0.33 **	0.309 **ns**	0.335 **	0.282 **	0.273 **	0.244 **ns**	0.325 **	0.037 ns	x			
SYK	F_{ST}	0.136 **	0.179 **	0.099 **	0.144 **	0.136 **	0.158 **	0.132 **	0.218 **	0.227 **	0.205 **	0.049 **	0.021 **ns**	x	0.15 *	0.099 *

Table 5: Continued

		DAY	YON	LIZ	NDR	LIN	MAR	MYR	PIT	TRU	BRI	ORP	OTI	SYK	POL	BRO
		Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}	Φ_{ST}
SYK	R_{ST}	0.76	0.437	0.489	0.521	0.494	0.518	0.502	0.516	0.479	0.519	0.172	-0.007	x		
		**	**	**	**	**	**	**	**	**	**	**	ns			
POL	F_{ST}	0.182	0.222	0.136	0.119	0.186	0.192	0.189	0.276	0.268	0.241	0.108	0.107	0.141	x	0.297
		**	**	**	**	**	**	**	**	**	**	**	**	**		**
	R_{ST}	0.516	0.116	0.097	0.134	0.144	0.166	0.10	0.144	0.107	0.172	0.017	0.095	0.315	x	
		**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	ns	**ns**	ns	ns	**		
BRO	F_{ST}	0.175	0.212	0.099	0.107	0.188	0.192	0.191	0.276	0.277	0.257	0.086	0.057	0.092	0.063	x
		**	**	**	**	**	**	**	**	**	**	**	*	**	**ns**	
	R_{ST}	0.681	0.255	0.266	0.307	0.293	0.317	0.269	0.307	0.268	0.333	0.022	0.08	0.306	0.01	x
		**	**	**	**	**	**	**	**	**	**	ns	ns	**	ns	

Significant of comparisons indicated as follows: ** = $P < 0.001$, * = $0.05 < P$, ns = non-significant comparisons (in bold if insignificant following Bonferroni correction)

Table 6: Average genetic differentiation within regions among markers and mutational models

Region	Mean Φ_{ST} (95% CI)	Mean F_{ST} (95% CI)	Mean R_{ST} (95% CI)
North	0.38 (0.13)	0.06 (0.01)	0.13 (0.05)
Central	0.41 (0.11)	0.10 (0.05)	0.09 (0.07)
South	0.28 (0.17)	0.08 (0.03)	0.13 (0.11)

Table 7: Strength of genetic differentiation among older and younger populations. Location abbreviations follow Table 1.

Marker	Older	Younger	Youngest	Support
Northern region	DAY-YON	LIZ–NDR	MAR-LIN	
Φ_{ST}	0.146	0.236	0.33	Yes
F_{ST}	0.042	0.011	0.024	No
R_{ST}	0.345	-0.014	-0.02	No
Central region	PIT-MYR		ORP-TRU	
Φ_{ST}	0.357		0.689	Yes
F_{ST}	0.066		0.216	Yes
R_{ST}	0.009		0.185	Yes
Southern region	POL-BRO		SYK-OTI	
Φ_{ST}	0.297		0.459	Yes
F_{ST}	0.063		0.021	No
R_{ST}	0.01		-0.001	No

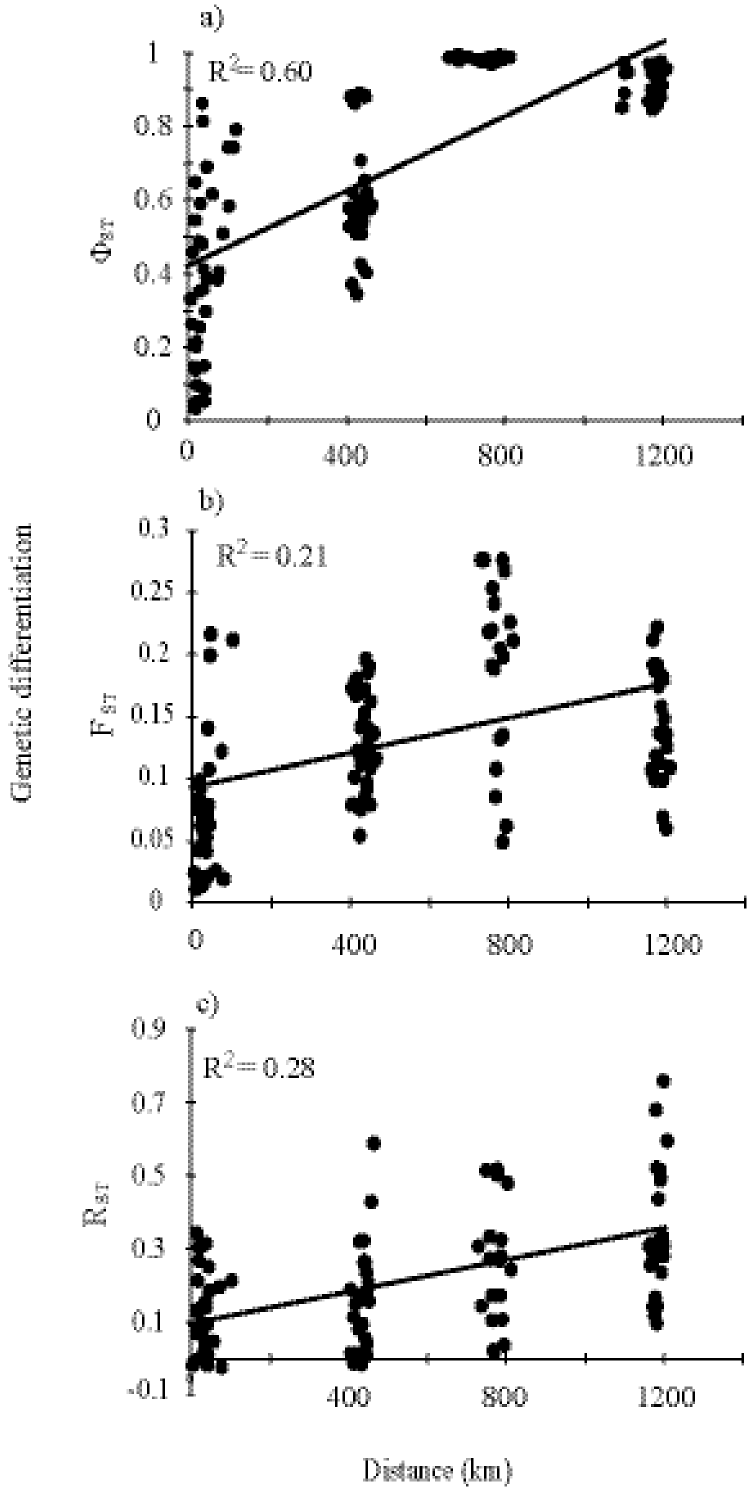

Fig. 2: Relationship between genetic differentiation and linear distance based on a) mtDNA (Φ_{ST} = 0.00051 (0.00043 – 0.00058)km + 0.41 (0.36 – 0.47) b) microsatellites (IAM) (F_{ST} = 0.00007 (0.000044 – 0.000096)km + 0.09 (0.07 – 0.11) and c) microsatellites (SMM) (R_{ST} = 0.00022 (0.00015 – 0.00029)km + 0.087 (0.04 – 0.13).

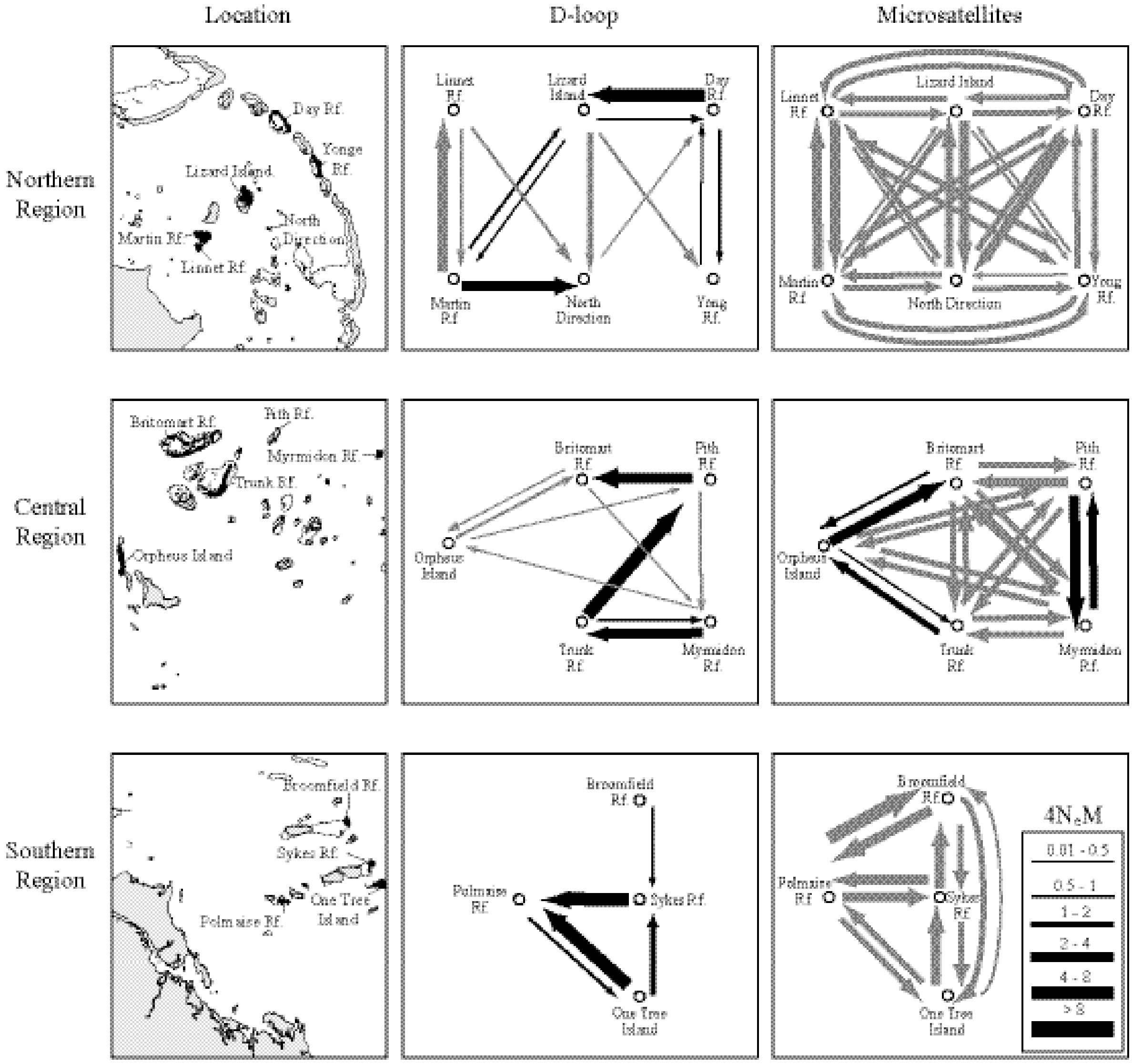

Fig. 3: Reciprocal migration rates (4N$_e$m) among reefs in the northern, central and southern regions estimated for the mtDNA (Control region) and the microsatellites. The thickness of the arrows indicate the migration rates and the colour indicates statistical difference between reciprocal migration rates (black = 95% confidence intervals of estimates did not overlap; grey = 95% confidence intervals of estimates overlapped).

For both markers, the frequency of significantly different migration rates between populations increased in a north – south direction and all regions were characterised by one or two mitochondrial migration rates being significantly higher (4N$_e$m ~ 4) than all other estimates. Migration rates based on mtDNA and microsatellites were significantly different in 80% of northern, 75% of central and 58.3% of southern comparisons (Fig. 3). The microsatellites consistently indicated higher migration rates (north: 91.6%, central: 93.3 %, south: 71.4%) compared to those estimated based on mtDNA, although higher mtDNA migration rates were occasionally found (north: 8.3%, central: 6.7%, south: 28.8%).

Reductions in microsatellite sample sizes by one third and one sixth, to make them comparable to the sample size used for the mtDNA (i.e., one marker, one haploid marker) did not substantially affect estimates of migration (although variances increased as expected) (Appendix 1). When significant differences occurred there was no consistent pattern in which data set indicated higher or lower migration rates (Appendix 1).

Discussion

This study revealed strong genetic structure among populations of *A. polyacanthus* on the GBR. There were substantial differences in the spatial structure and migration rates within and among regions and molecular markers. This suggests that the genetic structure of this species is complex and that understanding the evolution of the structure revealed here will require knowledge of the operation of processes operating in a scale-dependent fashion.

Genetic structure among regions

Strong genetic structure among northern, central and southern regions of the GBR was revealed by analyses of both mtDNA and nuclear markers. This pattern conforms to previous findings for this species of strong structure between northern and southern regions based on allozyme markers (Doherty et al. 1994; Planes et al. 2001) and indicates the presence of further strong structuring among bi-coloured morphs between northern and central locations. My analyses of both mitochondrial and nuclear markers indicated that this structure followed an isolation-by-distance model of dispersal where genetic exchange is more likely among neighbouring locations (Fig. 2). Consequently, at this spatial scale, *A. polyacanthus* does not appear to function as a metapopulation. While patterns of isolation-by-distance have been reported at large spatial scales in marine organisms (e.g., Palumbi et al. 1997; Planes and Fauvelot 2002) this study reports one of very few examples of such dynamics across small spatial scales (see also Planes et al. 1996). The relationships between geographical and genetic distances among these populations varied within and among markers, but in general, the within population divergence (i.e., intercepts) estimated by the IAM and SMM models were similar, and less than that estimated from mtDNA. Genetic divergence accumulated more quickly with distance (i.e., slopes) when estimated using the mtDNA compared to microsatellites (Fig. 2). This difference would be expected given the slower fixation rates of co-dominant compared to haploid markers and indicates that this may be occurring here (see below).

Genetic structure within regions

Strong genetic structuring was revealed within all three regions (Table 5). This variation was attributable to shelf position for the mtDNA marker in the northern region and for the microsatellite data under the SMM model in the central region (Table 4). No evidence of isolation-by-distance was found within either the northern or the central region. Therefore, although initial colonisation of the continental shelf may have occurred from the outer to inner continental shelf in the northern and central regions, other processes have erased any signature of this process in these genetic markers. Genetic differentiation among populations within regions was similar among regions (Table 6) and generally very high particularly in the mtDNA analyses (Table 5). For example, Lizard Island (LIZ) and North Direction Island (NDR) are separated by less than 10 km but have a Φ_{ST} value of 0.26 and Martin Reef (MAR) and Linnet Reef (LIN) are separated by less than 6 km and have a Φ_{ST} value of 0.33. Such differentiation is among the highest recorded for any coral reef fish at such small spatial scales (e.g., Doherty et al. 1995; Dudgeon et al. 2000; Chapter 5) and is comparable to values obtained in other studies of direct-developing reef fishes at similar spatial scales (Bernardi 2000; Bernardi and Vagelli 2004; Hoffman et al. 2005; van Herwerden and Doherty 2006). This finding suggests that the spatial patterns described by this study may be broadly applicable to direct developing reef fish species.

The propagule-pool model was supported in all comparisons dominated by pairwise fixation indices significantly greater than 0 (Table 7). The strength of fixation indices increased from older to younger populations in these comparisons, however, sample sizes did not permit statistical testing of these results (Table 7). These results add to only a handful of empirical investigations that have explored the predictions of these models (reviewed by Giles and Goudet 1997). The majority of these previous investigations reported that younger populations displayed greater genetic differentiation than older ones (but see Dybdahl 1994) which is congruent with the results of this study.

Asymmetric migration rates

There were considerable asymmetries in migration rates among locations based on mtDNA data. All regions were characterised by high frequencies of significantly different reciprocal migration rates and one or two migration rates that were much greater than the rest (Fig. 3). Insignificant pairwise Φ_{ST} values (based on mtDNA) were often (e.g., Day – Lizard Island; Britomart – Pith) but not always (e.g. North Direction – Linnet) associated with significantly asymmetric migration rates. Conversely, higher and asymmetric migration rates were occasionally detected between locations with low but significant genetic structuring (e.g., North Direction – Martin; One Tree Island – Polmaise; Sykes - Polmaise). Consequently, migration rates based on mtDNA data were complex and gene flow occurred, although generally at low rates, both uni- and bi-directionally among the sampled locations. Examples of asymmetric migration rates based on genetic evidence are emerging (e.g., Fraser et al. 2004) and emphasise the potential role of such variation in the dynamics of metapopulations (Hanski and Gilpin 1997; Stacey et al. 1997). In contrast, migration rates based on the microsatellites were generally high, mostly symmetrical and uniform among locations. There were no clear differences in migration rates between locations with significant genetic structure compared to those without. These results suggest that migration patterns may be sex-biased (discussed below).

Differences among markers

In general, patterns of lower genetic differentiation and higher migration rates among populations, shelf position and regions were estimated for nuclear as compared to mtDNA markers. Such differences in estimates of fixation may arise due to differences in the levels of heterozygosity between markers (Hedrick 1999, 2005). In addition at least another three processes may have contributed to this difference between the results obtained with these two different classes of markers.

Differences in migration rates and genetic structure between markers may have been due to the larger sample sizes of the microsatellites (3 loci, 2 alleles per locus) compared to the mtDNA (1 allele, 1 locus). However, substantial reduction of the microsatellite sample size did not materially change the migration rates estimated from them (although variances did increase substantially) (Appendix 1). It is, therefore, unlikely that these observed differences were due to sampling issues alone.

Slower fixation rates of neutral nuclear markers compared to mitochondrial ones because of greater effective population sizes (Birky et al. 1989; Neigel 1997, 2002; Balloux et al. 2000) may have contributed to the patterns observed here. This difference in fixation rates may also explain the pattern of pairwise genetic distances in the northern region where there was a westward trend of decreasing difference coinciding with the presumed age of the populations. Consequently, slower fixation of nuclear markers may have affected estimates of migration. The significant structure found by the microsatellites in a large proportion of the comparisons (75%), however, indicates that other processes may also be operating. It is also possible that the low number of loci, high heterozygosity and moderate sample sizes did not permit accurate estimation of the population genetic structure. If so, genetic differentiation estimates from all populations should be equally affected (assuming equal N_e and genetic diversity among populations). Levels of genetic structure varied greatly, from very high to very low, among populations separated by approximately equal distances. It is, therefore, unlikely that differences in fixation rates alone were responsible for the observed pattern.

The third possibility is that the higher migration rates estimated based on microsatellites compared to mtDNA is the results of male-biased dispersal. Sex-biased dispersal may evolve where there are differential fitness consequences with respect to sex associated with acquiring and defending reproductive resources such as territorial space (e.g., Greenwood 1980; Clarke et al. 1997). Dispersal tends to be female biased in birds (Greenwood 1980) and male biased in mammals (Dobson 1982) and fishes (Hutchings and Gerber 2002; Fraser et al. 2004). Differences in pairwise genetic differentiation and migration rates observed here suggest that male-biased dispersal is occurring in this species. If so, this study provides the first documented example of sex-biased dispersal in coral-reef fishes. However, until the effects of male-biased dispersal and slower fixation of microsatellites can be separated unequivocally some uncertainty of this interpretation remains.

Differences among mutational models

Pairwise genetic estimates of R_{ST} and F_{ST} among populations differed greatly (Table 5) despite apparent conformity of the data with the assumptions of both models (Table 2). While it is not always possible to evaluate which statistic provides a better estimate (Balloux and Goudet 2002), R_{ST} calculations incorporate more biologically realistic assumptions by taking into account the evolutionary relationships among alleles (Estoup and Cornuet 1999), and may provide better estimates when mutation contributes significantly to allelic differences between populations (Goldstein et al. 1995; Pérez-Lezaun et al. 1997). R_{ST} estimates, however, may also be associated with greater variances, especially if analyses are based on a low number of loci (Nei 1995; Takezaki and Nei 1996; Neigel 2002). In contrast, F_{ST} may provide a potentially biased estimate (because the effects of migration and mutation cannot be separated) but are typically associated with less error (Balloux and Goudet 2002). My results are consistent with these patterns; F_{ST} estimates were generally smaller but less variable than R_{ST} estimates (Table 5). While an extensive analysis of which mutational model better describes the data is beyond the objectives of the current study, the high mutation rates of microsatellites may have affected estimated levels of fixation here and 'true' fixation levels are probably somewhere between those estimated by both models, that is, low but in many instances significantly greater than zero.

Conclusion

The population genetic structure of *A. polyacanthus* on the GBR contained significant scale-dependent variation consistent with isolation-by-distance and metapopulation models. Within regions, there was high levels of population structure and low and asymmetric migration. While the population structure of *A. polyacanthus* on the GBR strongly suggests that this species displays metapopulation dynamics, a crucial distinction between such a genetic system and others including island (Wright 1931) and patchy population models (Harrison 1991; Planes et al. 1996) lie in the occurrence and frequency of local extinctions. I examine the evidence for population bottlenecks and extinctions in a subsequent investigation of the genetic structure of this species (Chapter 3).

Chapter 3: Genetic diversities, demographic bottlenecks and coalescence times in a marine metapopulation.

Publication: Bay LK Crozier RH and Caley MJ (In Review) Genetic diversities, demographic bottlenecks and coalescence times in a marine metapopulation. Journal of Evolutionary Biology

Abstract

Metapopulation dynamics are often invoked to explain the complex genetic structure and the evolution of distributional borders of species living in naturally fragmented ecosystems. The habitats of coral reef fishes are highly fragmented, but the evolution of their spatial genetic structure has rarely been examined in a metapopulation context. Here I use a mtDNA sequence marker (control region, n = 296) and three microsatellite loci (n = 316) to examine the evolution of spatial genetic structure in an abundant species of coral reef fish with direct development (*Acanthochromis polyacanthus*). I examine patterns of genetic diversity, historical demography, including local population reduction and/or extinctions, and coalescence times among populations and regions of this species on the Great Barrier Reef (GBR), Australia. Genetic diversities, mismatch and coalescence analyses all identified large variation in the demographic histories in this species among populations and regions. Evidence of genetic bottlenecks was detected by mismatch analysis in the majority of populations sampled (mismatch means = 0.7 – 12.3). In most populations, these bottlenecks appeared to be relatively old since genetic diversities (e.g. $h > 0.6$) and coalescence based population growth estimates ($g < 1000$) did not indicate recent genetic bottlenecks. In contrast, three populations displayed low genetic diversities (e.g., $h < 0.6$) and large population growth rates ($g > 1500$) indicating more recent genetic bottlenecks. Reductions in genetic diversities of local populations resulted in overall lower genetic diversity (e.g., $h = 0.83$, $\pi = 0.007$) and a higher regional expansion rate ($g = 1936$) in the southern region located towards a geographic margin of this species. These results suggest that *A. polyacanthus* exists as a metapopulation within regions on the GBR and that local populations experience periodic genetic bottlenecks and/or extinctions. These fluctuations in local populations have the potential to affect the evolution of the metapopulation and geographical range of this species.

Introduction

Metapopulation theory is often invoked to explain the evolution of spatial genetic structure of populations and the geographical limits of species (Lennon et al. 1997; Holt and Keitt 2000). Many models based on spatially structured arrays of ephemeral populations that interact and persist through time via migration, extinction and re-colonization have been developed to describe such systems (Levins 1970; Hanski and Gilpin 1997; Hanski and Simberloff 1997). Models of metapopulations also often incorporate additional detailed information about fluctuations in population size (Lande et al. 1998). Therefore, within metapopulations, broadly defined, local populations may experience fluctuations in size ranging from small and transient changes to local extinction, and such fluctuations should increase towards the distributional margin of species (Lennon et al. 1997; Holt and Keitt 2000; Holt et al. 2005). The effects of these metapopulation dynamics on spatial genetic structure have typically been measured in terms of genetic differentiation among populations, but may also be evident in patterns of genetic diversity within local populations (Pannell and Charlesworth 1999, 2000; Pannell 2003). As such, important information about the role of local extinctions in a metapopulation and its importance in determining the species range may be gained by examining patterns of genetic diversity, demographic history and genetic differentiation in sets of local populations.

In general, metapopulation dynamics should reduce total genetic diversity (π_T) at the level of the metapopulation and genetic diversity within the sub-populations (π_S) making up a metapopulation compared to a panmictic population equal in size to the metapopulation. The relative magnitude of this difference, however, may vary greatly depending on the frequency and intensity of effective population size reductions among the sub-populations, and the mode of subsequent re-colonization and migration (Pannell and Charlesworth 1999 and references therein). For example, reductions in genetic diversities may be large where reductions in effective size of sub-populations is frequent and large, and if re-colonisation obeys a propagule-pool model, where colonisers originate from a single population. Coalescence times within sub-populations are also reduced under this scenario because of genetic bottlenecks associated with propagule colonisation (Pannell and Charlesworth 1999). If sub-populations experience minor fluctuations in population size, or if colonisation obeys a migrant-pool model, where colonisers originate from a range of populations, sub-populations may not experience genetic bottlenecks and π_S and π_T may not be affected to a measurable extent (Pannell and Charlesworth 1999, 2000; Pannell 2003).

Coral reef fishes generally display high levels of gene flow, low population genetic structure and large effective population sizes (Palumbi 1994). Consequently, many marine fishes are characterised by relatively shallow population genetic structures with coalescence times indicating the presence of genetic bottlenecks often associated with Pleistocene climate variation (reviewed by Grant and Bowen 1998; Fauvelot et al. 2003). With the exception of a single study, information on demographic bottlenecks and their effects on coalescence times in coral reef fishes are restricted to interspecific comparisons (e.g., Dudgeon et al. 2000; Fauvelot et al. 2003; but see van Herwerden and Doherty 2006). Consequently, we do not have a good understanding of metapopulation dynamics in coral reef fishes.

Coral reef fish species with low dispersal and high genetic structure are the mostly likely to display metapopulation dynamics, and thereby provide opportunities to examine the roles of local extinction and demographic bottlenecks in natural marine systems. One such species is *Acanthochromis polyacanthus.* This species is unusual among coral reef fishes in not having a pelagic larval stage. Instead, it rears its broods within parental territories (Thresher 1985; Kavanagh 2000). Dispersal, which occurs most commonly during the larval phase in coral reef fishes, is restricted in this species and strong genetic and morphological (colouration) differentiation is apparent among reefs and regions (Doherty et al. 1994; Planes et al. 2001; van Herwerden and Doherty 2006; Chapter 2). Populations of *A. polyacanthus* on the Great Barrier Reef (GBR) operate as a metapopulation at intermediate spatial scales (within regions) (Chapter 2). At this spatial scale, populations display high genetic structure in both mtDNA and microsatellite markers. Populations are characterised by low and asymmetric migration and colonisation appears to conform to a propagule-pool model (Chapter 2). Genetic bottlenecks may therefore play an important role through founder effects in producing the strong genetic differentiation observed among reefs in this species (Planes et al. 2001; van Herwerden and Doherty 2006). However, genetic variabilities and demographic histories of this species have only been examined at the regional level. These previous studies suggest that the populations in the southern region of the GBR display lower genetic diversities than in the central and northern regions of the reef when estimated by both allozymes (Doherty et al. 1994) and mtDNA markers (van Herwerden and Doherty 2006) but more ancient population expansion times (van

Herwerden and Doherty 2006). To date, however, analyses of the population genetic structure of this species have been restricted to outer shelf locations on the Great Barrier Reef, offshore reefs, and northern and southern hybrid zones (Planes et al. 2001; van Herwerden and Doherty 2006). Consequently, the genetic structure of the populations of this species remains unexplored throughout large parts of its range on the GBR (but see Chapter 2).

Here I examine patterns of coalescence and demographic bottlenecks within and among regions on the Great Barrier Reef based on both mtDNA and nucDNA markers. I examine the phylogenetic structure of *A. polyacanthus* populations sampled across the continental shelf in three regions on the Great Barrier Reef using a maximum likelihood approach. I then examine the evidence for local population size reductions and/or extinctions by examining genetic diversities among populations, regions and phylogenetic clades. Lastly, I employ two different methodologies, mismatch analysis and a maximum likelihood coalescence approach, to examine the demographic histories of populations and regions. I then interpret these results in the context of metapopulation dynamics at the regional level.

Methods and Materials

Sampling and Laboratory Procedures

A total of 17 populations of *A. polyacanthus* from 3 regions of the GBR, north, central and south, and three individuals from the Solomon Islands were sampled during 2000 – 2003 following the methods outlined in Chapter 2 (Fig. 1). The latitudinal range of *A. polyacanthus* extends from the southern Philippines (10°N) to southern Queensland, Australia (26°S) and the southern region was therefore close to the southern distributional margin of this species. Limited sample sizes were obtained for two other locations (Great Keppel Island n = 10 and Solomon Island n = 3). These two locations were included in the phylogenetic analysis but excluded from all other analyses. DNA was extracted and 356 base pairs of the mtDNA hyper variable control region 1 and three microsatellite loci (Miller-Sims et al. 2005) were amplified, scored and aligned following the methods outlined in Chapter 2.

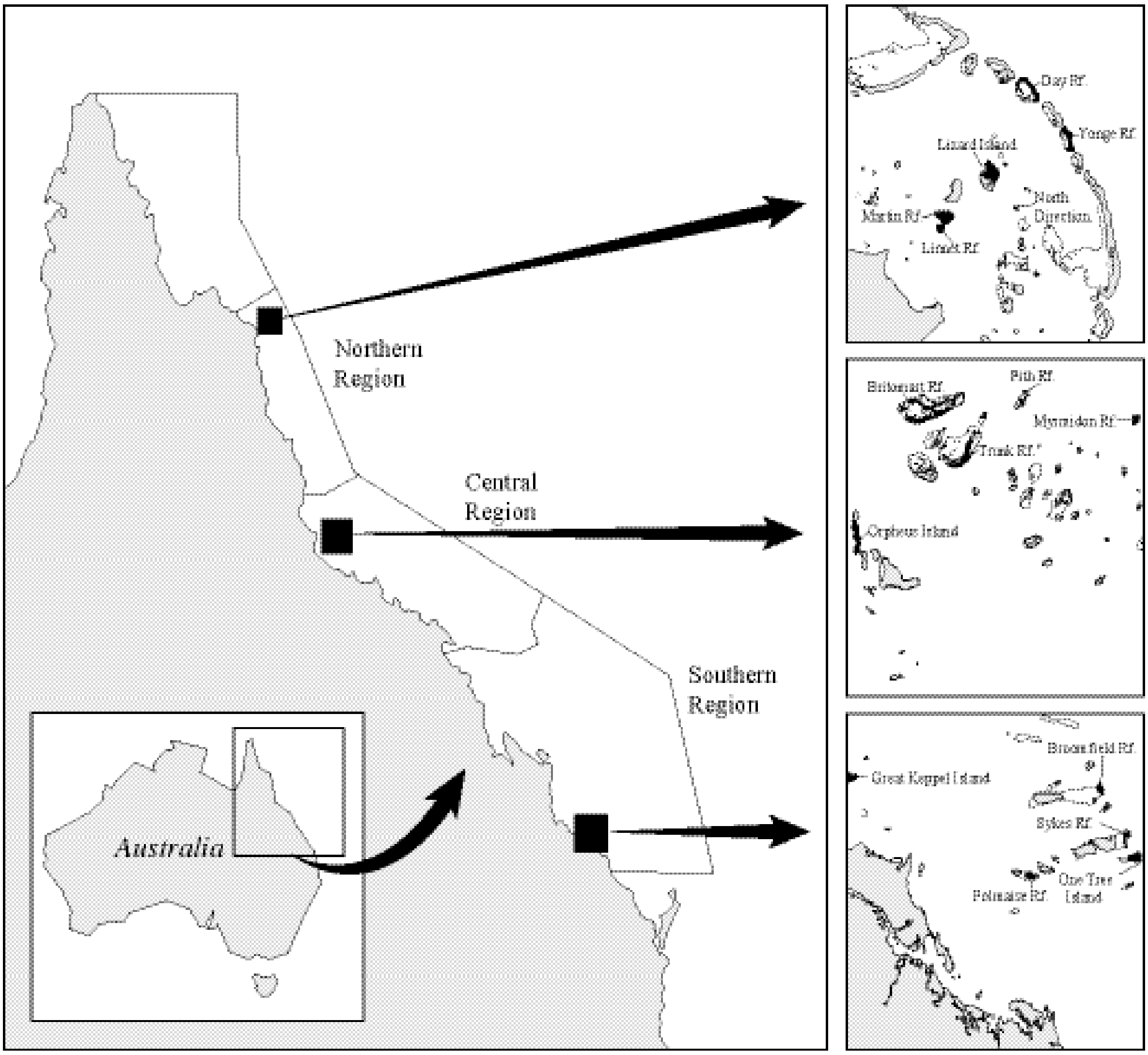

Fig. 1: The sampling locations of *Acanthochromis polyacanthus* on the Great Barrier Reef.

Sequences have been deposited in GenBank under accession numbers DQ199666 – DQ199947, DQ204725 – DQ204734, DQ206818 – DQ206820.

Population genetic investigations commonly use a single mitochondrial marker, which introduces some uncertainty about whether results are gene specific or representative of population level processes (Avise 2000). Therefore, the analysis of the microsatellites here was intended to provide an assessment of population structure independent of the mtDNA. Because of the relatively low number of microsatellite loci screened, interpretations based on these data should be regarded with some caution.

Phylogenetic Analysis

The best model of nucleotide substitution was determined using MrModeltest 2.2 (Nylander 2004) and Paup* 4.0b10 (Swofford 1998). The hierarchical likelihood tests and Akaike Information Criteria agreed that the GTR model with a $\gamma = 0.507$ best fitted the data (-LogLikelihood = 1914.9; AIC = 3847.8). This model and rate heterogeneity estimate was used in the phylogenetic analysis. The phylogenetic structure of *A. polyacanthus* was explored using Bayesian inference implemented in MrBayes 3.0B4 (Huelsenbeck and Ronquist 2001). The analysis was performed using a Markov Chain Monte Carlo search with four chains for one million generations. Trees were sampled every 100 generations and the first 100,000 generations were discarded as burn-in. The tree was rooted by an outgroup consisting of two closely related species, *Amphiprion melanopus* and *A. akindynos.* Credibility values were obtained from a majority rule consensus tree of the last 2000 trees and values greater than 90% are indicated on the major nodes of the tree. For the population level analyses of the ingroup the best model of nucleotide substitution was determined using Modeltest 3.5 (Posada and Crandall 1998) and Paup* 4.0b10 (Swofford 1998). The hierarchical likelihood tests and Akaike Information Criteria agreed that the Tamura and Nei model (Tamura and Nei 1993) with a $\gamma = 0.301$ best fitted the data (-LogLikelihood = 1220.7; AIC =2453.3). This model and rate heterogeneity estimate was used in all population level analyses.

The phylogenetic analysis of the mitochondrial control region sequences identified the presence of three divergent lineages on the GBR and the mitochondrial and nuclear population genetic datasets were categorised on the basis of these. Five groups were obtained of which three groups were based on the three major phylogenetic clades and two groups were based on geographic location within one of the clades (Clade 3). The “South” group contained all Clade 1 fish. The “Clade 2” group contained all northern region fish encompassed in the mtDNA Clade 2. The “Clade 3” group contained all Clade 3 individuals from the northern and central regions. The “Clade 3N” group was a subgroup of Clade 3 and contained all Clade 3 fish from the northern region. The “Central” group was also a subgroup of Clade 3 and contained all the fish from the central region. Because the nucDNA did not display any significant structure based on the mtDNA clade structure (Clade 2 vs. Clade 3N: Pairwise $R_{ST} = 0.003$, $P = 0.35$) the microsatellites were analysed on the basis of geographic location only.

Genetic Diversities

Estimates of mitochondrial haplotype and nucleotide diversity (Tajima 1983, 1993; Nei 1987) and their associated standard deviations were calculated using Arlequin 2.000 (Schneider et al. 2000) for each population and region. Standard deviations were converted to 95% confidence intervals as 95% CI = $\pm 1.96*(SD/\sqrt{(n)})$. For each population also, allele frequency and richness and the frequency of private alleles were estimated using Fstat 2.9.3.2 (Goudet 2001), while allelic diversity and heterozygosity were calculated using Arlequin. Mean genetic diversities and 95% confidence intervals were plotted and interpreted as being statistically different when error estimates did not overlap.

Demographic History

A range of analytical techniques are available for reconstructing past demography based on the identity and frequency of genotypes within and among populations (Knowles 2004). Existing techniques employ different methodologies including frequentist, cladistic and Bayesian approaches, but do not always calculate error estimates associated with models and their parameters. (Knowles and Maddison 2002). Because of the potential role of fixed effects in these statistical models, and the frequency with which natural systems may violate their assumptions, these methods often produce historical demographic parameters with large error estimates making conclusions about the demographic histories of populations difficult to draw (Knowles and Maddison 2002). The concurrent application of several of these analytical tools, and careful interpretation of error estimates where they do exist, can ameliorate some of the problems associated with the interpretation of such analyses (Knowles 2004).

Demographic histories were explored by mismatch analysis of mtDNA sequence data using Arlequin and 1000 bootstrap replicates. This analysis compares the distribution of pairwise nucleotide differences to a permuted distribution under the null hypothesis of sudden expansion. The age of population expansion (τ) is also estimated ($\tau = 2\mu t$, where μ = the mutation rate and t = generation time). Mismatch analysis was conducted based on geographical position (reef location and region) and phylogenetic clade membership (Clade 1, 2 and 3 from Fig. 2) independently. Because the mismatch mean of a population is inflated by genetic substructure, the spatial scale at which these comparisons were made was kept constant by analysing northern and central individuals of Clade 3 separately (Clade 3N and Central respectively). The age of population expansion (τ) values were considered significantly different when their 95% confidence intervals did not overlap.

Demographic bottlenecks in the microsatellites were investigated by examining the conformation to mutation-drift equilibrium (as identified by heterozygote excess) among populations and regions under the Infinite Allele Model (IAM) and the Stepwise Mutation Model (SMM) using one-tailed Wilcoxon tests (1000 bootstrap replicates) implemented in Bottleneck 1.2.02 (Piry et al. 1999).

The exponential population expansion parameter (*g*) was calculated among locations, regions and clades using a maximum likelihood coalescence approach implemented in FLUCTUATE 1.4 (Kuhner et al. 1998). A search strategy, each 10000 steps long using ten short chains, sampling every 20th step, followed by ten long chains each of 20000 steps sampled every 20th step, gave consistent results among runs and was used in all analyses. Estimates of *g* and their associated standard deviations were plotted and interpreted as significantly different if these error estimates did not overlap. Allele frequency data cannot presently be used in FLUCTUATE. This analysis was therefore restricted to the mtDNA data. The information obtained from all demographic history analyses were summarised into four demographic history models for interpretative purposes.

Results

Phylogenetic Structure

356 bases of the mtDNA control region I were obtained from 296 individuals sampled from 17 locations. The Bayesian analysis produced a well-resolved tree (-ln likelihood = 2146.39) and the ingroup was monophyletic (Fig. 2). Three clades were well resolved with credibility values greater than 90%. One clade (Clade 1) was further split into two clades of which one was well supported (Clade 1, credibility value = 100%) and the other less well supported (Clade 2, credibility value 72%). Clades 1 and 2 are sister clades to Clade 3 (credibility value = 100%). Clade 1 (credibility value = 100%) is composed of all individuals from the southern region and does not contain individuals from other regions. Clade 2 is comprised of individuals from the northern region with a high proportion of individuals from the two inner shelf (Martin and Linnet) and one midshelf reef (North Direction) (Fig. 2). Clade 3 (credibility value = 95%) is the largest clade in the tree and contains individuals from all reefs from the northern and central regions (although the three primary reefs in Clade 2 (Martin, Linnet, North Direction)

are represented by few individuals) (Fig. 2). Clade 4 (credibility value = 100%) is the most basal clade in the ingroup and the sister clade to Clades 1 - 3. It was exclusively composed of three individuals from the Solomon Islands (Fig. 2).

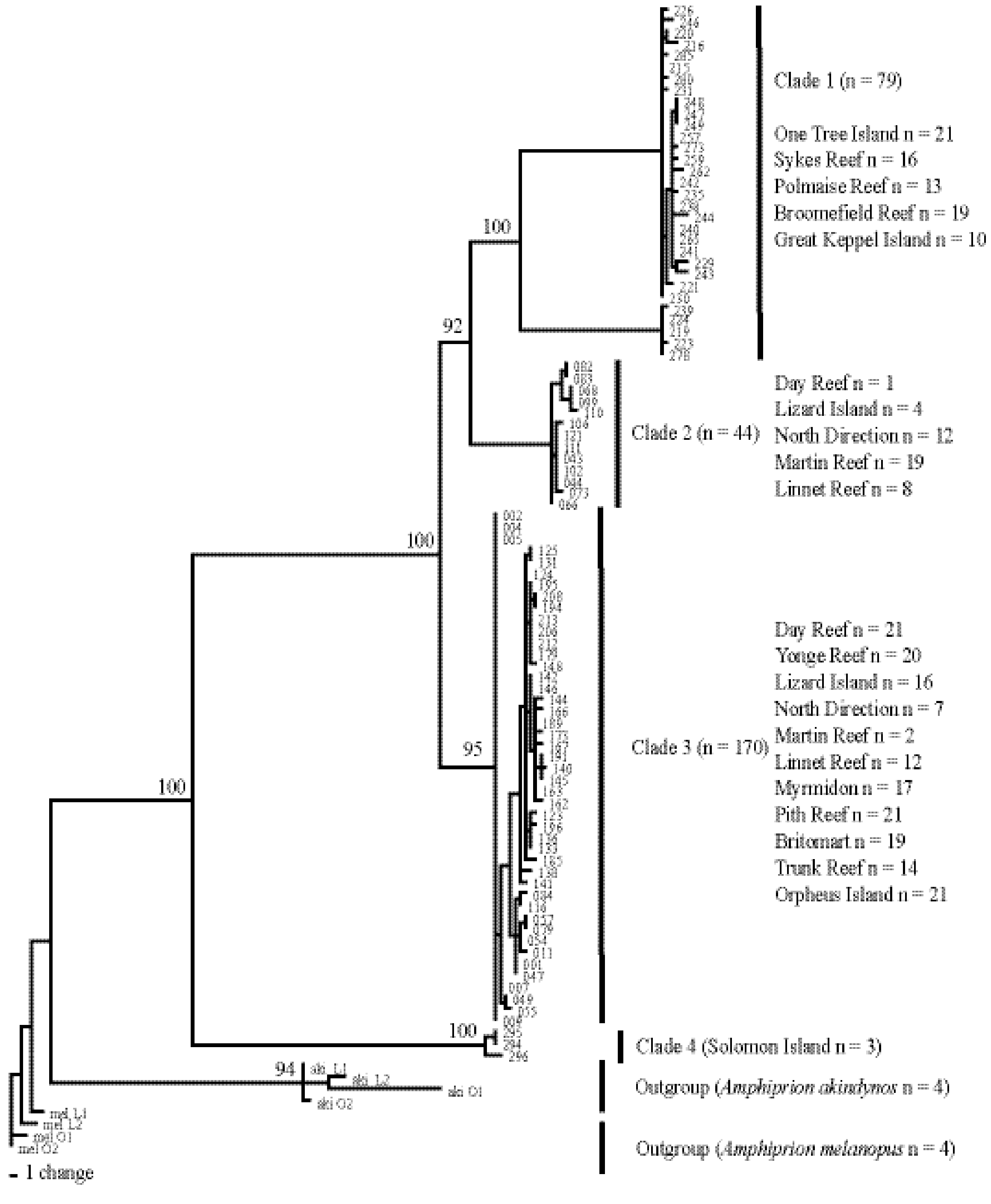

Fig. 2: Bayesian tree of 100 unique sequences from *A. polyacanthus* and outgroups. Internal branch support estimates greater than 90 are shown.

Patterns of Genetic Diversity

Genetic diversities of both mtDNA and microsatellite markers were generally high and variable among regions (Fig. 3). Haplotype diversities were very high when summed over all populations (total (± 95%CI) = 0.97 (±0.0003)) and high in the northern and central regions (and consequently also high in Clade 3 and its subgroup Clade 3N). Haplotype diversities, however, were significantly lower and much more variable among locations in the southern region and in Clade 2 (Fig. 3a). Nucleotide diversities were generally high (total (± 95%CI) = 6.6% (±0.37)), and highest and most variable, in the northern region. Nucleotide diversities were low in Clade 2 and generally declined toward the south (Fig. 3b). Allelic diversities displayed a very similar pattern to that of the nucleotide diversities: they were generally high (total (± 95%CI) = 1.00 (± 0)), greater in the northern region and declined with increasing latitude (Fig. 3c). Heterozygosities were high (total (± 95%CI) = 0.9 (±0.04)) and did not differ among regions (Fig. 3d).

Genetic diversities varied among reefs (Fig. 4). Each region contained one or two reefs with significantly lower haplotype diversities than the rest (i.e., North = Yonge, Central = Trunk, south = One Tree Island and Sykes) (Fig. 4a). In the southern region, the allelic diversity pattern was opposite to that of the mtDNA (lowest at Polmaise and Broomefield, highest at One Tree Island and Sykes) (Fig. 4c). Heterozygosity estimates were associated with large variances and did not differ among reefs within or among any of the regions (Fig. 4d).

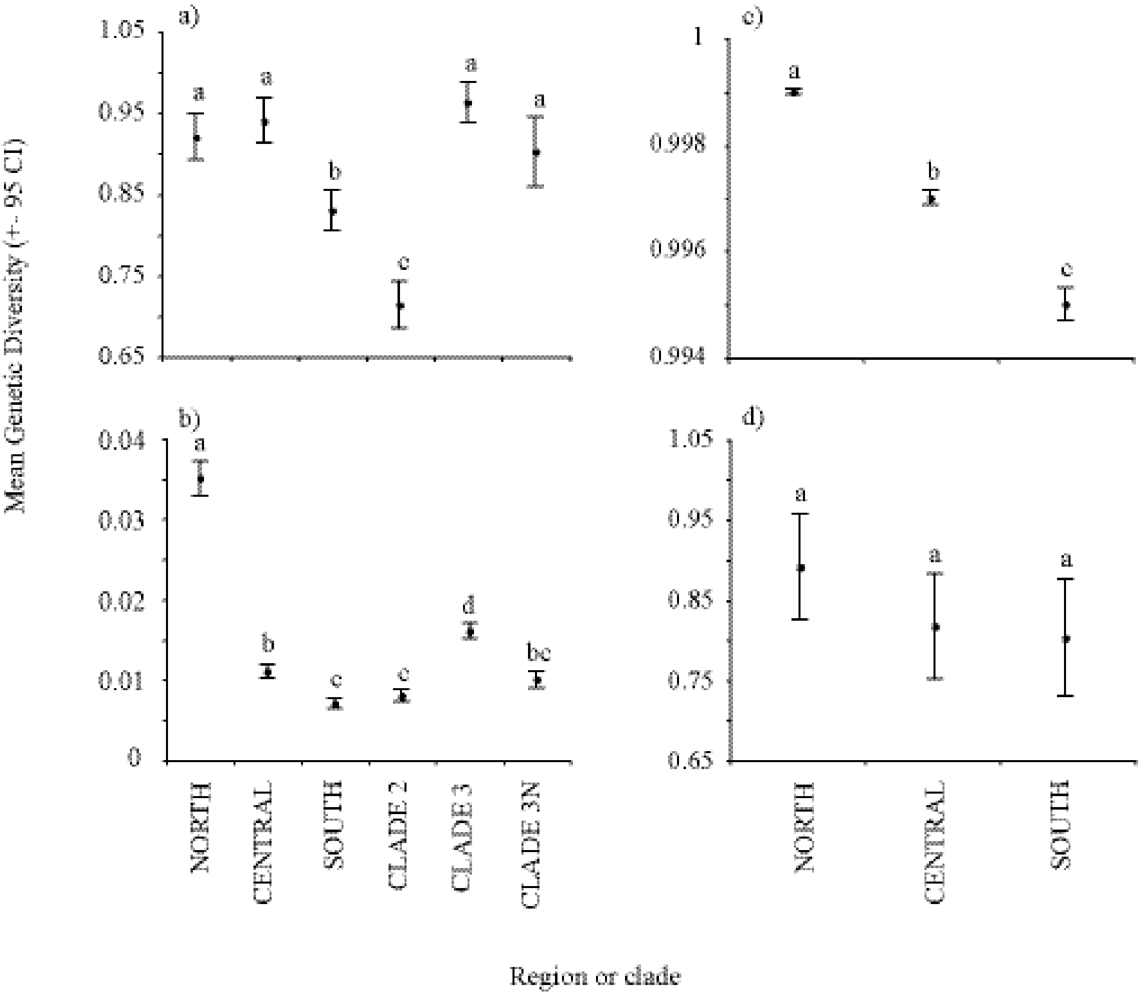

Fig. 3: Genetic diversities among regions and clades. a) Haplotype diversity and b) nucleotide diversity of mtDNA, c) allelic diversity and d) heterozygosity of the microsatellites. Statistical differences have been indicated with letters (shared letter = not significantly different) above means.

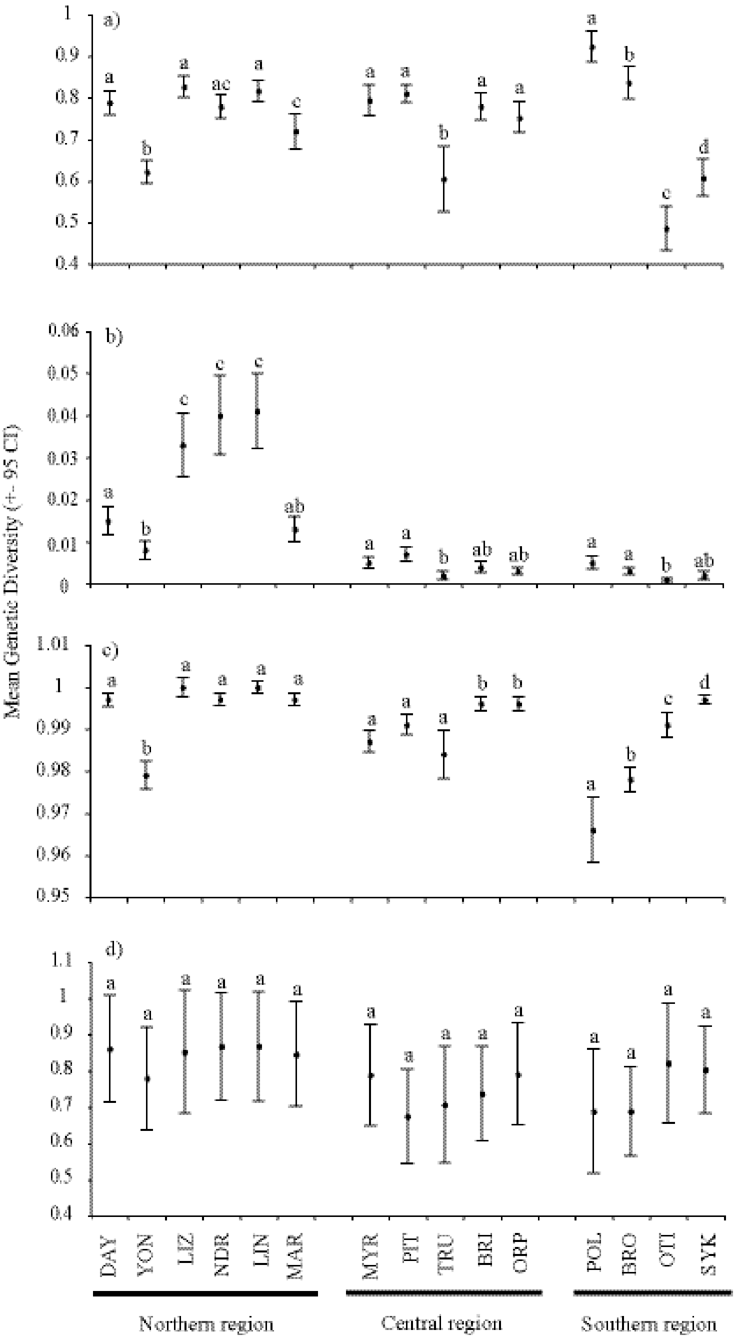

Fig. 4: Genetic diversities among reefs within regions. a) Haplotype diversity and b) nucleotide diversity of mtDNA, c) allelic diversity and d) observed heterozygosity of the microsatellites. Location abbreviations are: BRI = Britomart Reef, BRO = Broomefield Reef, DAY = Day Reef, LIN = Linnet Reef, LIZ = Lizard Island, MAR = Martin Reef, MYR = Myrmidon Reef, NDR = North Direction Island, PIT = Pith Reef, OTI = One Tree Island, ORP = Orpheus Island, POL = Polmaise Reef, SYK = Sykes Reef, TRU = Trunk Reef, YON = Yonge Reef. Statistical differences have been indicated for each region separately with letters (shared letter = not significantly different).

The number of alleles and allele richness were generally high and varied among populations and loci (Table 1). Microsatellite AC 42 had the highest number of alleles (24 at Martin) and highest allele richness (19.34 at Sykes). Microsatellite AC33 displayed the lowest number of alleles (3 at Britomart and Polmaise) and lowest allele richness (2.8 at Trunk). Private alleles were infrequent at most loci and populations, but one allele of microsatellite AC37 was more frequent at Yonge Reef (Table 1).

Historical Demography and Population Expansion

Mismatch analyses indicated that sudden expansion could not be rejected for any region or clade (Table 2). The mean number of pairwise differences ranged from 2.3 in the southern region to 12.3 in the northern one. There was, however, considerable uncertainty associated with the northern estimate (Fig. 5a). The age of population expansion (τ) ranged from 0.7 in Clade 2 (the southern region) to 6.5 in Clade 3. There were no statistically significant differences in this parameter among regions or clades. In the southern region, the lower bound of the 95% confidence interval could not be distinguished from 0 (Fig. 5b). Estimates of population expansion varied greatly among regions and clades (Fig. 5c). Estimates of population expansion were significantly greater in the southern region, intermediate in the central region, and close to 0 in the northern region (Fig. 5c). Population expansion rates were more variable in the two northern clades (Clade 2 and Clade 3N) and were significantly greater in Clade 3N compared to the northern region. Clade 3 was intermediate to the northern and the central regions.

The mismatch analysis indicated that the genetic architectures of all populations except two (i.e., North Direction and Linnet) were consistent with a model of sudden expansion (Table 2). These two populations were characterised by intermediate proportions of individuals contained in Clade 2 and 3, respectively (Fig. 2). The microsatellites did not reveal population bottlenecks under either the IAM or SMM models at any of the reefs (Table 2).

Mismatch distribution means were variable among reefs and ranged from 0.7 (Sykes) to 11.8 (Linnet). Reefs from the northern region that contained 20 – 65 % of individuals contained in Clade 2 had greater mismatch means, and larger uncertainty (Fig. 6a). Mismatch means were lower and more similar among reefs in the central and southern regions. The age of population expansion (τ) followed a similar pattern to that of the mismatch means.

Table 1: Genetic diversity indices from three microsatellite loci among 15 populations of *Acanthochromis polyacanthus* on the Great Barrier Reef including average number of alleles, mean allelic richness per population, number of private alleles and their frequencies, observed and expected heterozygosity.

	Number of alleles			Allelic richness			Private alleles (frequency)			Observed H			Expected H		
Locus	AC33	AC37	AC42	AC33	AC37	AC42	AC33	AC37	AC42	AC33	AC37	AC42	AC33	AC37	AC42
Reef															
Day Reef	7	13	26	5.964	10.22	17.75	0	1 (0.042)	2 (0.042)	0.625	0.708	0.833	0.737	0.845	0.944
Yonge Reef	6	8	11	5.52	6.36	8.65	0	1 (0.159)	0	0.636	0.636	0.636	0.755	0.753	0.777
Lizard Island	10	10	23	8.712	9.27	18.65	0	1 (0.056)	1 (0.028)	0.778	0.778	0.722	0.776	0.764	0.944
North Direction	12	11	20	9.36	8.64	15.01	1 (0.042)	0	1 (0.021)	0.958	0.542	0.625	0.845	0.774	0.928
Linnet Reef	7	15	23	5.762	11.99	17.42	0	1 (0.022)	3 (0.108)	0.739	0.783	0.522	0.713	0.891	0.940
Martin Reef	7	11	24	4.872	9.80	12.17	1 (0.021)	0	2 (0.084)	0.458	0.792	0.792	0.703	0.833	0.944
Myrmidon Reef	6	12	17	3.237	8.15	16.88	0	0	0	0.435	0.826	0.870	0.580	0.870	0.862
Pith Reef	4	11	22	3.853	6.83	16.46	1 (0.024)	0	0	0.286	0.571	0.905	0.323	0.715	0.934
Trunk Reef	4	7	18	2.795	9.67	16.82	1 (0.067)	0	1 (0.033)	0.4	0.333	1.000	0.429	0.700	0.920
Britomart Reef	3	11	23	6.807	8.74	13.93	0	0	2 (0.042)	0.292	0.833	0.917	0.369	0.851	0.943
Orpheus Island	9	11	19	3	7	10	0	0	1 (0.043)	0.217	0.870	0.826	0.553	0.854	0.911
Polmaise Reef	3	7	10	3.336	8.54	7.46	0	0	0	0.154	0.769	0.692	0.500	0.698	0.787
Broomefield Reef	4	10	9	6.133	9.01	16.35	0	0	0	0.042	0.917	0.708	0.376	0.845	0.799
One Tree Island	7	10	20	7.18	7.39	14.42	0	0	4 (0.167)	0.500	0.722	0.833	0.637	0.832	0.926
Sykes Reef	10	10	21	8.333	12.2	19.34	2 (0.048)	0	3 (0.048)	0.290	0.774	0.806	0.674	0.770	0.922

Error estimates from most reefs in all three regions overlapped to a great extent. Greater values with large variances were observed in two northern reefs (North Direction and Linnet), lower and less variable estimates were found in one central location (Trunk) and in two southern locations (One Tree Island and Sykes) (Fig. 6b). The age of population expansion (τ) could not be distinguished from 0 in four locations: Trunk and Orpheus Island in the central region and One Tree Island and Sykes in the southern region. Population expansion rates varied significantly among locations (Fig. 6c). All northern locations displayed negative growth rates close to 0. Reefs in the central region showed both positive and negative growth rates that were all close to 0 except Trunk that displayed a highly positive value. The high mean regional growth rate in the southern region (Fig. 5c) was contributed to by three of the four reefs having growth rates that were greater than all other reefs except one (Trunk in the central region) (Fig. 6c).

Discussion

The analyses of the mtDNA of *A. polyacanthus* indicated a complex phylogenetic structure with evidence of population expansion at most spatial and phylogenetic scales examined. There were substantial differences in the timing of these expansions and the population expansion rates among regions and reefs within regions. These results indicate that local populations of *A. polyacanthus* have experienced periodic reductions in population size, and possibly local extinctions. Taken together these results suggest that the evolution of the spatial dynamics of this species are best interpreted in a metapopulation context.

Population Demography among Regions

Patterns of haplotype, nucleotide and allelic diversity, mismatch analyses and population growth estimates all indicated substantial differences in the demographic histories of *A. polyacanthus* among regions on the GBR (Fig. 3a – c, Fig. 5a - c). The southern region, located close to the species' southern border, was characterised by lower nuclear and mitochondrial genetic diversity and a expansion rate 5 – 10 times greater than the central and northern regions (Fig. 5c).

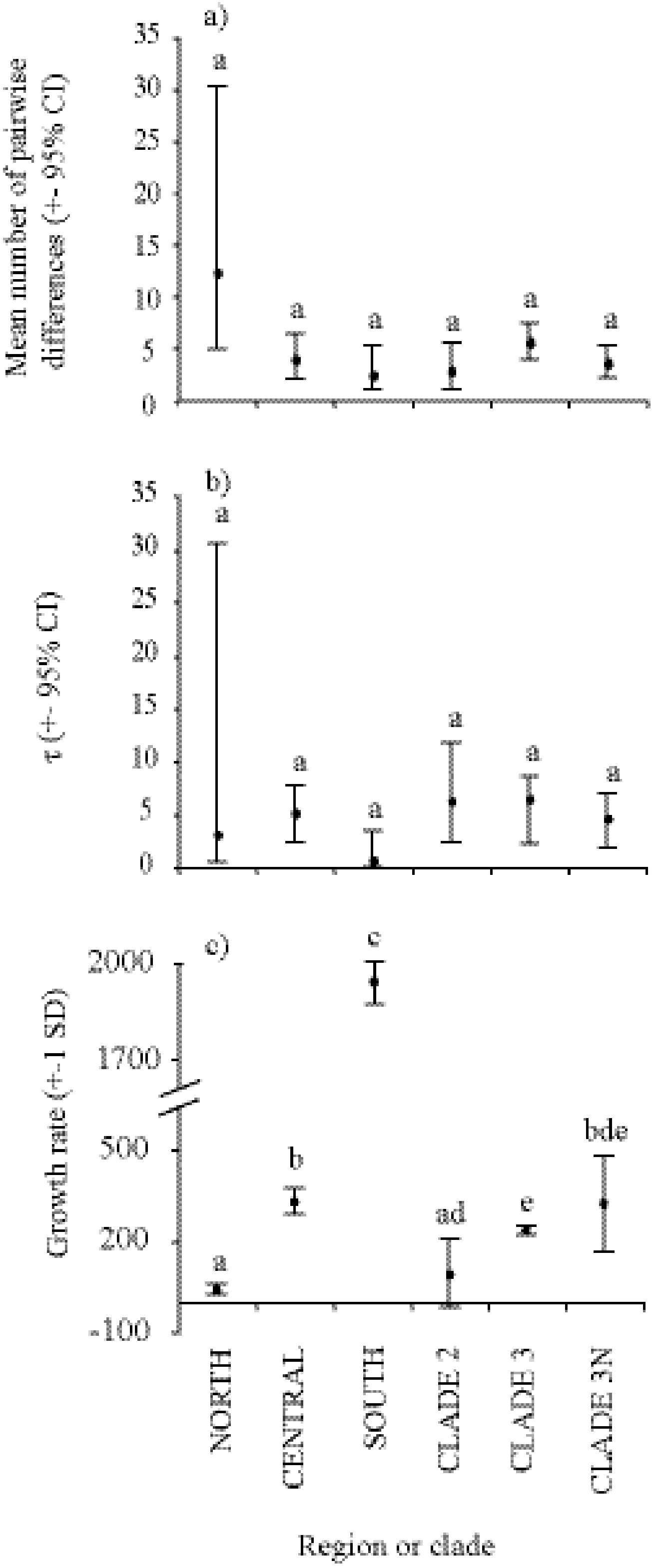

Fig. 5: Mismatch means, expansion parameter (τ) and population expansion rates (*g*) among regions and clades. Statistical differences have been indicated with letters (shared letter = not significantly different) above means.

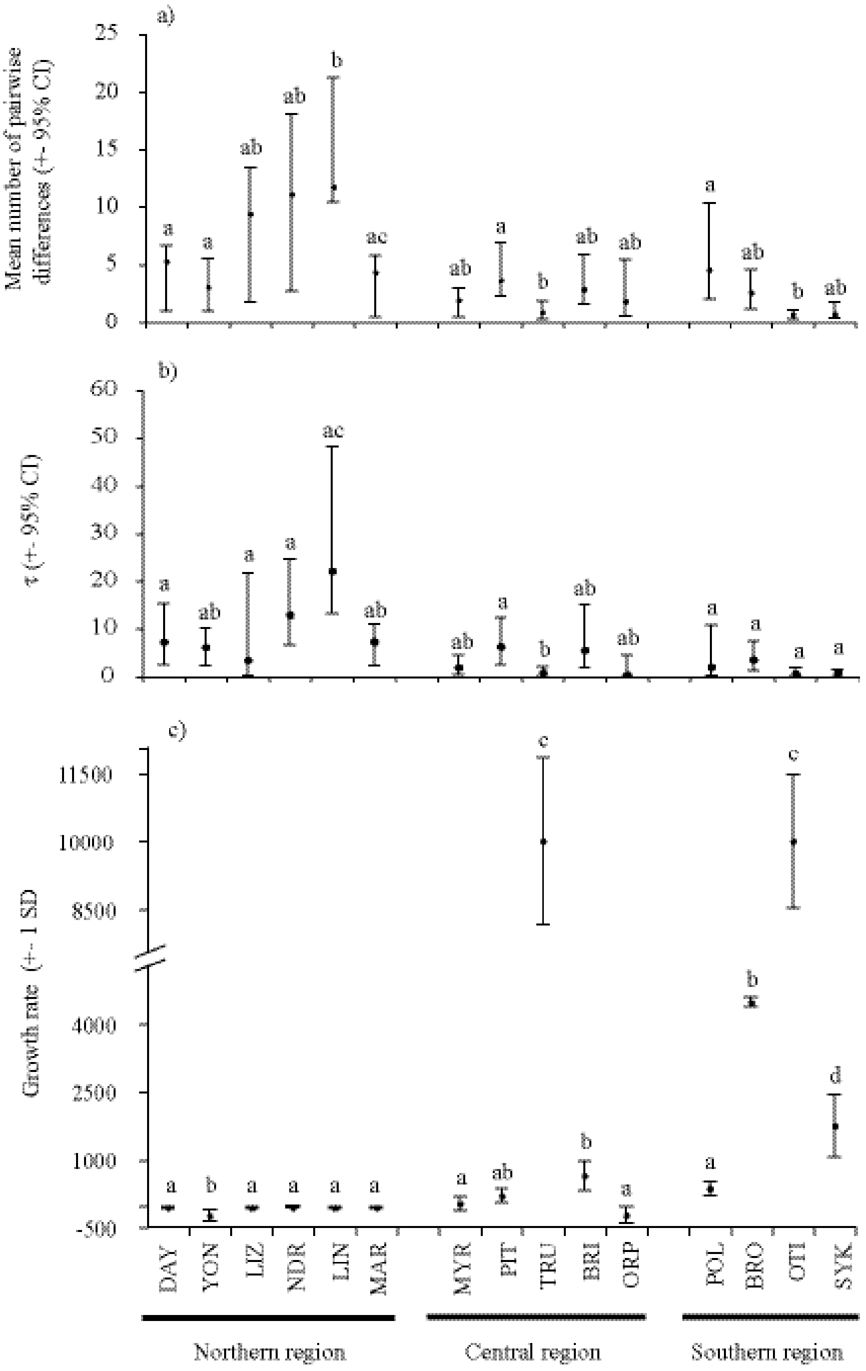

Fig. 6: Mismatch means, expansion parameter (τ) and growth rate (*g*) among reefs within regions. Location abbreviations follow Fig. 4. Statistical differences have been indicated for each region separately with letters (shared letter = not significantly different).

Table 2: Results of the demographic bottleneck analyses: mismatch means (mismatch), sums of squared deviations (SDD), probability (p) of sudden expansion, sequential Bonferroni corrected significance level (α), one-tailed probabilities of heterozygotic excess using a Wilcoxon test under the Infinite Allele Model (IAM) and the stepwise mutation Model (SMM).

	MtDNA				Microsatellites	
Region, clade or reef	Mismatch	SDD	p	α	IAM	SMM
NORTH	12.293	0.045	0.02ns	0.003	0.125 ns	1.00 ns
CENTRAL	3.939	0.007	0.33 ns	0.005	0.934 ns	0.938 ns
SOUTH	2.373	0.004	0.61 ns	0.01	0.813 ns	1.00 ns
Clade 2	2.801	0.055	0.22 ns	0.004		
Clade 3	5.618	0.002	0.64 ns	0.01		
Clade 3N	3.576	0.006	0.45 ns	0.006		
Day Reef	5.273	0.051	0.35 ns	0.005	0.063 ns	1.00 ns
Yonge Reef	3.032	0.198	0.01 ns	0.003	0.188 ns	0.938 ns
Lizard Island	9.395	0.050	0.24 ns	0.004	0.813 ns	0.938 ns
North Direction	11.11	0.140	0.000 *	0.002	0.125 ns	0.938 ns
Linnet Reef	11.76	0.097	0.000 *	0.003	0.063 ns	0.938 ns
Martin Reef	4.343	0.032	0.58 ns	0.007	0.063 ns	0.938 ns
Myrmidon Reef	1.919	0.017	0.39 ns	0.006	0.813 ns	1.000 ns
Pith Reef	3.619	0.074	0.03 ns	0.003	0.938 ns	1.000 ns
Trunk Reef	0.857	0.001	0.81 ns	0.03	0.938 ns	1.000 ns
Britomart Reef	2.830	0.051	0.16 ns	0.003	0.063 ns	0.875 ns
Orpheus Island	1.819	0.012	0.58 ns	0.008	0.813 ns	1.000 ns
Polmaise Reef	4.551	0.011	0.73 ns	0.02	0.875 ns	0.938 ns
Broomefield Reef	2.561	0.006	0.83 ns	0.05	0.125 ns	0.938 ns
One Tree Island	0.705	0.007	0.28 ns	0.004	0.813 ns	1.000 ns
Sykes Reef	0.692	0.0321	0.01 ns	0.003	0.813 ns	1.000 ns

Statistical significance: * = p <0.0001, ns = not significant.

The lower genetic diversities in the southern region identified here agrees with previous reports (e.g., Doherty et al. 1994; van Herwerden and Doherty 2006). However, the mean number of pairwise differences and the expansion parameter (τ) for the southern region identified here were not significantly different to estimates obtained from the central and northern regions. This contrasts with a previous report (van Herwerden and Doherty 2006) that the southern region had undergone a more ancient population expansion based on the mean number of pairwise differences and the expansion parameter (τ). The 95% confidence interval of their estimates, however, overlapped among regions indicating that they were not significantly different. I suggest that the southern region has undergone a more recent population expansion compared to the other two regions. This interpretation is based on the results of both the mismatch analysis (the lower bound of the 95% confidence interval of τ could not be distinguished from 0 in the southern region) and the population expansion analysis (population expansion rate was significantly greater in the southern region). These results support the predictions that local extinctions may affect the metapopulation dynamics towards the species margin to a greater extent than within more centrally located regions (Lennon et al. 1997; Holt and Keitt 2000).

Population Demography among Locations

There were substantial differences in the genetic diversities, mismatch means and population growth rates among reefs within regions (Fig. 4 and 6). Four general patterns were observed (Table 3). The majority of populations conformed to a model of mutation-drift equilibrium with no evidence of local extinctions. In contrast, two populations in the northern region (North Direction, Linnet) conformed to a model of migration-drift equilibrium with a departure from panmixia (Table 3). Very high nucleotide diversities in these locations (Fig. 4b, Fig. 6a) argue against a model of sudden expansion (Table 2). These high diversities were most likely the result of the presence of approximately equal numbers of individuals from two differentiated lineages at these locations (Fig. 2). Because the sudden expansion model was supported in both these lineages when analysed independently (Clade 2 and Clade 3N Table 2), it is likely that these differences were caused by a departure from panmixia, rather than long-term stability of the populations leading to the accumulation of diversity via mutation.

Such sub-structure within populations violates the assumption of mismatch analysis of panmixia (Slatkin and Hudson 1991) and this result highlights the potential role of departures from the assumptions of demographic history analyses (Knowles and Maddison 2002; Knowles 2004).

Only a single reef (i.e., Yonge) provided support for a model of a weak bottleneck or a large bottleneck followed by migrant-pool colonisation, where colonisers originate from many sources (Wade and McCauley 1988; McCauley 1991) (Table 3). Genetic diversity estimates were significantly lower at this reef than other northern reefs (Fig. 4a – c) but this low diversity was not associated with a higher population expansion rate (Fig. 6c). The genetic pattern observed at this reef may be expected if a local population reduction was followed by a migrant-pool colonisation event. If colonisers were few, but from a range of genetically differentiated populations, this could lead to reduced genetic diversities, but not necessarily a shorter coalescent history (Pannell and Charlesworth 2000; Pannell 2003). Conversely, it is possible that the observed reduction here in local genetic diversity (π_S) was not great enough to result in a bottleneck (Nei et al. 1975) although a large genetic bottleneck, identified by a high expansion rate, was detected in the population from Trunk Reef which displayed a similar reduction in local genetic diversity (π_S).

Populations on three reefs, Trunk, One Tree Island and Sykes, conformed to a model of a large bottleneck and/or extinction followed by propagule-pool colonisation, where colonisers originate from a single source (Wade and McCauley 1988; McCauley 1991) (Table 3). These locations were characterised by low local diversity (π_S) and high population expansion rates. In addition, the expansion parameter (τ) for these populations did not differ from 0 (Fig. 6b) and, therefore, their expansion times could not be distinguished from the present. In concert, these results provide strong evidence for recent population bottlenecks and/or local extinctions in these populations with re-colonisation most likely following the propagule-pool model. This finding suggests that the evolution of marine metapopulations may be more greatly affected by local extinctions than previously thought (Planes et al. 1996; Planes 2002). The southern region was characterised by a high frequency of local populations with reduced genetic diversity (π_S), which resulted in a reduction of regional diversity (π_T) for that region. Therefore, there appears to be considerable potential for local genetic bottlenecks and/or extinctions to affect regional genetic diversity (π_T), and consequently, the evolutionary potential and the metapopulation dynamics of this marine species.

Table 3: Models of the demographic histories of populations of *Acanthochromis polyacanthus* on the Great Barrier Reef and criteria including genetic diversity (π_S), conformation to the sudden expansion model and population growth used to assess support for each model.

Model of demographic history	π_S	Sudden expansion	Population growth	Examples
Migration-drift equilibrium	High	Retained	Low	DAY, MYR
Migration-drift equilibrium and departure from panmixia	High	Rejected	Low	NDR, LIN
Small population size reduction or extinction and migrant-pool colonisation	Low	Retained	Low	YON
Large population reduction and/or extinction and propagule-pool colonisation	Low	Retained	High	TRU, OTI, SYK

Nuclear vs. mtDNA Diversity and Historical Demography Analyses

Patterns of microsatellite allelic diversity largely matched those recorded by the mtDNA except among populations in the southern region (Fig. 3 and 4). In this region, there was an opposing pattern where locations displayed high mtDNA diversity associated with low allelic diversity and vice versa (Fig. 4a and c). It is possible that these discrepancies can, in part, be explained by variations in sample sizes given the sensitivity of allelic diversity estimates to sample size (Leberg 2002). Indeed, the population here with the lowest allelic diversity was also the population with the smallest sample size (18 alleles). In contrast, however, allelic diversities were significantly different between Broomefield and One Tree Island despite similar sample sizes (34 and 36). Therefore, differences in sample sizes are unlikely to be the only source of variation in the allelic diversity estimates reported here.

None of the regions, or populations within regions, were characterised by heterozygote excess, regardless of the model of microsatellite evolution used (Table 2). The absence of a bottleneck signal in the nuclear markers may be a result of differences in the effective population sizes of nucDNA and mtDNA (Avise 2000). Because of the four-fold difference in effective population size a smaller population reduction may result in a bottleneck in the mtDNA but not in the nucDNA. Furthermore, if present, the genetic signal of demographic bottlenecks may be rapidly erased in nuclear markers (Cornuet and Luikart 1996). It is, however, also possible that the genetic bottlenecks observed in some of populations examined here were restricted to the mitochondrial genome. For example, a selective sweep on functional mtDNA genes, physically linked to the Control region (Rand 2001), could result in a similar pattern but without the severe reduction in population size characteristic of a bottleneck. It is also possible, that the number of loci used in this study was insufficient to detect bottlenecks that were indeed present. Concomitant reductions in allelic and mtDNA diversities found in some populations (e.g., Yonge and Trunk Fig. 4a – c) suggest that the bottlenecks indicated by the control region data were not restricted to the mitochondrial genome. Instead, the absence of genetic bottlenecks in the microsatellites are likely to have been caused by the number of microsatellite loci used here, the greater effective population size and the slower fixation of nuclear genes.

Phylogenetic Structure and Species Status

The phylogenetic analysis presented here resolved four highly divergent lineages of *A. polyacanthus* in the set of sampled populations. Two of these lineages consisted exclusively of the bicoloured morph in the northern region of the GBR (Fig. 2). The two lineages of bicoloured morphs co-occurred on a large proportion of sampled reefs in the northern region and the frequency of Clade 2 individuals increased across the continental shelf toward the west. According to our analyses, Clade 1 (black southern fish) and Clade 2 (northern bicoloured fish) were more closely related to each other than were Clade 2 and Clade 3 (northern and central bicoloured fish) and Clade 3 and Clade 4 (Melanesian bicoloured fish). This contrasts with the findings of van Herwerden and Doherty (2006) who reported monophyly among all northern fish and Planes et al. (2001) who reported chromatic monophyly. Differences between this study and the previous ones may have resulted from the inclusion of both transitions and transversions in the current phylogeny. The sequences used here showed a low ts/tv ratio, indicating that transitions carried significant information, and that combined with the lack of compositional heterogeneity between sequences indicated that using all substitution types was appropriate for these data.

Previous authors (e.g., Planes et al. 2001; van Herwerden and Doherty 2006) have argued that the depth of genetic divergence between the southern black and the northern bicoloured morphs warrants species status and have recommended a taxonomic review of *Acanthochromis*. The sequence divergences between black (Clade 1) and bicoloured morphs (Clade 2) found here, however, appeared to be less than the divergence among geographically disjunct bicoloured populations Clade 2 or 3 vs. Clade 4). I also found deep sequence divergence between bicoloured individuals co-existing on the same reefs (Clade 2 and 3). I propose, therefore, that the considerable genetic structure of chromatically similar and geographically co-occurring individuals does not support the simple interpretation of two species congruent with colour morph. The presence and maintenance of two or more divergent lineages within populations across relatively small spatial scales is emerging as a feature of many coral reef organisms (e.g., Knowlton 1993; Barber et al. 2000; Bernardi et al. 2003; Bay et al. unpublished data). Exactly how many divergent lineages of *A. polyacanthus* co-occur on the GBR, and whether the degree of divergence that is present constitutes species status will require further study. In the meantime, however, the data presented here do not support a single division of what is currently recognized as *A. polyacanthus* into two species aligned with colour morphs.

Conclusion

The variation in genetic diversities and population expansion rates among populations reported here strongly indicate that *A. polyacanthus* functions as a metapopulation and that local extinctions may play an important part in the evolutionary dynamics of this species through founder effects. These conclusions contrast with previous suggestions that genetic bottlenecks and/or local extinctions are unimportant in the metapopulation dynamics of coral reef fishes. The development and application of highly sensitive molecular markers and within-region sampling have the potential to illuminate the presence of metapopulations and the role of local extinctions in marine species with higher gene flow.

Chapter 4: Intraspecific variation in the pelagic larval duration.

Publication: Bay LK, Buechler K, Gagliano M Caley MJ (In Press) Intraspecific variation in the pelagic larval duration of tropical reef fishes. Journal of Fish Biology

Abstract

The pelagic larval duration (PLD) of coral reef fishes is an important life-history trait that can both affect and record the ecology and evolution of these species. Although the importance of PLD has been previously recognized, PLDs are available from only a handful of papers and, as a result, a typological view of the PLD of species has developed. Emerging evidence, however, suggests considerable intraspecific variation of PLDs. Here I present additional estimates of PLD for ten species of Pomacentridae and two species of Gobiidae, and coupled with previously published estimates, examine spatial and temporal variation of PLDs within these species. In eight of the twelve species examined here, within-population mean PLDs differed between sampling times, locations within regions and among regions. In contrast, the range of these same PLD estimates overlapped at all spatial and temporal scales examined in eleven of the twelve species, but not between regions in one species (*Amphiprion melanopus*). Therefore, despite tight error estimates typically associated with estimates of PLD taken from a particular population at a particular time in some taxa, the overlapping ranges in PLD reported here indicate that the length of the pelagic larval phase is a much more plastic trait than previously appreciated. Improved understanding of within-species variation in PLD has considerable potential to provide further insights into the ecology and evolution of tropical reef fishes.

Introduction

The life cycle of nearly every species of coral reef teleost fish includes a pelagic larval phase and a benthic reef-associated one (Leis 1991; Leis and Carson-Ewart 2000). During the past few decades the importance of the dispersive larval phase for understanding aspects of these species' ecology (e.g., Swearer et al. 2002; Sponaugle et al. 2002) and evolution (e.g., Shulman 1998; Bonhomme and Planes 2000) has begun to be appreciated. For example, processes acting on pelagic larvae may affect recruitment rates (Caley et al. 1996), biogeographical distributions (Thresher et al. 1989; Wellington and Victor 1989; Victor and Wellington 2000; Zapata and Herron 2002; Robertson et al. 2004), connectivity among populations (Doherty et al. 1995; Shulman and Bermingham 1995; Shulman, 1998; Riginos and Victor 2001), individual condition (McCormick 1998a; Searcy and Sponaugle 2000), post-recruitment growth (McCormick and Hoey 2004) and survival (Shima and Findlay 2002). Because of these important links between the pelagic and benthic phases, it is important to have reliable estimates of the spatial and temporal variation in pelagic larval durations (PLD) (Leis 1991; Victor 1991; Cowen and Sponaugle 1997; Victor and Wellington 2000). For example, understanding interspecific spatial variation in PLDs may provide insights into processes that vary at geographical scales. Species recruiting to remote locations may display longer PLDs than those recruiting to well connected ones (e.g., Brothers and Thresher 1985; Victor 1986a), if selection favours connectivity, or shorter PLDs if self-recruitment is favoured. Similarly, geographical patterns in larval growth and dispersal may be informed by inter- and intraspecific spatial patterns of PLDs (Cowen and Sponaugle 1997). Temporal variation in PLDs may provide insight into environmental effects on larval duration and recruit quality (e.g., Searcy and Sponaugle 2000; Shima and Findlay 2002; Sponaugle and Pinkard 2004).

PLD can be reliably estimated using pre-settlement counts of daily rings deposited in otoliths (Pannella 1971; Victor 1982; Pitcher 1988). Estimates of PLDs are now available for a large number of species (Brothers et al. 1983; Brothers and Thresher 1985; Thresher and Brothers 1985; Victor 1986a; Thresher and Brothers 1989; Thresher et al. 1989; Wellington and Victor 1989), but little attention has been paid to the possible implications of variation in PLDs either within or among populations. Where variation in PLDs has been reported, this has typically been a subsidiary outcome of research studying other phenomena, not its primary focus (e.g., Murdoch and Doherty 1997; Kerrigan 1996, Sponaugle and Cowen 1997; Wilson and McCormick 1997;

Searcy and Sponaugle 2000; Sponaugle and Pinkard 2004; but see Thorrold and Milicich 1990; Radtke et al. 2001; Wellington and Victor 1992; McCormick 1994). Because the estimates of PLD are being used in a variety of applications such as predicting genetic differentiation among populations (e.g., Doherty et al. 1995; Shulman and Bermingham 1995) and explaining the evolution of larval durations (Bonhomme and Planes 2000), it is imperative to know the spatial and temporal variation of such estimates.

Sources of intraspecific variation in PLDs are potentially diverse. For any species, a range of PLDs might be expected with the lower limit of this range indicating the minimum time taken to attain competency to settle (Searcy and Sponaugle 2000) and the upper limit reflecting the maximum period of survival in the pelagic environment. Variation in PLDs within species should, therefore, reflect genotype X environment interactions influenced by the conditions encountered by larvae in the plankton such as food availability and temperature (e.g., McCormick and Molony 1992 1995; Meekan et al. 2003), plus any maternal effects (Kerrigan 1997; McCormick 1998b), and innate physiological capacities for growth and development (Victor 1986b; Cowen 1991; McCormick 1999). Alternatively, differences in PLDs in allopatric populations of the same species may indicate evolved mean differences in pelagic duration.

Here I document intraspecific variation in the means and ranges of PLDs estimated for 12 species of tropical reef fishes, both among populations separated in space, and within populations through time. The species compared include ten species of damselfishes (Family Pomacentridae) and 2 species of gobies (Family Gobiidae). Presented comparisons are based on combinations of new estimates of PLDs and previously published ones. These comparisons are interpreted as to whether the variation observed most likely reflects plasticity of PLDs, or evolved differences among populations.

Materials and methods

The PLDs of ten species of damselfishes (i.e., *Amphiprion melanopus* Bleeker*, Amphiprion akindynos* Allen*, Chrysiptera rollandi* (Whitley)*, Chrysiptera rex* (Snyder)*, Chromis viridis* (Cuvier)*, Pomacentrus wardi* Whitley*, Pomacentrus moluccensis* Bleeker*, Pomacentrus amboinensis* Bleeker*, Amblyglyphidodon curacao* (Bloch)*, Amblyglyphidodon aureus* (Cuvier)) and two species of gobies (i.e., *Amblygobius* *rainfordi* (Whitley), *Amblygobius phalaena* (Valenciennes)) were estimated in individuals collected from populations in the south (i.e., One Tree Island 23°30S; 152°05E) and/or north (e.g., Lizard Island 14°40S; 145°28E) of the Great Barrier Reef (GBR) during 2000. Fishes were collected using a range of methods. Fishes were measured (SL ± 0.1 mm) and then frozen. Otoliths were later removed (sagittae only), cleaned and stored and sagittal transverse sections were obtained following Wilson and McCormick (1997). Otoliths were read using a high-powered microscope (x40 magnification) and polarised transmitted light. In all species, a settlement mark was apparent as a dark ring followed by a marked decrease in increment width (i.e., Type 1, following Wilson and McCormick 1999). The pre-settlement rings were counted from the nucleus to the settlement mark along one axis. Three blind counts were done on consecutive days and percentage error (PE) estimates were calculated following Beamish and Fournier (1981):

$$\mathrm{PE} = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|Xij - Xj|}{Xj}\right]\times 100 \qquad \text{Eq. 1}$$

where N is the number of fish aged, R is the number of times increments on each otolith were counted, Xij is ith age determination for the jth fish and Xj is the average age estimated for the jth fish.

For all the species examined here, published estimates of mean PLD, and their associated error and range, where available, were used in combination with my data to sample variation in PLDs among times and locations. Because total ring counts of newly settled individuals tend to overestimate PLD by a few days (Wellington and Victor 1989), only estimates based on pre-transitional daily ring counts were used in the comparisons reported here. The techniques to quantify the pre-transitional phase are well established and these counts have been used most widely, and were therefore preferable for the purposes of this investigation. Where possible, mean, error (95% CI) and range were compared among studies on the same species. When 95% CIs could be calculated and did not overlap, means were deemed to be significantly different. In a number of cases from the literature, error estimates were not reported. In such cases, mean estimates were deemed to differ if they were not encompassed by the 95% confidence intervals calculated for other mean estimates.

Results

Sequential daily ring counts were very consistent with percentage error estimates of less than 4 % (range 1.47 – 3.35 rings) in the majority of species but 13.1 % in *P. wardi*. The mean larval duration displayed significant spatial and temporal variation in eight of the twelve species and these differences ranged from just over half a day (0.63 *A. aureus*) to more than 5 days in *P. wardi* (5.14) (Fig. 1). These differences were primarily associated with differences among locations, although *P. wardi* displayed significant temporal variation in PLD estimates.

The majority of species displayed a range of PLDs of approximately 5 – 6 days (mean 5.47 days ± SE 0.85) although this was much greater in the gobies (15.25 ± SE 2.50) than in the pomacentrids (4.17 ± SE 0.58). The range of larval durations among times and locations overlapped in all but one of the species examined. In *Amphiprion melanopus* the GBR locations ranged from 8 – 14 days whereas the Palauan population ranged from 15 – 22 days. In *P. wardi* the ranges of previously published PLD estimates (from the same location) were non-overlapping before the addition of our estimate.

Discussion

Previous authors have argued for the consideration of variation in PLDs in trying to understand the importance of the pre-settlement life-history stage of fishes to other aspects of their ecology and evolution (Leis 1991; Victor 1991; Wellington and Victor 1992; Cowen and Sponaugle 1997; Leis 2002), but their calls, to a certain extent, have been ignored. This study confirms significant intraspecific variation in PLDs in eight out of twelve species. Despite the high spatial and temporal variation in PLDs, variability at specific times and places (when it could be estimated) was generally low (Fig. 1). This indicates that although the processes affecting the length of larval life are complex, they tend to produce PLDs that vary little at specific times and places. Therefore single point estimates can underestimate considerably the capacity for intraspecific PLD variation.

The intraspecific range of larval durations observed here were about 5.5 days for pomacentrids and 15 days for gobies. This data confirm that the potential for variation in PLDs varies among taxonomic groups with pomacentrids being less variable than gobies (Thresher et al. 1989; Wellington and Victor 1989; Sponaugle and Cowen 1994;

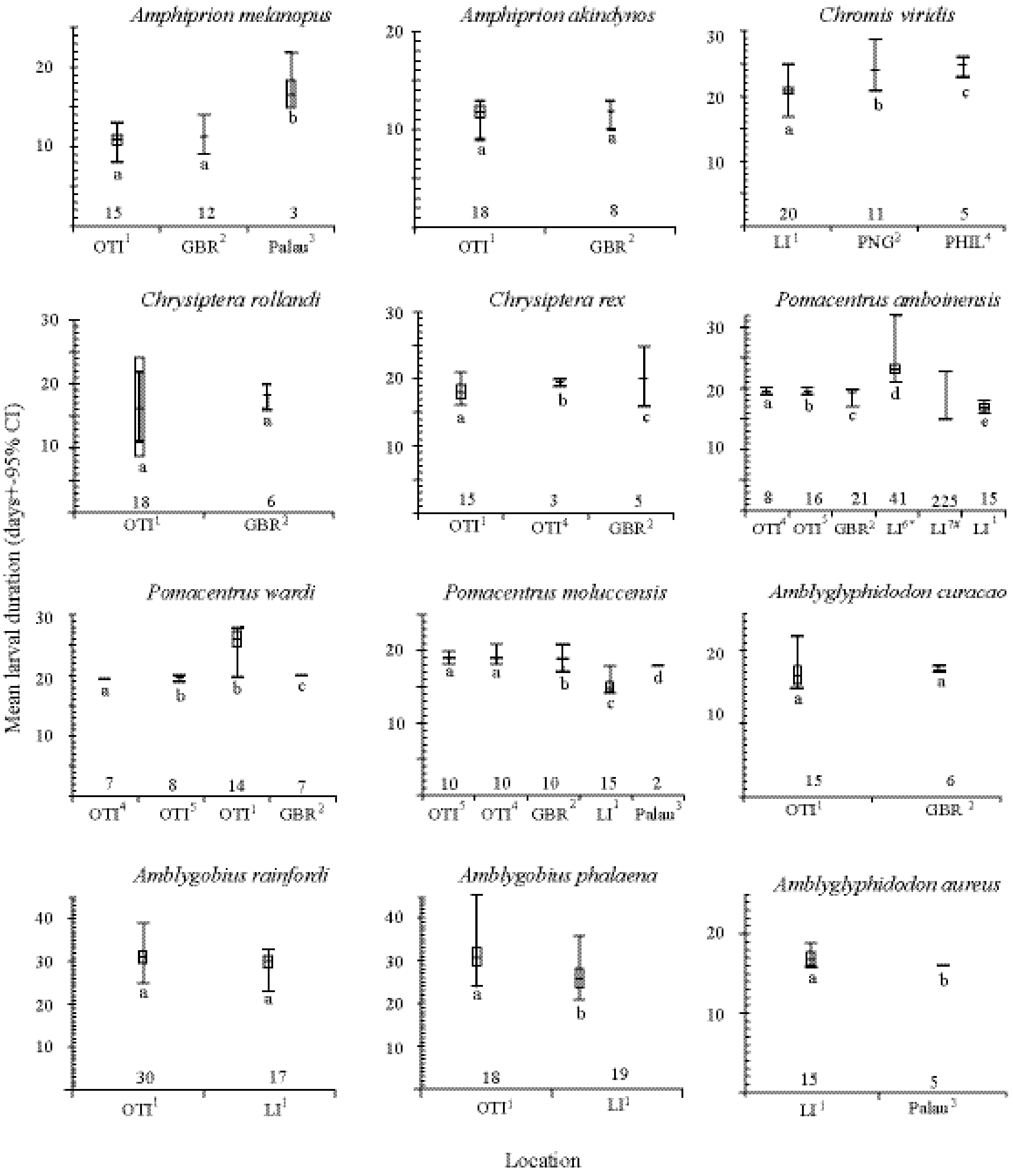

Fig. 1: Mean larval duration (horizontal bar) ± 95% confidence intervals (where available) (box) and range (whiskers) in twelve species of coral reef fish. Statistical significance and sample sizes are indicated below data points. OTI = One Tree Island, LI = Lizard Island, GBR = Great Barrier Reef (specific location not given), PHIL = Philippines, PNG = Papua New Guinea, Palau = Palau. Sources (year of sampling): [1]This study (2000), [2]Thresher et al. 1989 and Thresher and Brothers 1989 (1983), [3]Wellington and Victor 1989 (1987), [4]Brothers and Thresher 1985 (not indicated), [5]Brothers et al. 1983 (1976-77, 1978-99), [6]Wilson and McCormick 1997 (1994), [7]Kerrigan 1996 (1990-93). *1 Wilson and McCormick (1997) found no statistical difference in PLDs among 5 locations and for simplicity only one representative

location has been shown here. # Kerrigan (1996) found significant differences among seasons and season by pulses and only the overall range of PLDs has been indicated.

Cowen and Sponaugle 1997). The ranges of PLDs overlapped in all species examined except *A. melanopus* (Fig. 1).

The overlapping ranges of these PLDs may reflect environmentally mediated variation in growth rates during the pre-competent phase, and/or potentially behaviourally controlled delays in settlement (Cowen and Sponaugle 1997). Whatever the proximate cause of this observed variation in larval lifespan, the selective forces maintaining this plasticity remain to be understood.

Amphiprion melanopus, on the other hand, represents the first published example (to my knowledge) of intraspecific divergence of PLDs in coral reef fishes. This divergence suggests that local adaptation has occurred between Palauan and GBR populations of this species and that a longer larval duration has evolved in the apparently more isolated location (i.e., Palau) or shorter PLDs, among the well-connected locations (i.e., GBR). This conclusion, however, is based on a very small sample size from Palau (n = 3) and must remain tentative until more data are available. Similarly, it should be noted the non-overlapping pelagic larval durations are not necessary evidence of evolved differences in PLDs among populations. More subtle shifts in the distributions of PLDs among populations could also indicate evolved differences, but reliable estimations of such differences will require considerable additional sampling effort. These examples illustrate that conclusions derived from single point estimates of PLDs may fail to incorporate considerable intercohort variation in PLDs. Future investigations will need to consider this variation.

Geographical patterns in the length of larval life have primarily been investigated by interspecific or intergeneric comparisons (Brothers and Thresher 1985; Victor 1986a; Thresher et al. 1989; Wellington and Victor 1989; Victor and Wellington 2000) and several patterns have emerged from these analyses. Species, or genera, with shorter larval durations may have more localised populations (defined on the basis of colour pattern variation, Thresher et al. 1989), smaller ranges (Wellington and Victor 1989) and may also be characterised by considerable local genetic structure (Doherty et al. 1995; Nelson et al. 2000). Conversely, species at geographically isolated locations may have significantly longer PLDs compared to congeneric species at more central locations (Brothers and Thresher 1985; Victor 1986a). *A. melanopus* appears to have sufficiently restricted gene flow to allow for local adaptation in traits such as mean larval duration. Further investigations incorporating intraspecific variation in PLD among well-connected and isolated populations may provide new insights into the relationship between larval duration and geographical isolation. Pelagic larval duration has also been used as a proxy for dispersal ability (Victor and Wellington 2000) and, in turn, used to predict genetic differentiation among populations (Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Shulman 1998; Riginos and Victor 2001). Typically, studies of the relationship between PLD and genetic differentiation have used mean larval durations estimated at few times or places (Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Shulman 1998; Riginos and Victor 2001). Mean PLDs however, are clearly spatially and temporally variable (Cowen and Sponaugle 1997; this study). Because very low levels of migration can prevent genetic divergence through drift among locations (Wright, 1943), exploration of the relationship between PLD and genetic structuring of populations may be best done using maximum, rather than mean, larval durations (Leis 1991; Victor 1991), even if PLDs at a species' upper limit are only rarely expressed.

Conclusion

Variations in pelagic larval durations of coral reef fishes may be important in ecological and evolutionary contexts and provide significant information about many pre- and post-settlement processes that may be otherwise logistically difficult to document. By re-examining PLD in a range of coral reef fish species, this investigation has been able to provide three tangible examples of this. First, intracohort variability in PLDs can substantially underestimate intraspecific variability in this trait. Second, additional information presented here for one species, *P. wardi*, show that previously divergent ranges in PLDs at opposite ends of the GBR now appear to be overlapping at these locations. Third, with the addition of further estimates of PLD for *A. melanopus* on the GBR, the GBR and Palau populations of this species appear to have diverged. Understanding variation in larval duration is a worthwhile pursuit that has already provided many new insights, but one that can still yield more.

Chapter 5: The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef.

Publication: Bay LK Crozier RH and Caley MJ (In Press) The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Marine Biology

Abstract

Pelagic larval duration (PLD) is a commonly used proxy for dispersal potential in coral reef fishes. Here I examine the relationship between PLD, genetic structure and genetic variability in geographically widespread and ecological generalist species from one coral reef fish family (Pomacentridae) that differ in mean larval duration by more than a month. Genetic structure was estimated in eight species using a mitochondrial molecular marker (control region) and in a sub-set of five species using nuclear molecular markers (ISSRs). Estimates of genetic differentiation were similar among species with pelagic larvae, but differed between molecular markers. The mtDNA indicated no structure while the ISSR indicated some structure between the sampling locations. I found a relationship between PLD and genetic structure using both markers. These relationships, however, were caused by a single species, *Acanthochromis polyacanthus*, which differed from all the other species examined here in lacking a larval phase. With this species excluded, there was no relationship between PLD and genetic structure using either marker despite a range of PLDs of more than 20 days. Genetic diversities were generally high in all species and did not differ significantly among species and locations. Nucleotide diversity and total heterozygosity were negatively related to maximum PLD but again these relationships were caused by *A. polyacanthus* and disappeared when this species was excluded. These genetic patterns are consistent with moderate gene flow among well-connected locations and indicate that at this phylogenetic level (i.e., within family) the duration of the pelagic larval phase is unrelated to patterns of genetic differentiation.

Introduction

Identifying patterns of connectivity among populations and the extent of self-recruitment is important to the study of the population biology of marine species (e.g., Jones et al. 1999; Swearer et al. 1999; Taylor and Hellberg 2003) and to the effective management and conservation of marine resources (Palumbi et al. 2003; Palumbi 2004). Studies using genetic markers have become vital in this context due to the difficulties associated with directly observing movement among populations in the marine environment (e.g., Hedgecock 1986; Bohonak 1999; Hellberg et al. 2002). Although more genetic estimates of connectivity among marine populations are becoming available, generalisations about which species are likely to have genetically structured populations, and the processes driving such differentiation, are still hard to draw (Bohonak 1999). Comparative studies may be particularly important in filling this gap in our current knowledge since they allow for factors affecting dispersal to be isolated and controlled (Bohonak 1999).

Many marine organisms are relatively sedentary as adults, dispersing primarily during the larval phase (Leis 1991; Bonhomme and Planes 2000). The dispersal potential of these species may be related to traits such as egg type (e.g., pelagic vs. benthic: Shulman and Bermingham 1995; Shulman 1998) and larval development (direct vs. pelagic: Hunt 1993; Hellberg 1996; Arndt and Smith 1998; Ayre and Hughes 2000), length of the pelagic larval phase (PLD: Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001) and pelagic larval environment (inshore vs. offshore: Riginos and Victor 2001). In previous reports, species with shorter larval durations have generally displayed stronger genetic differentiation than species with longer larval durations (e.g., Waples 1987; Doherty et al. 1995; Riginos and Victor 2001). However, many exceptions, such as genetically differentiated populations of species with extensive larval durations have also been documented (e.g., Planes et al. 1998; Barber et al. 2000; Taylor and Hellberg 2003). Consequently, the relationship between potential and realised dispersal remains unclear (Shulman 1998; Bohonak 1999) and further study is warranted.

Behavioural, physiological and ecological factors may affect dispersal abilities, and these may vary among taxonomic groups (Bohonak 1999). For example, larval swimming ability, a potentially important determinant of dispersal distance varies considerably among reef-fish families (Stobutzki 1998; Leis 2002; Fisher 2005). Consequently, the distances that particular species may disperse during pelagic larval phases of the same length may differ among families with different swimming abilities. Likewise, the spawning mode and early developmental patterns of species may also affect larval dispersal (Bohonak 1999). Directly developing species generally display strong genetic structure across small geographical distances (Doherty et al. 1994; Bernardi 2000; Planes et al. 2001) and the inclusion of such species may greatly affect comparative investigations of PLD and gene flow (Bohonak 1999; Riginos and Victor 2001). Conversely, species that broadcast spawn pelagic eggs may display lower genetic structure, but it is currently unclear whether this occurs because of the early developmental state at which they enter the plankton, the distribution of these larvae in the water column or because of the longer larval duration generally associated with this suite of life history traits (Leis 1991; Victor 1991; Shulman 1998). Because examinations of the relationship between PLD and genetic structure in marine fishes to date have incorporated a range of distantly related species, displaying different spawning characteristics and adult ecologies (e.g., Waples 1987 (10 species, 9 families); Doherty et al. 1995 (7 species, 3 families); Shulman and Bermingham 1995 (8 species, 6 families); but see Riginos and Victor 2001 (3 species, 1 family)) current understanding of the relationship between genetic structure and pelagic larval duration in species that display little variation in their biology and ecology is poor.

Pelagic larval durations within species may vary greatly both spatially and temporally (Chapter 4). For example, the PLD of *Amphiprion melanopus* was reported as 16.7 days (±SE = 1.5) in a sample of 3 individuals from Palau (Wellington and Victor 1989) but 10.96 days (±SE = 0.32) in a sample of 15 individuals from One Tree Island on the Great Barrier Reef (Chapter 4). At present it is unclear whether such intra-specific variation in PLDs may affect the relationship with genetic structure because only mean larval duration has previously been used to predict genetic structure (e.g., Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001). Consequently, investigations that incorporate information about the mean pelagic larval duration and its variation have the potential to increase our understanding of the relationship between gene flow and larval duration further (Leis 1991; Victor 1991).

In order to minimise the potential effects of spawning strategy, adult ecology and phylogeny on any relationship between pelagic larval duration and gene flow, I examined the relationship between mean, minimum and maximum pelagic larval duration, genetic structure and genetic diversities among common, widespread and generalised species of the family Pomacentridae on the Great Barrier Reef (GBR). I estimated population genetic structure between northern and southern locations of the Great Barrier Reef, Australia, in eight species using a rapidly evolving mtDNA sequence marker (control region) and, in a sub-set of five species, using nuclear genetic fingerprints (ISSRs). In this technique semi-arbitrary banding profiles with bands corresponding to a DNA sequence deliniated by two microsatellites are amplified by PCR (Zietkiewicz et al. 1994) and compared among individuals. I examined the relationship between mean, minimum and maximum pelagic larval duration and genetic differentiation. I then examined patterns of genetic variability (heterozygosity, haplotype and nucleotide diversity) among species and locations and their relationships to mean, minimum and maximum pelagic larval duration. Lastly I re-analysed data from Doherty et al (1995) to examine the potential roles of using different estimates of PLD on the relationship between PLD and genetic structure.

Materials and Methods

Specimen collection and estimation of pelagic larval duration

Eight species that span a broad range of pomacentrid genera were selected. These species differ in their pelagic larval duration, but share characteristics such as benthic spawning, a generalised ecology in terms of habitat use, high local abundances and wide geographical distributions (Table 1). Based on their relationship between PLD and gene flow, Doherty et al. (1995) suggested that the genetic structure of species with larval durations of less than 9 days are likely to be primarily influenced by drift, and hence, display strong genetic structure, whereas, the population genetic structure of species with larval durations between 9 and 39 days are likely to be influenced by both drift and migration and species with larval durations longer than 39 days are likely to display low genetic structure due to high migration rates and minimal importance of drift (Doherty et al. 1995). Because I was interested in genetic structure that was under the influence of both drift and migration, I investigated species displaying pelagic larval durations of less than 39 days. No pomacentrid species with a larval duration of less than 9 days, other than *A. polyacanthus* (Table 1), was available for inclusion in this study.

Approximately 25 individuals per species were collected from each of the same two locations: Northern GBR (Lizard Island, 14°40S; 145°28E) and southern GBR (One Tree Island 23°30S; 152°05E). These locations are separated by more than 1200 km with no obvious habitat discontinuities, or other hard barriers to dispersal. Collections were made during 2000 and 2001 and all individuals from each species were collected in the same year. Fish were collected using hand spears, and clove oil and hand nets. Animals were placed in an ice slurry following capture, and then transported to shore where a tissue sample (fin clip preserved in 100% EtOH) was taken.

Table 1: Study species and their geographical, biological and ecological attributes. Markers used and sample sizes, latitudinal spread (Lat. spread), pelagic larval duration (PLD) (min – max), diet (P = planktivore, H = herbivore), reproductive mode (B = benthic spawning) and habitat use (L = live, D= dead).

	Sample sizes		Geography, Biology and Ecology				
Species	Control region	ISSR	Lat. spread[1]	PLD	Diet[1]	Rep.[1]	Habitat use[1]
Acanthochromis polyacanthus	41	46	40	0[2]	P	B	L/D coral
Amphiprion melanopus	42	46	40	11 (8 – 22)[3]	P	B	Anemones (3 spp.)
Pomacentrus moluccensis	46	48	55	15 (14 – 21)[3]	P/ H	B	L/D branching corals
Pomacentrus amboinensis	41		55	17 (15 – 32)[3]	P/ H	B	L/D coral, sand
Chromis atripectoralis	41	44	62	16 (10 – 24)[4]	P	B	L/D branching corals
Chrysiptera rex	46	48	57	18.2 (16 – 25)[3]	H	B	D coral
Amblyglyphidodon curacao	42		45	17 (15 – 22)[3]	P	B	L/D branching corals
Stegastes nigricans	39		60	28 (16 – 32)[5]	P/ H	B	L/D branching corals

Sources: [1]www.fishbase.org; [2]Kavanagh (2000); [3]Chapter 4; [4]Thorrold and Milicich (1990) and Murdoch (1995); [5]Thresher et al. (1989) and Wellington and Victor (1989).

All species were included in the mtDNA analysis and a sub-set of five of these species was included in the ISSR analysis (Table 1). In five species, published estimates of mean larval duration from counts of pre-settlement rings on otoliths of fishes from the GBR were used (Chapter 4) (Table 1). Estimates of mean larval duration were obtained for two additional species, *Chromis atripectoralis* and *Stegastes nigricans* by averaging published estimates from Thresher et al. (1989), Wellington and Victor (1989), Thorrold and Milicich (1990) and Murdoch (1995) (Table 1). Because point estimates of larval durations often display little variation (Chapter 4), the minimum and maximum larval durations used here were estimated by using the extremes of the range of pre-settlement ring counts published so far for each species (Table 1).

DNA extraction and amplification

Genomic DNA was extracted from approx 0.25 cm^2 of fin tissue (rehydrated by several TE washes) by a modified Phenol-Chloroform extraction procedure (Sambrook and Russell 2001, excluding the phenol-chloroform step) and resuspended in 50μl of TE. Concentrated DNA stock was diluted 1:50 yielding a final DNA concentration of approximately 5 ng/μL. A 400 base-pair region of the mitochondrial control region (hyper variable region I, HVR I) was amplified using the universal primers CR-A L15995 (5'-AATTCTCACCCCTAGCTCCCAAAG-3') and CR-E H16498 (5'-CCTGAAGTAGGAACCAGATG-3') (Lee et al. 1995). After a representative sample of all species had been sequenced, a specific forward primer was designed (dLoopF 5'-CATATATGTRTTATCAACATTA-3'), and this was used with CR-E in all further PCR and sequencing reactions. PCR reactions were carried out on a PE Applied Biosystems 9700 in 25μl containing 1x PCR Buffer (Promega), 3.5 mM $MgCl_2$, 200 μM each dNTP, 0.4 μM each primer, 10 ng template DNA and 0.1 unit of *Taq Polymerase* (Promega). Amplification using the polymerase chain reaction (PCR) was conducted with a cycling profile of 30 s at 94°C, 45 s at 48°C and 60 s at 72°C for 30 cycles. The cycling profile was flanked by an initial 2 min denaturing step (94°C) and a 10 min terminal extension phase (72°C). Using this procedure, only a single product ranging in size from 335 – 555 base pairs was amplified in most species, although two products (approximately 550 and 350 base pairs) were occasionally amplified in *Pomacentrus amboinensis*. Analysis of these fragments in *P. amboinensis* confirmed that the presence of the smaller fragment was due to a repeat in the t-RNA Pro end of the Control region as previously described for a pomacentrid species (Bernardi et al. 2002). Only the larger fragment was sequenced. PCR products were cleaned up using PCR clean up columns (Qiagen) and resuspended in 20μL of ddH2O. Two μL of the cleaned product was sequenced in the forward and reverse direction using a dyenamic ET dye terminator kit (Megabase) chemistry (Amersham Biosciences). Sequence products were cleaned using sephadex G-50 columns. Labelled extension products were sequenced on a Megabase 1000 (Amersham Biosciences) at the Genetic Analysis Facility of James Cook University. Representative sequences have been deposited in GenBank under accession numbers DQ199708 – DQ199726, DQ199879 – DQ199899, DQ212199 – DQ212495.

Genetic fingerprints were obtained from 21 – 24 individuals per location using Inter Simple Sequence Repeats (ISSR). In this technique, semi-arbitrary banding profiles (where each banding position corresponds to a DNA sequence delimited by two microsatellites) are amplified by PCR (Zietkiewicz et al. 1994; Bornet and Branchard 2001). Because the PCR reaction is primed by a specific but universal primer, it allows highly reproducible fingerprints to be rapidly obtained across distantly related taxa. Five 5 fluorolabelled universal primers were used (809: $(AG)_8G$, 834: $(AG)_8YT$, 841:$(AG)_8YC$, 864: $(ATG)_6$ and 880: $(GGAGA)_3$). Reactions were carried out in 15μL reactions containing 1x High Fidelity PCR Buffer, 2 mM $MgSO_4$, 200 μM each dNTP, 0.8 μM primer, 1 ng template DNA (diluted in water) and 0.3 unit of *Taq Polymerase* (Invitrogen Life Technologies). All amplifications were conducted on the same Peltier thermal cycler (DNA Engine Tetrad 2) following a cycling profile with an initial step of 96°C for 5 min then 35 cycles of denaturing at 96°C (1 min.), primer annealing at 50°C (30 sec.), then extension at 68°C (1 min.) and a final extension step of 68°C (10 min). PCR products were cleaned using sephadex G-50 plates (Whatman) and multiplexed into 10μL volumes containing 0.5μl of each product and 0.25μl standard (Amersham ET 900). Labelled extension products were genotyped on a Megabase 1000 (Amersham Biosciences) at the Genetic Analysis Facility in the Advanced Analytical Center (James Cook University). Presence and absence of bands between 50 and 850 base pairs were scored using MegaBACE Fragment Profiler 1.2 (Amersham Biosciences), then converted into binary data matrices and concatenated. Raw binary data matrices are available from the author upon request.

Statistical Analyses

mtDNA: Forward and reverse sequences were aligned using Sequencher 4.2 (GeneCodes Corp. Michigan USA). The best fitting substitution model and associated rate heterogeneity were estimated separately for each species using Modeltest 3.5 (Posada and Crandall 1998) and PAUP* 4.0b10 (Swofford 1998) and these, where possible, were implemented in all subsequent analyses. Genetic diversity measures (haplotype and nucleotide diversity) (Tajima 1983; Nei 1987) and their associated standard deviations were calculated using Arlequin 2.000 (Schneider et al. 2000). Standard deviations were converted to 95% confidence intervals (95% CI = $\underline{+}1.96*(SD / \surd(n))$) and genetic diversities were interpreted as statistically different when 95% confidence intervals did not overlap. Estimates of genetic structure were calculated as pairwise Φ_{ST} values following the methods implemented in Arlequin and significance levels were corrected for multiple comparisons following the Dunn-Sidak method (Sokal and Rohlf 1995). Transition–transversion ratios indicated that saturation may be occurring in some of the species, and therefore, all analyses were repeated using transversions only.

ISSR data: Due to the large number of fragments amplified by the 5 primers, only bands with minimum frequencies of 0.25 within species were analysed. No differences were found among analyses of 0.05, 0.1 and 0.25 minimum frequency data sets conducted for a subset of species (Bay, unpublished data). Mendelian segregation of fragments with a single dominant (amplified) and recessive (absent) allele at each banding position was assumed. Because dominant data do not allow within-individual heterozygosities to be estimated, Hardy-Weinberg equilibrium was assumed in the analysis of these data. Given the large population sizes and pelagic larval dispersal in all species except *A. polyacanthus,* this assumption was deemed reasonable in this study. Analogues of codominant genetic diversity measures including within-population diversity H_e (here H_j), total heterozygosity H_t and mean heterozygosity across populations H_s (here H_w) were calculated following the methods of Lynch and Milligan (1994) using AFLP-Surv 1 (Vekemans et al. 2002). Their associated standard errors were converted to 95% confidence intervals as above. Genetic differentiation (Φ_{PT}) was estimated using Genalex 5 (Peakall and Smouse 2001) and significance levels were corrected for multiple comparisons (Sokal and Rohlf 1995).

The relationships between genetic differentiation, genetic diversities and larval duration were explored independently for each marker using mean, minimum and maximum PLD both including and excluding *A. polyacanthus,* by fitting least-squares regressions. Estimates of fixation (Φ_{ST} and Φ_{PT}) showed some departure from normality, however, transformations failed to rectify these and untransformed data were analysed. Genetic diversity estimates between locations for each species separately were interpreted as statistically different when confidence intervals did not overlap. The non-parametric Mann Whitney U test was used to test for differences in genetic diversities between locations and the Kruskal-Wallis test was used to test for differences among species. The results of Doherty et al. (1995) were reanalysed using log transformed mean F_{ST} values from their Table 5, mean, minimum and maximum PLD values from their Table 1 and the mean, minimum and maximum PLD values used here (Table 1).

Results

Genetic differentiation and PLD

Control region: 335 – 555 base pairs at the 5' end of the control region were resolved in 39 – 46 individuals from each of eight species of pomacentrid fishes on the Great Barrier Reef (Table 2). Pairwise genetic distances were generally low and insignificant in most species (although near significant in *A. curacao* and *C. rex*), but high and significant in *A. polyacanthus* (Table 3). A significant negative relationship between mean, minimum and maximum PLD (Mean PLD: $F_{(1,6)} = 17.23$, $P = 0.006$; Minimum PLD: $F_{(1,6)} = 11.21$, $P = 0.015$; Maximum PLD: ($F_{(1,6)} = 15.85$, $P = 0.007$) and Φ_{ST} was evident (Fig. 1). Mean, minimum and maximum PLD explained 70, 59 and 68% of the variation in Φ_{ST}, respectively. These relationships were not significantly different with the 95% confidence intervals of all slopes and intercepts overlapping (mean PLD: $y = -0.037\ (-0.06 – -0.02)\ x + 0.679\ (0.304 – 1.05)$; Min PLD: $y = -0.038\ (-0.07 – -0.01)\ x + 0.611\ (0.203 – 1.019)$; Max PLD: $y = -0.027\ (-0.04 – -0.01)\ x + 0.689\ (0.299 – 1.079)$. Consequently, only the relationship between mean PLD and Φ_{ST} is presented (Fig. 1). All three relationships, however, were driven primarily by *A. polyacanthus.* With this species removed, little variation in genetic structure was explained (Mean PLD: $F_{(1,5)} = 2.89$, $P = 0.15$; Min PLD: $F_{(1,5)} = 1.804$, $P = 0.237$; Max PLD:$F_{(1,5)} = 2.76$, $P = 0.16$).

Table 2: Sample sizes, Number of transitions (ts) vs. number of transversions (tv), ts/tv ratio, substitution model selected and among rates variation (gamma) estimated by Modeltest and model implemented for the eight species.

Species	No. base pairs	No.Ts/ No.Tv	ts - tv ratio	Model	-ln Likelihood	Gamma	Model[1]
Acanthochromis polyacanthus	351	34/24	8.5	TN(93)	1220.65	0.3012	TN(93) + G
Amphiprion melanopus	335	17/3	6.3	HKY	576.73	0	TN(93)
Pomacentrus moluccensis	349	21/4	5.9	HKY	652.70	0	TN(93)
Pomacentrus amboinensis	555	26/7	3.4	HKY	983.98	0	TN(93)
Chromis atripectoralis	349	42/7	7.8	HKY	924.56	0	TN(93)
Amblyglyphidodon curacao	398	73/5	12.5	HKY	989.57	0.1687	TN(93) + G
Chrysiptera rex	348	31/3	14.4	HKY	751.84	0.0069	TN(93) + G
Stegastes nigricans	423	51/5	16.1	HKY	1065.54	0.0146	TN(93) + G

[1] Implemented in Arlequin.

Transitions accounted for the majority of substitutions in all species (Table 2) although they accounted for less of the variation in *A. polyacanthus*. Therefore, the population genetic structure of these species may be affected by saturation. Estimates of genetic differentiation based on transversions alone were low and insignificant in all species except *A. polyacanthus* (Table 3). The exclusion of transitions did not appear to affect the relationship with PLD. Using the transversion data alone, PLD explained less variation in Φ_{ST} (Adj. $R^2 = 0.54$) and was not significantly different to the relationship between PLD and Φ_{ST} obtained with both transitions and transversions because the 95% confidence intervals of both slopes and intercepts overlapped (Mean PLD: $F_{(1,6)} = 9.128$, $P = 0.02$, $y = -0.033\ (-0.059 - -0.006)x + 0.64\ (0.18 - 1.10)$). This relationship also became statistically insignificant with *A. polyacanthus* removed ($F_{(1,5)} = 0.76$, $P = 0.42$).

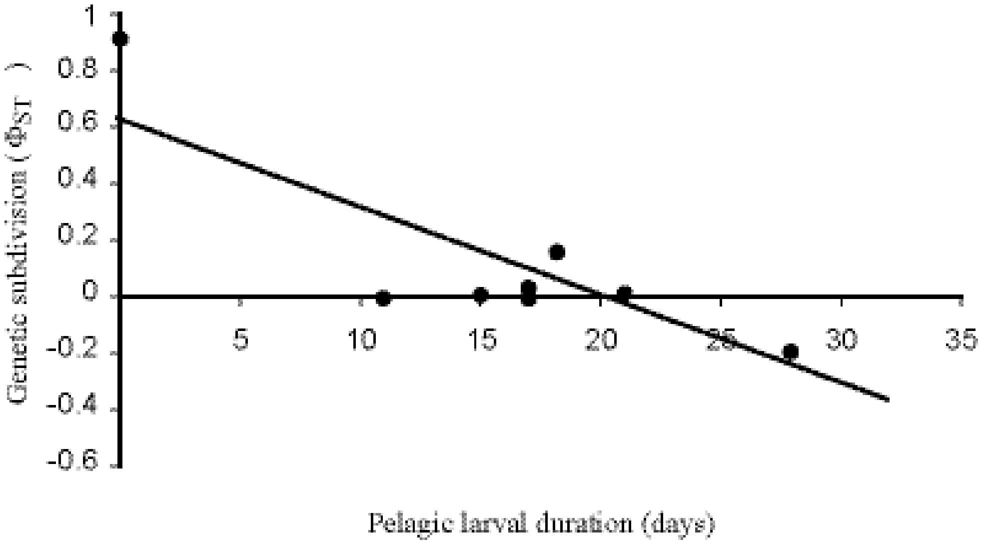

Fig. 1: The relationships between mean pelagic larval duration and genetic structure of the mtDNA (Φ_{ST}) in eight species of coral reef fishes.

Estimates of genetic differentiation based on the ISSR markers were higher compared to estimates based on the mtDNA control region and indicated significant genetic structure between sampling locations in all species (Table 3). There was a significant negative relationship between minimum PLD and genetic subdivision explaining 85% of the variation ($F_{(1,3)} = 22.81$, $P = 0.01$) (Fig. 2).

Table 3: Estimates of genetic differentiation based on MtDNA control region (Φ_{ST}) including and excluding transitions and ISSRs (Φ_{PT}) and their significance levels.

Species	Transitions and transversions		Transversions		ISSR	
	Φ_{ST}	p	Φ_{ST}	P	Φ_{PT}	p
Acanthochromis polyacanthus	0.91	<0.001[1]	0.89	<0.001[1]	0.426	0.001[2]
Amphiprion melanopus	-0.005	0.47	-0.001	0.42	0.188	0.001[3]
Pomacentrus moluccensis	0.006	0.25	0.01	0.18	0.018	0.022[4]
Pomacentrus amboinensis	-0.008	0.60	-0.002	0.45		
Chromis atripectoralis	0.012	0.23	0.011	0.25	0.272	0.001[5]
Amblyglyphidodon curacao	0.030	0.06	0.024	0.07		
Chrysiptera rex	0.158	0.28	0.044	0.07	0.027	0.007[6]
Stegastes nigricans	-0.195	0.97	0.014	0.17		

Bonferroni corrected α: [1] 0.006, [2] 0.01, [3] 0.02, [4] 0.05, [5] 0.01, [6] 0.03.

However, neither mean nor maximum PLD was significantly related to Φ_{PT} (Mean PLD: $F_{(1,3)} = 5.76$, $P = 0.1$; Max PLD: $F_{(1,3)} = 4.00$, $P = 0.14$). The relationship between minimum PLD and Φ_{PT} became statistically insignificant upon exclusion of *A. polyacanthus* ($F_{(1,2)} = 4.98$, $P = 0.16$).

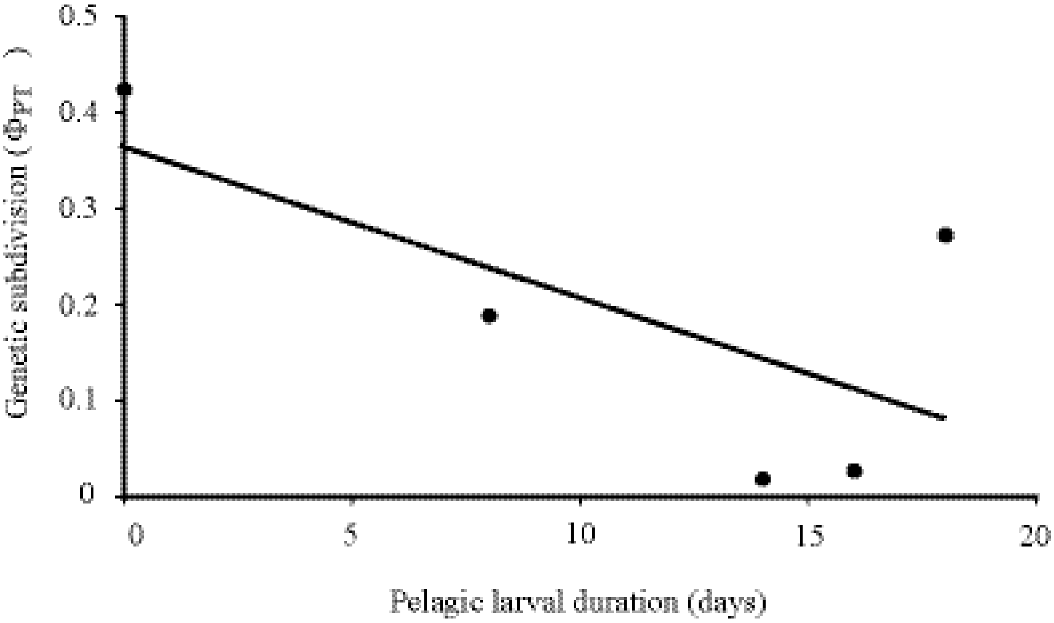

Fig. 2: The relationships between minimum pelagic larval duration and genetic structure of the nucDNA (Φ_{PT}) in five species of coral reef fishes.

A re-analysis of the results of Doherty et al. (1995) found that their relationship between PLD and gene flow remained significant when using their estimate of minimum PLD ($F_{(1,5)} = 22.26$, $P = 0.005$, Adj. $R^2 = 0.78$) but became insignificant when maximum PLD was used ($F_{(1,5)} = 4.95$, $P = 0.07$, Adj. $R^2 = 0.39$). The relationships between mean and minimum PLD and genetic structure were not significantly different and the 95% confidence intervals of both slopes and intercepts overlapped (Mean PLD: $y = -0.04\ (-0.06 – -0.02)x -- 0.313\ (-0.95 – 0.32)$; Min PLD: $y = -0.04\ (-0.07 – -0.02)x - 0.343\ (-1.04 – 0.35)$). The relationship between mean PLD and genetic structure of the benthic spawning species (including the brooding species) became insignificant when mean PLD values estimated in Chapter 4 were used (($F_{(1,3)} = 4.07$, $P = 0.14$). Maximum PLD values (Chapter 4) produced a significant relationship with genetic differentiation, whereas minimum PLD did not (Max PLD: $F_{(1,3)} = 13.51$, $P = 0.035$; Min PLD: $F_{(1,3)} = 2.08$, $p = 0.245$).

Genetic diversity and PLD

None of mean, minimum or maximum PLD could predict haplotype diversity (Mean PLD: $F_{(1,6)} = 3.51$, $P = 0.11$; Min PLD: $F_{(1,6)} = 1.74$, $P = 0.24$; Max PLD: $F_{(1,6)} = 3.14$, $P = 0.13$). Mean and maximum PLD were negatively related to nucleotide diversity (Mean PLD: $F_{(1,6)} = 6.49$, $P = 0.04$, Adj. R^2 0.44; Max PLD: $F_{(1,6)} = 18.18$, $P = 0.005$, Adj. $R^2 = 0.53$) but again this relationship was reliant on *A. polyacanthus* (with *A. polyacanthus* excluded: Mean PLD: $F_{(1,5)} = 0.58$, $P = 0.48$; Max PLD: $F_{(1,5)} = 0.02$, $P = 0.89$). Minimum PLD did not predict nucleotide diversities (Min PLD: $F_{(1,6)} = 5.89$, $P = 0.051$). Minimum PLD could predict total heterozygosity (H_t) ($F_{(1,3)} = 12.39$, $P = 0.039$, Adj. $R^2 = 0.74$), but neither mean nor maximum PLD could predict H_t (Mean PLD: $F_{(1,3)} = 6.97$, $P = 0.078$; Max PLD: $F_{(1,3)} = 2.57$, $P = 0.21$). Again, this relationship became insignificant upon exclusion of *A. polyacanthus* ($F_{(1,2)} = 4.59$, $P = 0.17$). Neither mean, minimum nor maximum PLD could predict mean heterozygosity among locations (H_j) (Mean PLD: $F_{(1,3)} = 1.99$, $P = 0.25$; Min PLD: $F_{(1,3)} = 3.77$, $P = 0.15$; Max PLD: $F_{(1,3)} = 2.89$, $P = 0.19$).

Genetic diversities among species and locations

Haplotype diversities ranged from 0.8 – 1, other than *A. polyacanthus* at One Tree Island which was 0.49. Haplotype diversities were similar between sampling locations for all species except two (*A. polyacanthus* and *C. rex*). In these species, the northern populations (Lizard Island) were more diverse than the southern ones (One Tree Island) (Fig. 3a). Nucleotide diversities ranged from 0.1 – 3.2% (although most species were less than 2.5%) and were similar between sampling locations of all species except two. In *A. polyacanthus,* the northern location was more diverse, and in *P. amboinensis* the southern location was more diverse (Fig. 3b). Heterozygosities ranged from 0.3 – 0.4 among species and locations, and were significantly different between sampling locations in two species (*A. melanopus* and *C. atripectoralis*) (Fig. 4). There were no significant differences between haplotype diversity (Fig. 3a Mann-Whitney $U = 23.5$, $Z = -0.89$, $P = 0.37$), nucleotide diversity (Fig. 3b Mann-Whitney $U = 29.5$, $Z = -0.26$, $P = 0.79$) or heterozygosity (Fig. 4 Mann-Whitney $U = 12$, $Z = -0.104$, $P = 0.92$) and geographical location, nor were there any significant differences in genetic diversities among species (Haplotype diversity: Kruskal-Wallis $H_{(7,\ 16)} = 13.08$, $P = 0.07$;

Nucleotide diversity Kruskal-Wallis H $_{(7, 16)}$ = 9.26, P = 0.23; H_w: Kruskal-Wallis $H_{(4, 10)}$ = 5.345, P = 0.25).

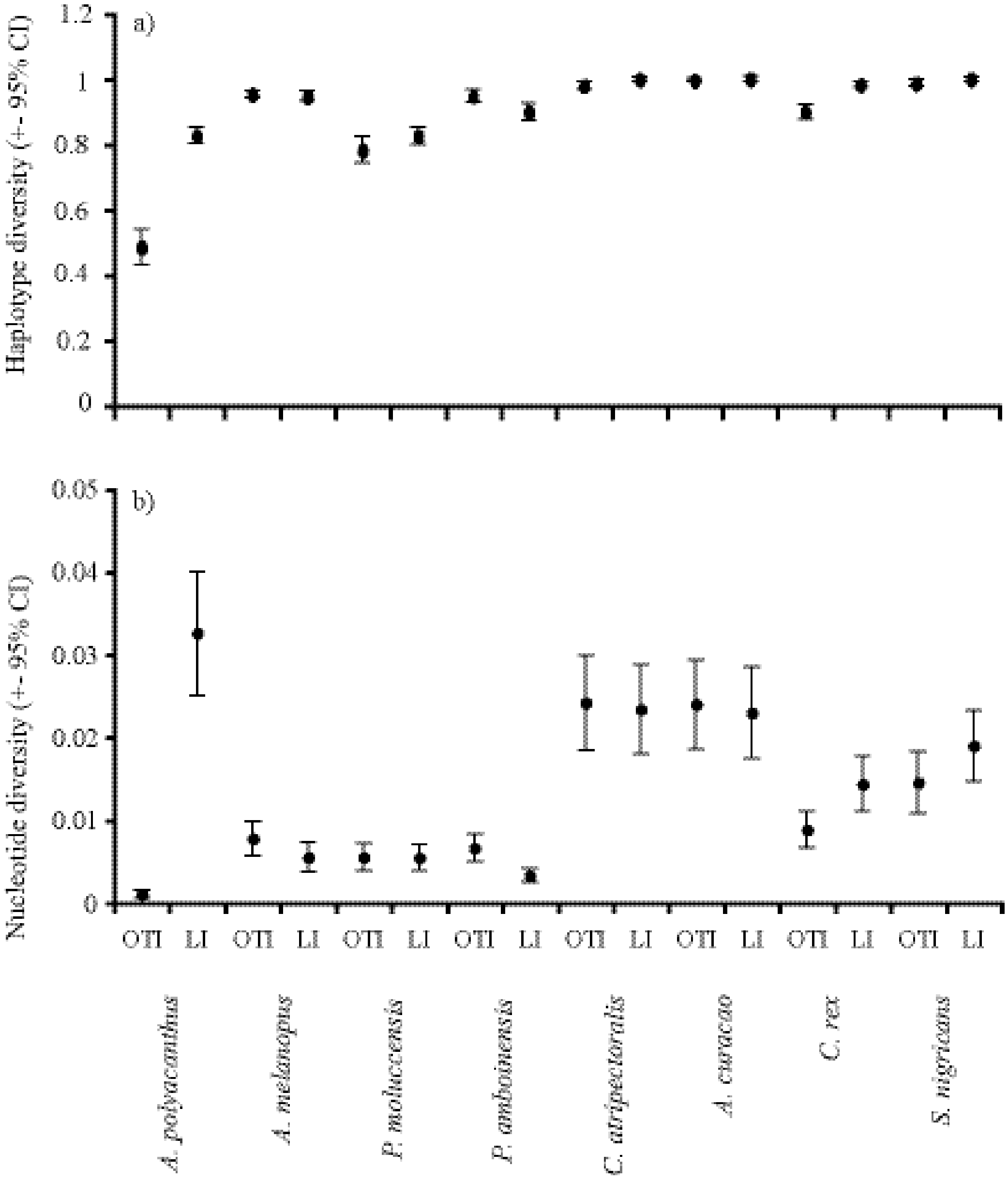

Fig. 3: Genetic diversities of the mtDNA in southern (One Tree Island, OTI) and northern (Lizard Island, LI) populations of eight species of coral reef fishes.

Discussion

Genetic structure on the GBR

Estimates of genetic differentiation differed between molecular markers, but were consistent among species. In both datasets, *A. polyacanthus* displayed much stronger genetic differentiation than all other species, although less structure was indicated by the ISSRs (Table 3). This result was expected given the strong genetic structure often recorded in this species (Doherty et al. 1994; Doherty et al. 1995; Chapter 2). It also adds support to the notion that directly developing marine species generally display more genetic structure than ones with pelagic larvae (e.g., Hunt 1993; Hellberg 1996; Arndt and Smith 1998).

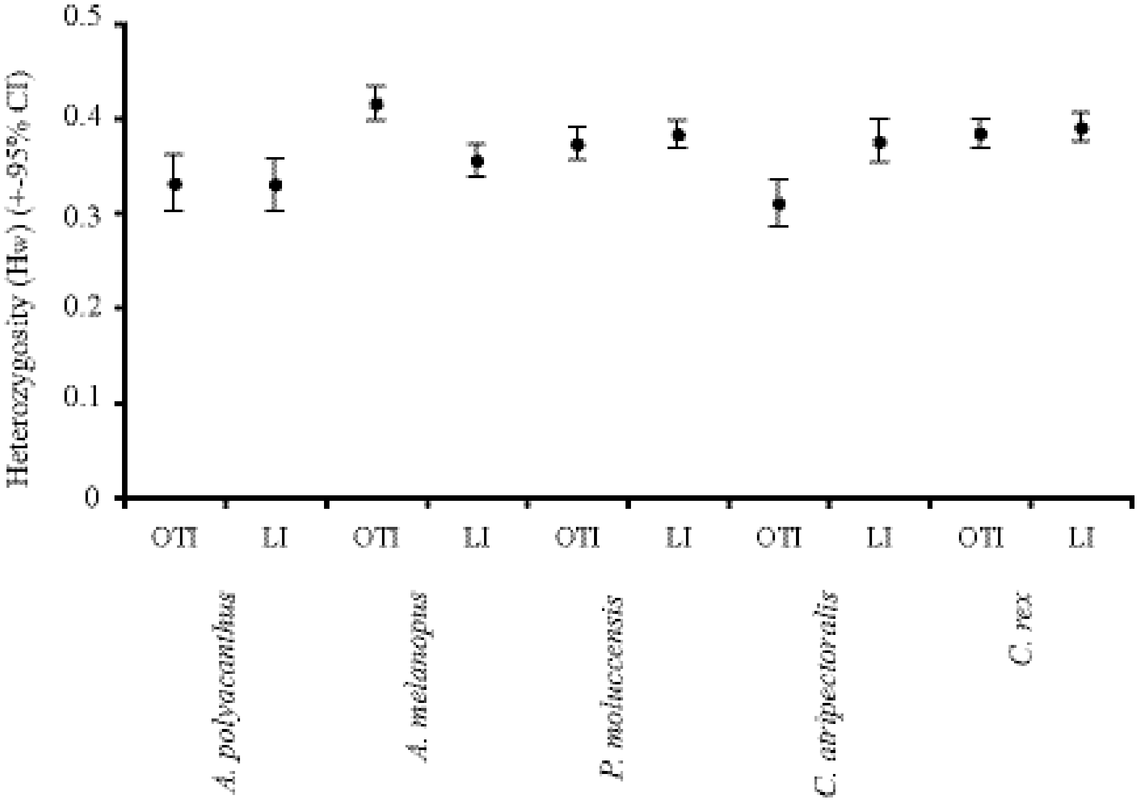

Fig. 4: Within population heterozygosity (H_w) in southern (One Tree Island, OTI) and northern (Lizard Island, LI) populations of five species of coral reef fishes.

The mtDNA marker revealed no genetic structure between northern and southern locations in all species with pelagic larvae. These species all displayed very small and insignificant Φ_{ST} estimates (Table 3). In contrast, the ISSRs revealed significant structure between the sampling locations with Φ_{PT} values ranging from 0.02 – 0.27 (Table 3). Such differences in the population structure indicated by the two markers may arise because of the large effective population size (reducing the effect of genetic drift) and the higher variability of the nucDNA marker compared to the mtDNA marker. The higher level of fixation indicated by the mtDNA may also be indicative of sex-biased dispersal. Sex-biased dispersal may evolve when there are sex-biased fitness consequences associated with acquiring and defending reproductive resources (Greenwood 1980; Clarke et al. 1997). Where sex-biased dispersal has been reported in fishes, it tends to be male biased (Hutchings and Gerber 2002; Fraser et al. 2004). Sex-biased dispersal can be identified directly by a comparison of the population genetic structure of males and females (Mossman and Waser 1999), or indirectly, by a comparison of maternally inherited molecular markers and those with bi-parental inheritance (Avise 2000). If males migrate at higher rates than females, maternally inherited mitochondrial genes should show more genetic structure, compared to bi-parentally inherited nuclear genes. This is the pattern observed in *A. polyacanthus* (Table 3). Conversely, if dispersal is female biased, mitochondrial markers would be expected to be less differentiated than nuclear markers. This pattern was observed in all species with pelagic larvae (Table 3). Male-biased dispersal has recently been suggested for *A. polyacanthus* on the GBR (Chapter 2) and the higher genetic structure of the mtDNA marker compared with the nucDNA marker reported here is consistent with this hypothesis (Φ_{ST} = 0.90; Φ_{PT} = 0.43). The other species included in this study are all thought to disperse primarily during the pelagic larval phase (Leis 1991; Bonhomme and Planes 2000). It is unclear whether these larvae are sex differentiated (Fishelson 1998) and if so, how female larvae would realise greater dispersal than male larvae. Furthermore, the adults of many of the species included here change sex (e.g., *A. melanopus* Godwin and Thomas 1993) and reproductive resources are defended by males in most of the species (but females in *A. melanopus*). Therefore, I consider that the differences in genetic differentiation between the mtDNA and nucDNA markers for these species with pelagic larvae are unlikely to be caused by sex-biased dispersal.

It is also possible that the differences in population structure indicated by the mtDNA and nucDNA markers were caused by differences in mutation rates between markers. The ISSRs record microsatellite variation (Zietkiewicz et al. 1994; Bornet and Branchard 2001) and should therefore evolve at a rapid rate. If so, under low levels of migration, such rapidly evolving markers should show local genetic structure because of a high mutation rate, whereas markers evolving at a slower rate might not (Avise 2000). Consequently, all species with pelagic larvae included in this study display genetic structures consistent with some migration between populations in the northern and southern parts of the GBR. This conclusion is compatible with previous results based on allozyme markers (Doherty et al. 1995) where significant genetic structure was found in five of the same species among locations on the GBR.

Genetic diversities may differ within and among species due to differences in their evolutionary histories (Fauvelot et al. 2003). However, genetic diversities recorded here were similar within and among species. Haplotype diversities were generally high and comparable with those observed in other coral reef fishes on the GBR (e.g., *Chlorurus sordidus* $h = 0.98$ Bay et al. 2004; *Gobiodon histrio* $h = 0.87$ Munday et al. 2004; *Pseudochromis fuscus* $h = 0.79$ Messmer et al. 2005). The very low haplotype diversity in the southern population of *A. polyacanthus* is most likely due to a recent mitochondrial or population bottleneck (van Herwerden and Doherty 2006; Chapter 3). Nucleotide diversities were also generally high and comparable to estimates from other coral reef fish species on the GBR (e.g., *Chlorurus sordidus* $\% \pi = 3.0$ Bay et al. 2004; *Gobiodon histrio* $\% \pi = 1.23$ Munday et al. 2004; *Pseudochromis fuscus* $\% \pi = 0.36$ Messmer et al. 2005). The very high nucleotide diversity from the northern population of *A. polyacanthus* appears to be the result of the presence of two differentiated mtDNA lineages in this region (Chapter 3). Estimates of heterozygosity were similar between sampling locations within species (except in *A. melanopus* and *C. atripectoralis*) and among species (Fig. 4). ISSRs have not previously been used to investigate the population genetic structure of fishes and could therefore not be compared to previous estimates. Consequently, the estimates of genetic variability of both mtDNA and nucDNA markers indicated that recent evolutionary histories did not differ substantially among locations or species.

PLD and population genetic structure

I identified a relationship between PLD and genetic structure using both the mtDNA and the nucDNA markers (Fig. 1 and 2). However, this relationship was strongly influenced by the inclusion of the directly developing species (*A. polyacanthus*) that displayed strong genetic differentiation between locations. Once this species was removed from the analysis, little variation was explained. Riginos and Victor (2001) found a significant relationship between PLD and gene flow in three species of blennies with mean larval durations spanning 32 days (range = 28 – 50 days). It is possible that the range of larval durations exhibited by the species included in the present study (28 days) was not great enough to reveal measurable differences in genetic differentiation.

However, given the significant genetic structure revealed by the ISSRs here and the analysis by Doherty et al. (1995) indicating that the genetic structure of species with larval durations between 9 – 39 days should be determined by the opposing forces of drift and migration, such a possibility appears unlikely. Instead, the species with pelagic larvae included in this study more likely displayed very similar levels of genetic structure unrelated to the length of their larval life. Because these species are closely related, and have relatively generalised ecologies and similar spawning behaviours (Table 1), this result may indicate that biological and ecological traits, some of which may be associated with phylogeny, may be important in producing differences in gene flow, independent of the duration of the pelagic larval phase.

Population genetic structure may result from a range of causes that were not explored here. For example, ecological specialisation may play an important role in shaping patterns of genetic structure even in species with extensive larval durations (e.g., Taylor and Hellberg 2003; Rocha et al. 2005). The inclusion of such species may have affected previous investigations of the relationship between genetic structure and spawning strategy. For example, Shulman and Bermingham (1995) reported high genetic structure in *Halichoeres bivittatus* indicating low migration rates contrary to expectations in a species with pelagic eggs and a relatively long larval duration (i.e., 24.1 days). The high population genetic structure of this species was more recently attributed to its level of ecological specialisation (Rocha et al. 2005). With the removal of this species, the data of Shulman and Bermingham (1995) were consistent with the idea of higher gene flow in pelagically spawning species. Consequently, examinations of the relationship between gene flow and PLD or spawning mode need to account, where possible, for such biological and ecological factors. Future investigations comparing the relationship between PLD and genetic differentiation among groups of species with different biological, ecological or biogeographical attributes have the potential to illuminate this issue further.

PLDs may vary substantially within species (e.g., Chapter 4). Such variation could weaken any relationship between PLD and genetic differentiation (Leis 1991; Victor 1991) and may vary among datasets generated using different molecular markers that evolve at different rates. For example, the population genetic structure recorded by more slowly evolving molecular markers may be greatly affected by low levels of migration (Wright 1931). Individuals displaying a longer PLD may, therefore, influence the population genetic structure of species, even if such maximum PLDs are only rarely expressed. In contrast, allele frequencies of rapidly evolving molecular markers should not be as strongly affected by rare long distance dispersers because of the rate at which new genetic variation is generated by mutation within local populations. My analyses, and the re-analysis of the results of Doherty et al. (1995), indicated that the relationship between PLD and genetic structure may differ depending on the estimate of PLD used and its variation. Using mean, minimum or maximum PLD did not affect the relationship between PLD and genetic structure estimated using mtDNA. Only minimum PLD could predict gene flow and total heterozygosity using the more rapidly evolving nucDNA marker. The estimates of genetic structure based on allozymes could be predicted from their estimates of mean and minimum PLD, but not from my mean and maximum PLD estimates. The low number of species included in the ISSR analysis could potentially have restricted my ability to detect such relationships using this marker. However, it is also likely that the population genetic structure of the species investigated here based on the nucDNA, and presumably faster evolving marker, were more strongly influenced by individuals displaying a shorter PLD, whereas the relationships based on the mtDNA, and presumably slower evolving marker were more strongly affected by mean and maximum larval durations.

Conclusion

I identified a relationships between PLD and population genetic structure using both mtDNA and nucDNA markers. These relationships, however, were dependent on the inclusion of a directly developing species with high genetic structure. With this species removed PLD could not predict population genetic structure in the species examined here. The relationship between PLD and genetic structure varied depending on the estimates of PLD and genetic structure used. These results suggest that observed relationships between the population genetic structure and pelagic larval duration may be highly dependent on the molecular marker, estimator of PLD and species used. Further consideration of such variation has the potential to provide additional insights into the relationship between population genetic structure and dispersal potential.

Chapter 6: Greater genetic diversity on the edges of species’ ranges: Secondary contact among differentiated lineages?

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Abstract

Using mtDNA (control region) and nuclear (ISSR) markers, I investigated the population genetic structure of three congeneric species pairs of pomacentrid reef fishes (Pomacentridae) in the context of species’ borders theory. Each species pair consisted of one species sampled at two central locations within its geographic range, and one species sampled at the same locations, but for which these locations constituted one location toward the centre of its range and another close to its edge. Theory predicts that populations located on the periphery of a species’ range should be smaller and more fragmented and hence, display greater genetic structure among populations and lower genetic diversities within populations, compared to more centrally located populations. Estimates of genetic structure did not differ among central and peripheral species as expected. Similarly in contrast to predictions from theory, genetic diversities were greater in species whose sampling included a population toward the edge of its geographic range compared to species sampled at two locations toward the centre of their range. In two of the three species pairs, the distribution of genetic variation indicated secondary contact among differentiated lineages in the species sampled towards its periphery, but not in its congener that was not sampled towards a range edge. Elevated mutation rates mediated by environmental stress on the species’ margin may have contributed further genetic variability in these species.

Introduction

What limits the geographic ranges of species has long interested biologists (Darwin 1859, Mayr 1963), yet many issues in this field remain unresolved (Holt and Keitt 2005; Holt et al. 2005). Ultimately, the distributional range of a species will be determined by vital demographic rates and their variation across geographic ranges and through time, with borders forming where population growth rates approach zero beyond some point (Holt et al. 2005). Zero growth rates may result from physical barriers to dispersal, or because of discontinuities in suitable physical and biological environments (Gaston 2003; Holt et al. 2005). Species borders, however, commonly occur in the absence of such barriers, suggesting that range edges form in response to other demographic processes that result in a decline in fitness from the centre to the periphery or through changing metapopulation dynamics towards the species' margin (Lennon et al. 1997; Holt and Keitt 2000).

Generally, biological and environmental conditions are assumed to be optimal in the centre of a species' distribution and to decline towards its periphery (Hoffmann and Parsons 1991). As a result, population density should be highest in the centre of the species range and decline towards range edges (Brown 1984; Vucetich and Waite 2003; Guo et al. 2005 and references therein). Marginal populations should, therefore, become smaller and more fragmented (Vucetich and Waite 2003). They should also experience lower levels of migration among populations and hence display stronger genetic structure (Holt 1987; Lennon et al. 1997). Greater genetic structure has been reported towards species margins in some species (e.g., Gapare and Aitken 2005; Ayre and Hughes 2004) but not in others (Grant and Antonovics 1978). Consequently, the role of reduced gene flow towards the edge of the range in determining species borders is at present unclear.

The demographic processes operating on at a species border should be evident in the effective population sizes and genetic diversities of such populations. At migration – drift equilibrium, genetic diversity can be expressed as the effective number of alleles:

$$H = \frac{4N_e\mu}{1 + 4N_e\mu} \qquad \text{(Eq. 1)}$$

where N_e is the effective population size and μ is the mutation rate per site per generation. $N_e\mu$ is multiplied by 4 to account for the biparental origin of a diploid marker. Assuming mutation rates are equal among populations, genetic diversity becomes a function of the effective population size and generation time. The effective population size is almost always smaller than the actual size of the population (N) (Hartl and Clark 1997). Differences between N and N_e may arise when N fluctuates (Hartl and Clark 1997). Under such circumstances and because it is the harmonic mean of N, N_e tends to be the most affected by the smallest value of N. N_e will be low in populations that have undergone a severe reduction in size, or that have been founded by a small number of individuals. Marginal population should, therefore, display lower effective population sizes and lower genetic diversities compared to more centrally located populations (Holt 1987). Empirical investigations of variation in genetic diversities across a species' range have reported lower genetic diversities in peripheral populations in some species (e.g., Jain et al. 1981; Kat 1982; Schnabel and Hamrich 1990; Palumbi et al. 1997; Bowen et al. 1997; Durka 1999; Pedersen and Loeschcke 2001; McCauley and Ballard 2002; Hoffman and Blouin 2004; Lecomte et al. 2004) but not in others (Tigerstedt 1973; van Rossum et al. 1997; Betancourt et al. 1991; Planes and Fauvelot 2002; Garner et al. 2004; Gapare et al. 2005). Consequently, empirical support for this relationship remains equivocal and warrants further study.

Estimates of genetic diversity may also be affected by the mutation rate (Eq. 1). Mutation rates may vary among markers (Nei and Graur 1984), but are commonly assumed to be constant within markers among populations and closely related species (Avise 2000). Indeed, constant mutation rate is an explicit assumption in most commonly used population genetic analyses (e.g., AMOVA, Weir and Cockerham 1984). Emerging evidence, however, suggests that mutation rates of neutral genetic markers may be increased by environmentally induced stress (Parsons 1987; Hoffmann and Parsons 1991). This may occur through a variety of processes including a stress-induced error prone DNA repair mechanism (Walker 1984; MacPhee 1984). Elevated mutation rates following sub-lethal stress has only been demonstrated under controlled laboratory conditions (Lindgren 1972; Kerkis 1975; Belyaev and Borodin 1982). Consequently, it is not clear how widespread this mechanism is and how it may affect the population genetic structure of wild populations.

Coral reef fishes provide an excellent model for examining the evolution of species borders because they are speciose and their ranges are relatively well known. Their diversity enables the design of comparative studies that allow issues of species borders evolution to be addressed. Biological and ecological factors which may affect patterns of genetic variation such as ecological specificity (Nevo 1978; Smith and Fujio

1982), demographic and reproductive characteristics (e.g., Selander and Kaufman 1973; Mitton and Lewis 1989) can be controlled through the selection of species that display similar ecological and biological attributes. Despite this, coral reef fishes have not previously been used as a model system to test species border theory.

Here I examine the evolution of species borders in coral reef fishes on the Great Barrier Reef (GBR) using a comparative design. Using a mtDNA sequence marker (Control region) and nuclear genetic fingerprints (ISSR) I examine the population genetic structure of species sampled towards their range margin and compare this to congeneric species sampled in the centre of their ranges. I test the hypotheses that 1) species experience higher genetic structure towards the species' margin and 2) demographic processes such as smaller and more fragmented populations result in decreased genetic diversity in peripheral populations.

Materials and methods

Study species and locations

Three congeneric species pairs were selected for use in this study. Each species pair was selected from a different pomacentrid genus and was collected from a combination of three locations separated by 800-1200 km. There are no known dispersal barriers separating any of these locations. From each species pairs, one species had a distribution that allowed it to be collected from a location toward the centre of that species' geographic range and a location close to a geographic range limit. These species are hereafter referred to as the peripheral species. The congeneric species of each of the peripheral species had a geographic range that extended well beyond the sampling locations allowing it to be collected from two central locations. These species are hereafter referred to as the central species. Species pairs were also selected to control for other biological and ecological attributes that could otherwise confound the population genetic structure of these species. Species were selected that had similar dispersal potentials (Chapter 5), habitat use, diets, reproductive modes and generation time (Table 1). Distributional information was obtained from guidebooks (e.g., Randall et al. 1997; Kuiter 1993), Fishbase (www.fishbase.org) and the Australian Museum fish distribution database. Approximately 25 – 30 individuals per species were collected from each of the two locations used for each species (Table 1).

Table 1: Sampling locations, biological and ecological attributes of the six species. Sampling locations: One Tree Island (OTI) 23°30S; 152°05E; Orpheus Island (OI) 18°38S; 146°28E; Lizard Island (LI) 14°40S; 145°28E. Position in the species range (C = centre, P = peripheral) and sample sizes for (mtDNA/ nucDNA) data sets, latitudinal spread (Lat. spread) (W = widespread, R = restricted), pelagic larval duration (PLD) (min – max), diet (P = planktivore, H = herbivore), reproductive mode (Rep.) (B = benthic spawning), generation time (years) and proportional local abundance (Prop. abund) of widespread species (W) vs. restricted (R) species. = indicates approx. equal abundance of widespread and restricted species within a genus and W > R indicate a greater local abundance of the widespread species.

Sampling locations				Geography, biology and ecology					
Species	One Tree Island	Orpheus Island	Lizard Island	Lat. spread [1]	PLD	Diet [1]	Rep. [1]	Gen. time [1]	Prop. abun
Amphiprion melanopus	C (22/22)		C (20/24)	40 (W)	11 (8 – 14)[2]	P	B	1.4 – 4.4	W > R [5]
Amphiprion akindynos	C (24/24)		P (20/23)	22 (R)	11 (9 – 13)[2]	P	B	1.25	
Pomacentrus moluccensis	C (21/ 24)		C (25/24)	55 (W)	15 (14 – 21)[2]	P	B	1.25	W > R [6]
Pomacentrus wardi	C (20/23)		P (22/20)	22 (R)	26.1 (19 – 28)[2]	H / P	B		
Chromis atripectoralis	C (20/ 20)	C (22/ 24)		62 (W)	21.2 (18 – 22)[3]	P	B	1.25	W = R [7]
Chromis nitida	C (17/ 23)	P (17/ 17)		23 (R)	16 (10 – 24)[4]	P	B	1.4 – 4.4	

Source: [1] www.fishbase.org; [2] Chapter 4; [3] Bay unpubl. data; [4] Doherty et al. 1995, Thorrold and Milicich (1990); [5] Srinivasan unpubl. data; [6] Fulton unpubl. data; [7] Eagle unpubl. data

Fish were collected by hand-held spears, fence nets, clove oil and hand-held dip-nets. Fishes were transported live, or on ice, to the nearest shore where a sample (fin clip) was preserved in 100% EtOH for later analysis.

Molecular techniques

MtDNA: DNA was extracted, 335 to 398 base pairs of the mitochondrial hyper variable control region I were amplified, sequenced in the forward and reverse directions and aligned in 39 to 46 individuals from all species following methods outlined in Chapter 5. Representative sequences have been deposited in GenBank under accession numbers DQ250449 – DQ250526, DQ212240 – DQ212281, DQ212323 – DQ212410.

ISSR: Genetic fingerprints were obtained from 17 – 24 individuals per location using Inter Simple Sequence Repeats (ISSR) using 5 universal primers following the methods outlined in Chapter 5. Presence and absence of bands between 50 and 850 base pairs in length were scored using MegaBACE Fragment Profiler 1.2 (Amersham Biosciences), then converted into binary data matrices and concatenated. Raw binary data matrices are available from the authors upon request.

Statistical procedures

Sequence data: The best fitting substitution model and associated rate heterogeneity were estimated separately for each species using PAUP* 4.0b10 (Swofford 1998) and Modeltest 3.5 (Posada and Crandall 1998) and these, where possible, were implemented in all subsequent analyses (Table 2). Genetic diversity estimates for haplotype and nucleotide diversity (Nei, 1987, Tajima 1983) and their associated standard deviations were calculated using Arlequin 2.000 (Schneider et al. 2000). Standard deviations were converted to 95% confidence intervals (95% CI = $\pm 1.96*(SD/\sqrt{(n)})$). Estimates of genetic structure were calculated as pairwise Φ_{ST} values following the methods implemented in Arlequin and significance levels were corrected for multiple comparisons following the Dunn-Sidak method (Sokal and Rohlf, 1995). Transition – transversion ratios indicated that saturation may have occurred in some species (Table 3). Therefore, all analyses were repeated using transversions only. The demographic history of species was analysed using mismatch analysis using Arlequin and 1000 bootstrap replicates.

Table 2: Number of base pairs, transition – transversion ratios (ts-tv) substitution models, gamma distribution shape parameter (γ), invariable sites and their likelihoods (determined by Modeltest) for the six species included in this study

					Among-site rate variation		
Species	No base pairs	Ts-Tv ratio	Model selected	-ln Likelihood score	Invariable sites	γ	Model implemented
Amphiprion melanopus	335	6.3	HKY	576.73	0	0	TN(93)
Amphiprion akindynos	354	9.8	HKY	956.9848	0	0.1410	TN(93) +G
Pomacentrus moluccensis	349	5.9	HKY	652.70	0	0	TN(93)
Pomacentrus wardi	359	8.1	HKY	1211.3286	0	0.3002	TN(93) +G
Chromis atripectoralis	349	7.7	HKY	924.56	0	0	TN(93)
Chromis nitida	347	6.2	HKY	1058.0591	0.6167	0.7053	TN(93) +G

The distribution of genetic variation within species was compared among species using haplotype networks and the frequency distribution of pairwise differences among individuals pooled from the two sampling locations.

ISSR data: Due to the large number of fragments amplified by the 5 primers, only bands with a minimum frequency of 0.25 within any particular species were analysed. No differences were detected among analyses of 0.05, 0.1 and 0.25 minimum frequency data sets conducted for a subset of species. Therefore, this data reduction did not appear to have affected the results significantly. Mendelian segregation of fragments with a single dominant (amplified) and recessive (absent) allele at each banding position was assumed. Because dominant data do not allow within-individual heterozygosities to be estimated, Hardy-Weinberg equilibrium was assumed in the analysis of these data. Given the large population sizes and the potential for considerable pelagic larval dispersal, this assumption was deemed reasonable in this study. Analogues of codominant genetic diversity measures including within-population diversity H_e (here H_j), total heterozygosity H_t and mean heterozygosity across populations H_s (here H_w) were calculated following the methods of Lynch and Milligan (1994) using AFLP-Surv 1 (Vekemans et al. 2002). Genetic structure (Φ_{PT}) was estimated using Genalex 5 (Peakall and Smouse 2001) and significance levels were corrected for multiple comparisons (Sokal and Rohlf 1995). Genetic diversity estimates were interpreted as statistically different when confidence intervals did not overlap.

Results

Estimates of genetic differentiation based on the mtDNA were generally low (Φ_{ST} - 0.005 – 0.01) and statistically insignificant in all species when based on transitions and transversions or on transversions alone (Table 3). In contrast, estimates of genetic differentiation were higher when based on the ISSR data (Φ_{RT} 0.018 – 0.188) and indicated significant genetic structuring between northern and southern GBR locations in all species except *P. moluccensis* (Table 3). Patterns of gene flow were significantly lower in the peripheral species compared to central species when based on transversions of the Control region data ($Z_{3,3} = 1.964$, $P = 0.0495$) but not when including transitions ($Z_{3,3} = 0.655$, $P = 0.654$) or when based on ISSR data ($Z_{3,3} = 0.577$, $P = 0.564$).

Table 3: Estimates of genetic differentiation in the six species. Mitochondrial fixation indices (Φ_{ST}) based on transitions and transversion (ts-tv) and transversions alone (tv) and fixation index based on ISSR data (Φ_{PT}). Significance: * P < 0.01, ns = not significant (insignificance following sequential Bonferroni correction in bold).

Species	Φ_{ST} (ts – tv)	p	Φ_{ST} (tv)	p	Φ_{PT}	p
Amphiprion melanopus	-0.00487	0.47 ns	-0.0019	0.38 ns	0.188	0.001*
Amphiprion akindynos	-0.02405	0.65 ns	-0.0186	0.51 ns	0.119	0.001*
Pomacentrus moluccensis	0.00553	0.25 ns	0.0049	0.04 **ns**[1]	0.018	0.022 *
Pomacentrus wardi	-0.02021	0.75 ns	-0.0249	0.93 ns	0.034	0.002*
Chromis atripectoralis	-0.01551	0.70 ns	0.0131	0.17 ns	0.159	0.001*
Chromis nitida	0.01328	0.26 ns	-0.0366	0.89 ns	0.114	0.001*

[1] Bonferroni corrected significance level $\alpha = 0.008$

Mitochondrial (haplotype and nucleotide) and ISSR diversities (H_w) varied among species and locations (Fig. 1 – 3). Haplotype diversities were generally high (but lower in *P. moluccensis*) and significantly lower in the peripheral population in a single species (*A. akindynos*) when transitions and transversions were included (Fig. 1a). Haplotype diversities were low when considering only transversions and were similar between populations of all species except two. In *A. melanopus* and *P. wardi* haplotype diversities were greater in the northern population coinciding with the northern range margin in *P. wardi* (Fig. 1b). Haplotype diversities were consistently greater in both populations of the peripheral species compared to haplotype diversities of both populations of central species (Fig. 1b). Nucleotide diversities were generally high and did not vary between locations in any of the species except *A. akindynos* where nucleotide diversities greater in the peripheral population (Fig. 2a). When based on transversions, nucleotide diversities did not differ between populations of any of the species, but were generally higher in the peripheral species compared to their central congeners (Fig. 2b). Expected heterozygosities (H_j) were significantly lower in the northern location of *A. akindynos* and *A. melanopus* but similar between locations of the other four species (Fig. 3).

Significant differences were observed in overall genetic diversity (haplotype, nucleotide and mean heterozygosity) among species (Fig. 4 - 6). Haplotype and nucleotide diversities were significantly higher in the peripheral species compared to their central congeners when based on transitions and transversions (Fig. 4a, 5a) and this pattern was particularly evident when based on transversions only (Fig. 4b, 5b). Likewise, levels of heterozygosity (H_w) were significantly greater in the peripheral species compared to the central congeners in two genera (Fig. 6). Intraspecific variation in H_j of two species was large (*A. akindynos* and *A. melanopus* Fig. 3) and hence, the estimate of H_w was variable in these species. Nucleotide diversities were significantly greater in peripheral compared to central species (ts - tv: $Z_{3,3} = -1.963$ p = 0.0495; tv: $Z_{3,3} = -1.96$, P = 0.0495) but haplotype and expected heterozygosities were not (ts - tv: $Z_{3,3} = -0.65$, P = 0.51; tv: $Z_{3,3} = 1.527$, P = 0.126; H_w: $Z_{3,3} = 1.547$, P = 0.248).

The haplotype networks and mismatch distributions revealed large and consistent differences between peripheral (Fig. 7a – c) and central species (Fig. 7d – e). The haplotype networks of peripheral species were complex; central haplotypes were less frequent and distal haplotypes were separated by many mutations.

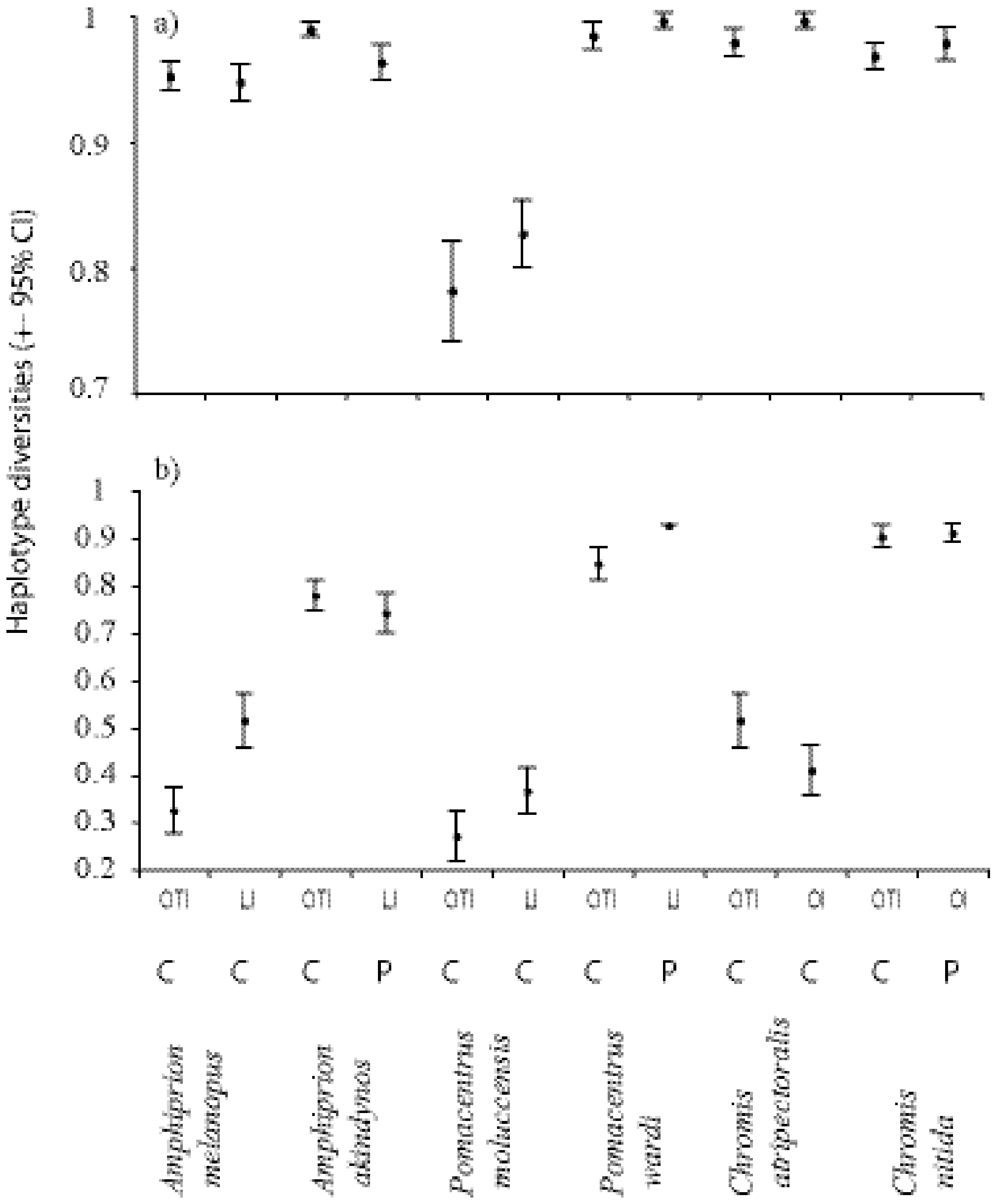

Fig. 1: Haplotype diversities (± 95% confidence intervals) based on a) transitions and transversions and b) transversions alone in central (C) and peripheral (P) populations of the six species. LI = Lizard Island, OTI = One Tree Island.

Mismatch distributions were characterised by larger means (13.7±SE 0.09) and were bimodal in two of the three species (*A. akindynos* and *P. wardi* Fig. 7 a, b). The mismatch distribution in *C. nitida* was unimodal with a large mean and variance (13.57 ±SD 6.96). In contrast the haplotype networks of the central species were characterised by one, or a few, central haplotypes of higher frequency with distal haplotypes being separated by one or a few mutations. Mismatch distributions were unimodal with small means (4.6 ±SE 2.19) (although a slightly higher mean of 8.99 was obtained for *C. atripectoralis*). This pattern was also evident in comparisons using only transversions, although mismatch means and their variation were lower (unpublished data).

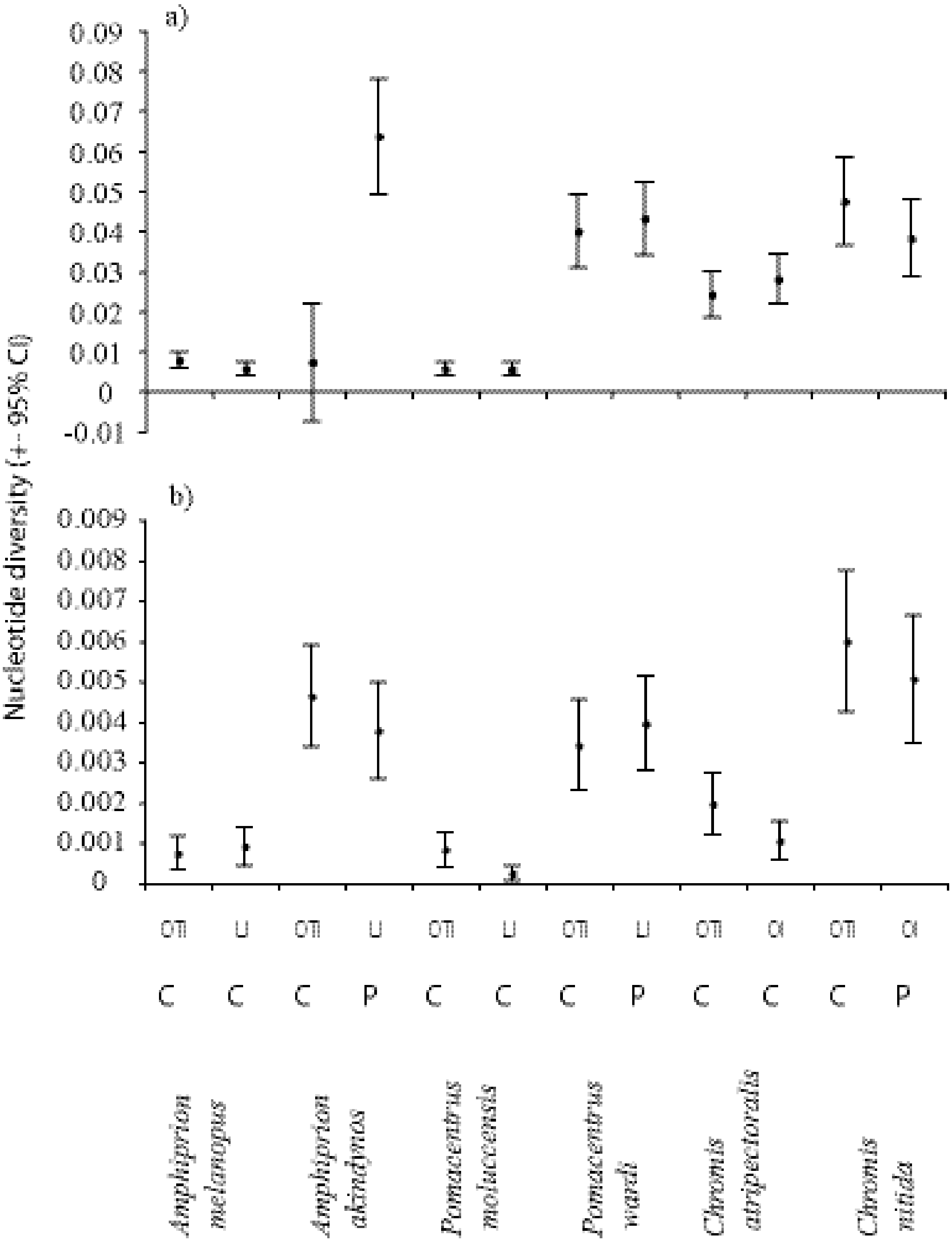

Fig. 2: Nucleotide diversities (± 95% confidence intervals) based on a) transitions and transversions and b) transversions in central (C) and peripheral (P) populations of the six species. LI = Lizard Island, OTI = One Tree Island.

These differences in genetic diversities could not be explained by differences in demographic histories as all species appeared to have a signal of demographic expansion (the null hypothesis of sudden expansion was retained in all species, Table 4) and experienced similar expansion times, regardless of positions in the species' range of the populations sampled (Table 4).

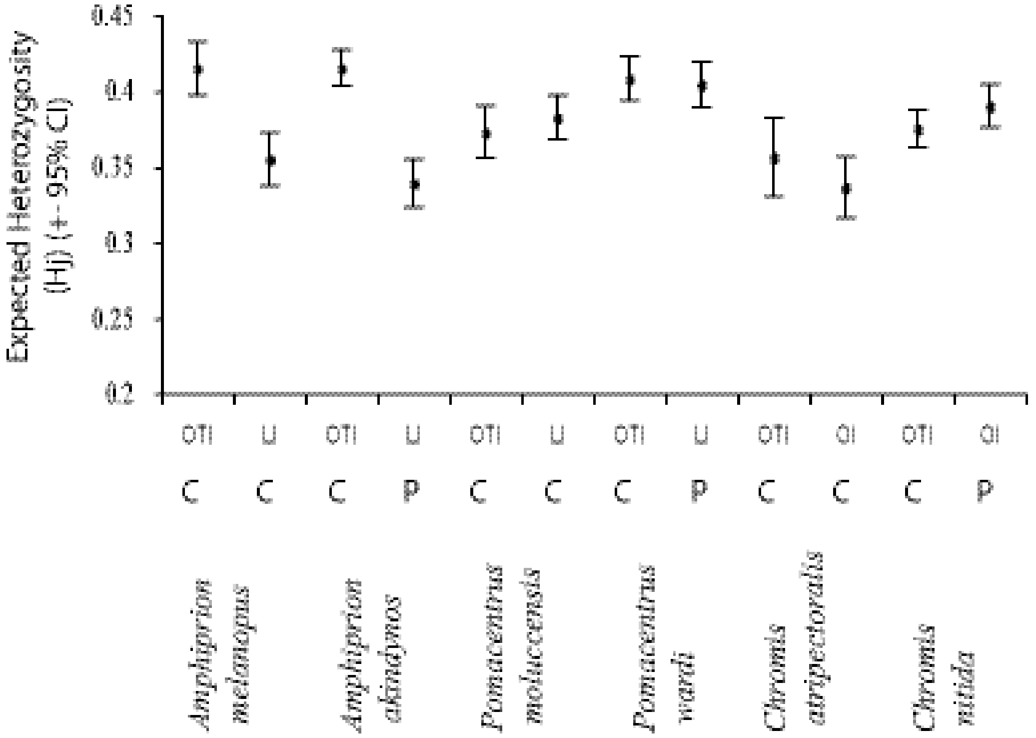

Fig. 3: Expected heterozygosities (± 95% confidence intervals) in central (C) and peripheral (P) populations of the six species. LI = Lizard Island, OTI = One Tree Island.

Discussion

Gene flow and genetic diversities on the species margin

Although the population genetic effects of species' borders determined by demographic processes are well established in theory, empirical support remains equivocal. In general, this study found no evidence to support the predictions from species' borders theory of greater genetic structure and lower genetic diversities towards the species margin. All species displayed high levels of gene flow although the ISSRs indicated that some genetic isolation was present (Table 3). Reduced gene flow towards the edge of the range was only evident from analyses of transversions (Table 3).

Table 4: Demographic history analysis of all species including mismatch mean, summed square deviations (SSD) and Bonferroni corrected p (ns = not significant, bold indicates insignificance following sequential Bonferroni correction), expansion parameter (τ) and its 95% confidence interval.

Species	Mismatch mean	SSD	p	τ	Lower bound of 95% CI	Upper bound of 95% CI
Amphiprion melanopus	2.416	0.007	0.091 ns	2.59	1.147	3.369
Amphiprion akindynos	13.853	0.022	0.035 **ns**[1]	5.023	1.312	26.895
Pomacentrus moluccensis	2.416	0.007	0.091 ns	2.896	0.727	5.652
Pomacentrus wardi	13.804	0.011	0.329 ns	3.589	1.481	16.545
Chromis nitida	13.569	0.002	0.954 ns	13.774	9.059	23.071
Chromis atripectoralis	8.988	0.001	0.966 ns	9.986	6.096	12.753

[1] Bonferroni corrected significance level $\alpha = 0.008$

This result may indicate that historical gene flow was lower than present day patterns, although the reduction in data may have influenced this result. Consequently, all species, regardless of position in the species range, were characterised by relatively low levels of genetic structure consistent with moderate gene flow between sampling locations.

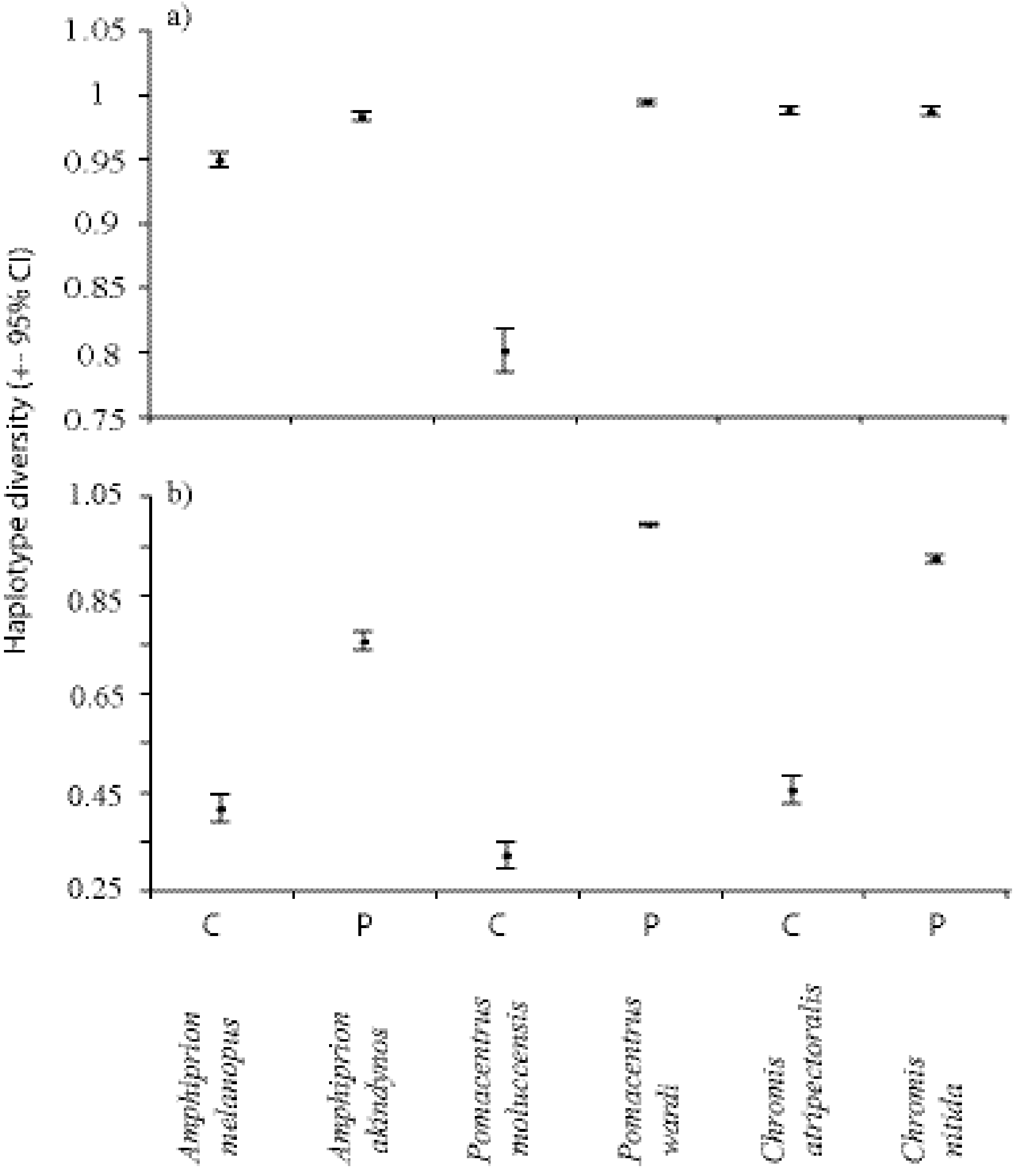

Fig. 4: Haplotype diversities (± 95% confidence intervals) based on a) transitions and transversions and b) transversions in the three central (C) and the three peripheral (P) species.

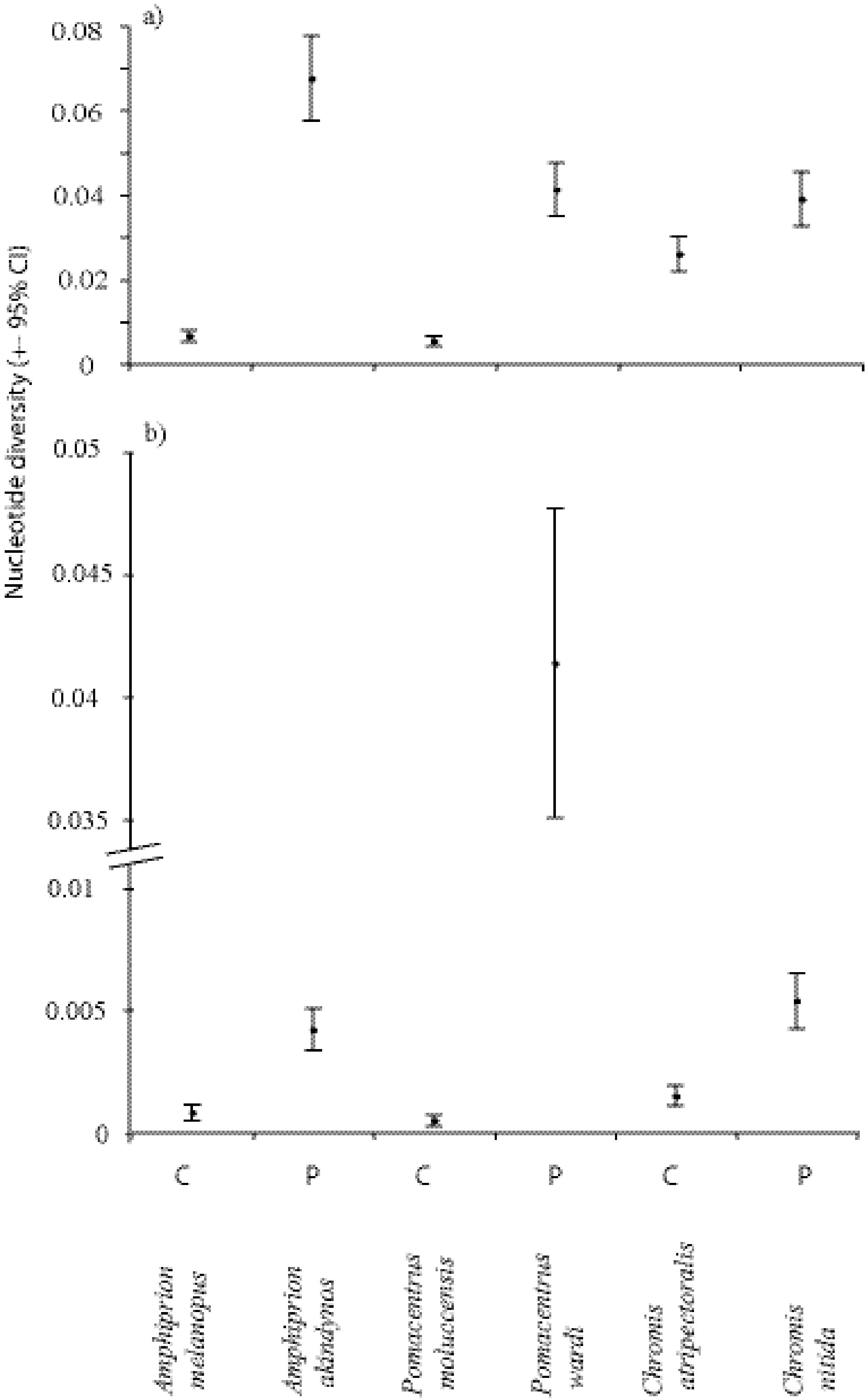

Fig. 5: Nucleotide diversities (+ 95% confidence intervals) based on a) transitions and transversions and b) transversions in the three central (C) and the three peripheral (P) species.

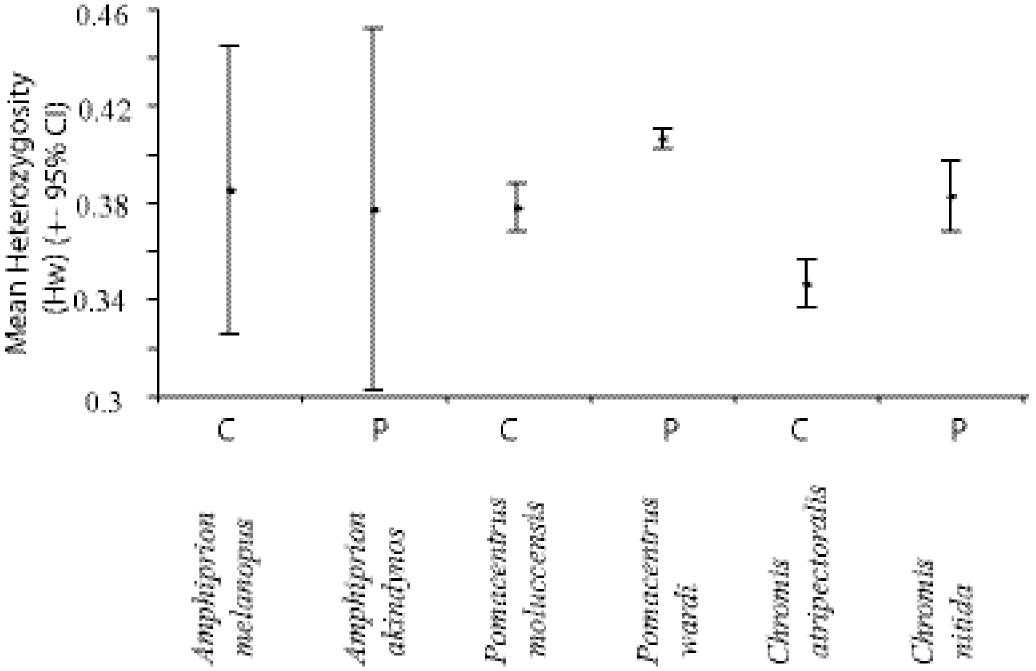

Fig. 6: Mean heterozygosity (± 95% confidence intervals) in the three central (C) and the three peripheral (P) species.

Genetic diversities varied among species and locations, but were not consistently lower in the populations sampled close to their geographic limits. For example, *A. akindynos*, a peripheral species, had lower ts-tv haplotype (Fig. 1a) and expected heterozygosity (Fig. 3) in the peripheral population, but nucleotide diversities were higher at this location (Fig. 2). Similarly, *A. melanopus*, a central species, had lower haplotype diversity (Fig. 1b) and lower expected heterozygosity in the northern population (Fig. 3), centrally located in its range. Therefore, the lower genetic diversities in the *Amphiprion* spp. appear to be associated with the northern location rather than the peripheral position in *A. akindynos*. While it is possible that I did not detect a genetic signature of declining populations at the species margin because I did not sample close enough to the border (Lennon et al. 1997), high genetic diversities can be maintained in peripheral populations, even if these are effectively sinks, by high levels of gene flow (Vucetich and Waite 2003). The estimates of genetic structure of the species included in this study all indicated relatively high levels of gene flow between central and peripheral populations. Consequently, the high genetic diversities maintained on the species margins recorded by this study may be maintained by high gene flow from more centrally located populations.

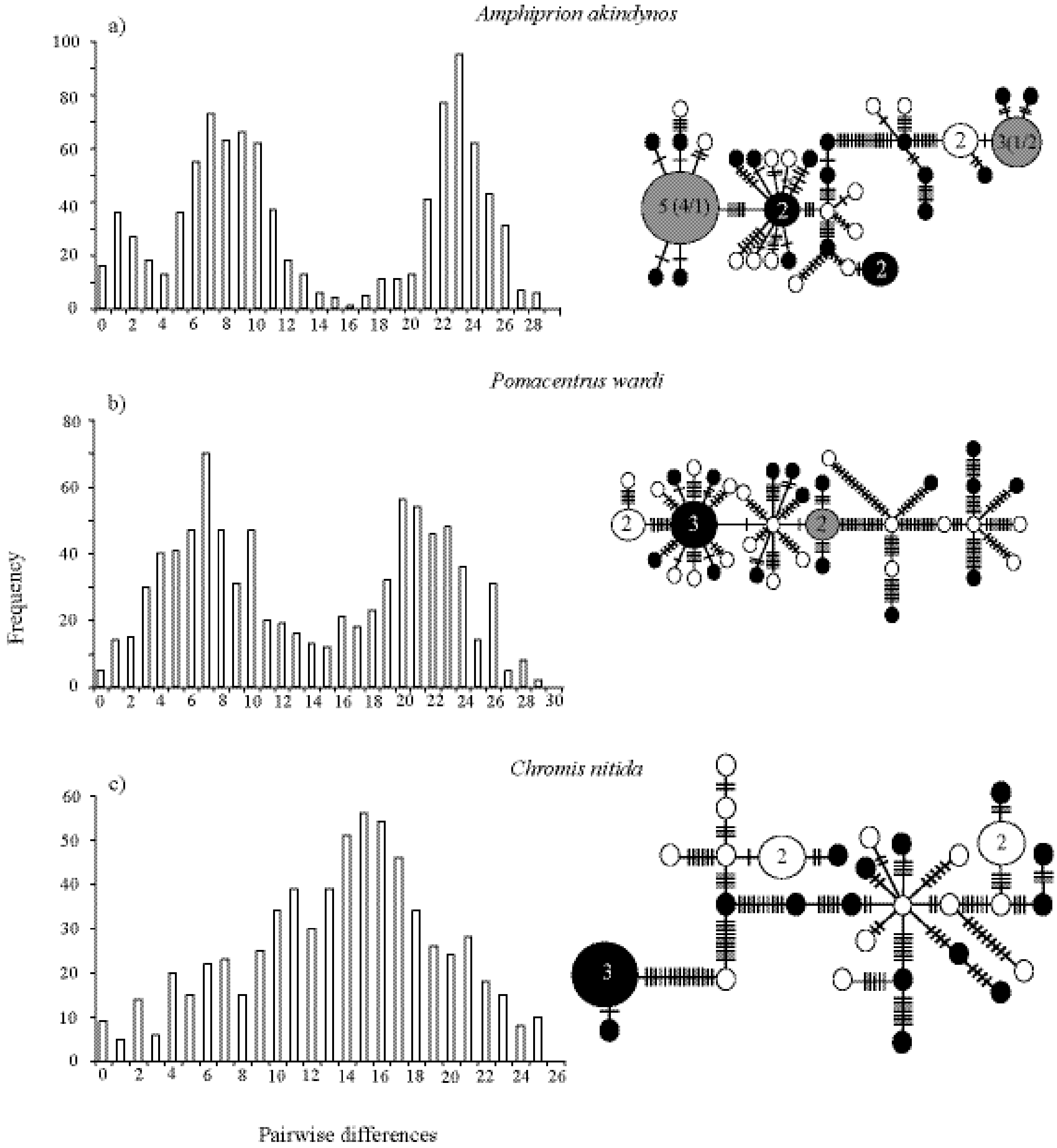

Fig. 7: Mismatch distributions and haplotype networks of the peripheral species (a – c) and the central species (d – f). Haplotype networks: Haplotypes indicated by circles where their size and the embedded number indicates its frequency with the frequency of shared haplotypes indicated in brackets (LI/OI, OTI), the location indicated by colour (white = LI/ OI, black = OTI, grey = shared) and the number of mutations separating haplotypes represented by bars.

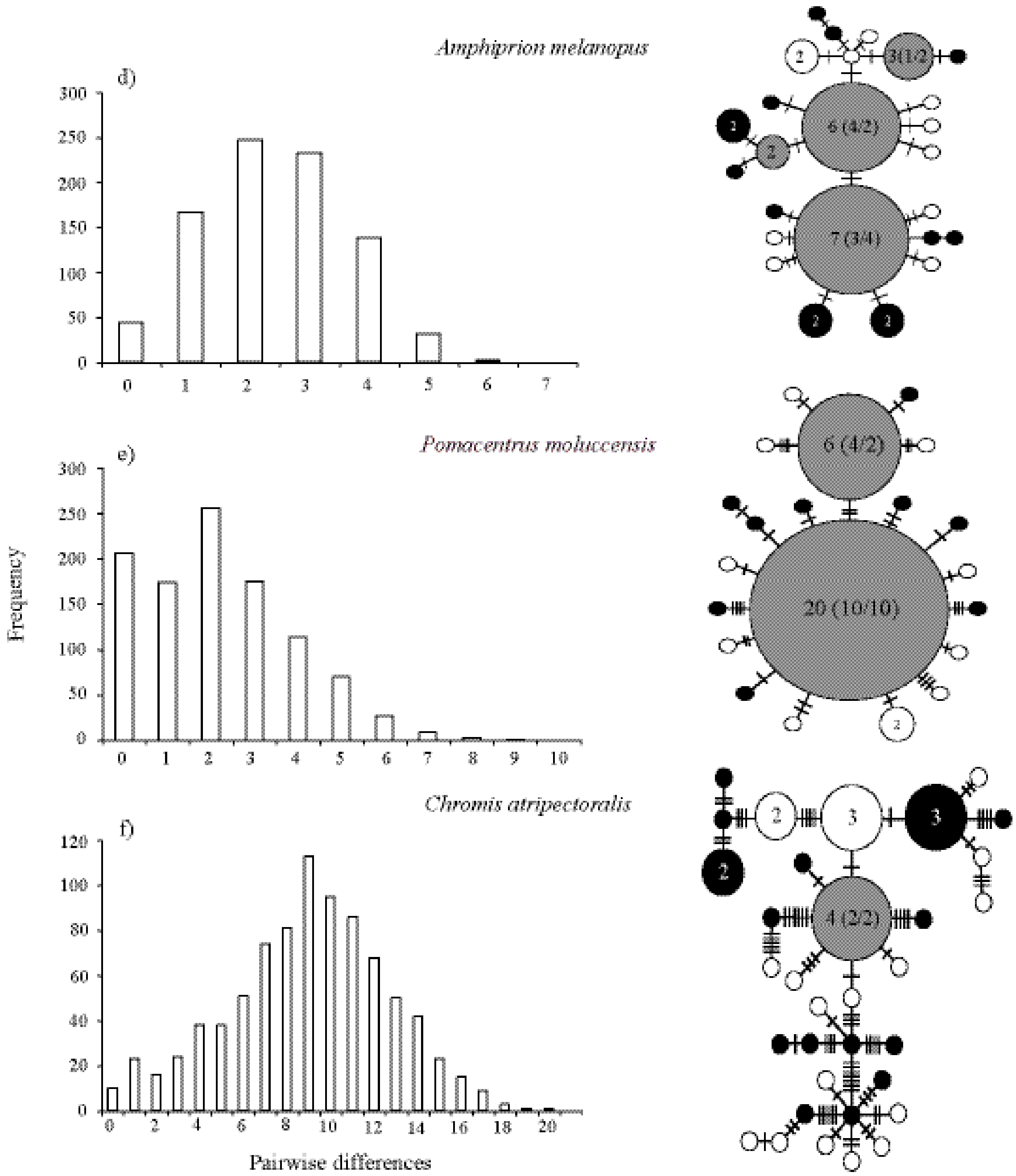

Fig. 7 Continued

Genetic diversities in peripheral and central species

Genetic diversities were consistently greater in the peripheral species compared to central species (Fig. 4b, 5, 6), despite very similar levels of gene flow in all species. Levels of genetic diversity in the central species were similar to those reported for widespread coral reef fishes that have experienced long stable evolutionary histories (e.g., Fauvelot et al. 2003). In contrast, the genetic diversities of the peripheral species were higher than most other values reported for reef fishes (e.g., Grant and Bowen 1998; Planes 2002; Fauvelot et al. 2003). The concordance of this pattern among species from three genera suggests that a general mechanism may be underlying this pattern. A number of possible explanations can be erected to explain the higher genetic diversities in the peripheral species and I consider these in turn below.

Large population sizes on the species' periphery

The higher genetic diversity in peripheral species could arise if they have higher local abundances (Soulé 1976) and if true would suggest that Lawton's universal rule (of a positive relationship between local abundance and geographic distribution) does not apply to coral reef fishes. Here, the central species were either similarly or more abundant than the peripheral species at all sampling locations (Table 1). Local abundance patterns do not therefore appear to provide an adequate explanation for the observed differences in genetic diversities.

Peripheral species are older or have inhabited the GBR for longer

If mutations accumulate at a constant rate then the higher genetic diversity in peripheral species may be expected if the taxa are older, or if they have occupied the GBR for longer (Soulé 1972). The phylogenetic relationships of the majority of species used here (except *P. wardi*) were examined by Quenouille et al. (2004). Branch lengths of peripheral species were not significantly longer than branch lengths of central species ($F_{2,\ 1}$ = 0.17, P = 0.13) indicating that these taxa are not older. Likewise, peripheral species did not appear to have occupied the GBR for longer than the central species; all species displayed a signal of sudden expansion and expansion times did not differ among species (Table 4). These results indicate that the populations of the species included in this study may not be at migration – drift equilibrium, however, the potential degree of disequilibrium did not appear to differ among species. The current population genetic structure of these species may have been affected by a genetic bottleneck potentially associated with the initial colonisation of the GBR following the last glacial maximum. If so, this did not appear to have had a greater effect on central species compared to peripheral ones. It therefore appears that neither taxon age, nor duration of local occupancy provide adequate explanations for the observed patterns.

Peripheral species have greater genetic diversity because of their evolutionary histories

High haplotype and nucleotide diversities may arise when populations that have diverged during historical isolation come into secondary contact, or if species have experienced a long and stable evolutionary history (Grant and Bowen 1998). Secondary contact among differentiated lineages should be evident as bimodal or multimodal distributions of pairwise differences whereas long stable evolutionary histories should produce broad unimodal mismatch distributions (Avise 2000). I observed strong bimodal mismatch distributions in two of the three peripheral species (i.e., *A. akindynos* and *P. wardi*) and a broad unimodal mismatch distribution in *C. nitida* (Fig. 7a - c) contrasting with the narrow unimodal distributions obtained for all three central species (Fig. 7d – f). This pattern indicates that the high genetic diversities in at least two of the peripheral species could be the result of secondary contact. It is possible that the peripheral species persisted and diverged in isolated off-shore refugia during the last glacial maximum (Davies 1989) and that these lineages came into contact when the GBR was formed approximately 6000 - 9000 years ago (Hopley and Thom 1983; Larcombe 2001). Consequently, the observed pattern in genetic diversities could have been produced if the GBR was colonised by several genetically differentiated lineages of the peripheral species, but only one of the central species. While this mechanism is plausible, it is not particularly parsimonious. It is unclear why such isolation would only apply to some of the species, given that all commonly co-occur on many reefs of the GBR. It is also plausible that this pattern could have been generated in sympatry if gene flow were historically lower in peripheral species compared to their central counterparts. I detected lower gene flow in the spatially restricted species based on analyses of transversions (Table 3). These rates, however, were still too high to allow for this level of divergence. Conversely, if historically the dispersal potential of the peripheral and central species were similar, patterns of genetic diversity may have been generated if they did not occupy the same Pleistocene refugia to the east of the GBR, or if the central species colonised the GBR from northern refugia. Genetic diversities commonly decline with increasing distance from Pleistocene refugia (reviewed by Gaston 2003; Briggs 2004). Consequently the differences in the genetic diversities between central and peripheral species may by explained by their contemporary proximity to such refugia. This explanation bears superficial resemblance to the centrifugal speciation hypothesis initially proposed by Brown (1957) and advocated by Briggs (2000). In this model species disperse out of the centre of diversity and populations at the periphery of the ranges become isolated and speciate in allopatry during repeated cycles of range expansion and contraction (Brown 1957; Briggs 2000). The peripheral species are not able to colonise the centre of diversity potentially due to interactions with already established species (Briggs 1974). Such unidirectional dispersal filters have been proposed for the east and west of the Indo-pacific centre of diversity (Briggs 1974), but have never previously been implicated in explaining the species distributions to the south of the centre of diversity. Some of the tenets of the centrifugal speciation hypothesis, such as peripheral species being plesiomorphic and extinction prone relics (Brown 1957; Briggs 1974) are not met by the current study. Here the peripheral species were not older and did not appear extinction prone given their very high effective population sizes. However, the predictions regarding the direction of dispersal and the presence of barriers erected by the centrifugal speciation hypothesis could explain the pattern of genetic diversity found by this study, although this explanation is not very parsimonious.

Species have higher mutation rates on the periphery of the range

The differences in genetic diversities between the peripheral species and the central species could be generated if mutation rates differ between the two groups. Mutation rates can be elevated by sub-lethal temperature stress (*Drosophila melanogaster,* Lindgren 1972) or by other stressful conditions that disrupt intracellular homeostasis (*Mus musculus*, Kerkis 1975; Belyaev and Borodin 1982). Environmental conditions are generally assumed to be effectively more extreme and stressful on populations at the species margin compared to populations closer to range centres (Hoffmann and Parsons 1991; Parsons 1991). If so, such a process may have led to a higher mutation rate at this location. The moderate levels of gene flow (Table 3) could have then distributed these mutations across the species' range. It is, however, unclear how elevated rates of mutation on the species margin could have produced the bimodal mismatch distributions observed in two of the peripheral species without genetic isolation among lineages (Fig 7 a – b). Consequently, elevated mutation rates on the species margin may play an important role in producing the very high genetic diversities in the peripheral species. This hypothesis alone, however, does not provide a satisfactory explanation for patterns of genetic diversities observed here.

The species' borders are the result of physical barriers or physiological stress

It is possible that the species' borders examined are not the product of declining demographic processes in peripheral populations but rather a result of a physical barrier to dispersal. If so, barriers would have to occur in at least two different locations and be species specific or unidirectional (i.e., permeable from the north but not the south) (Briggs 1974) given the distributional patterns of the species studied here. At present there is insufficient evidence of the population genetic structure of marine organisms at this spatial scale to evaluate this hypothesis. Further sampling incorporating a population genetic examination of central species from locations north of the GBR may elucidate the potential presence and role of such putative barriers.

It is possible that the species borders examined here are determined by physiological stress at the species margin, a lack of genetic variation in stress tolerance related traits and/or a failure of natural selection to produce local adaptive optima because of gene flow from central locations (Hoffmann and Parsons 1991). The examination of neutral genetic variation here does not permit an examination of these hypotheses, but the high levels of gene flow recorded indicate a potential important role of migration into marginal populations.

Conclusion

Contrary to expectations, gene flow did not differ among central and marginal species and genetic diversities were not less in peripheral populations compared to central populations or species sampled in the centre of their ranges. Indeed, genetic diversities were much higher in the spatially restricted border species compared to their more widespread counterparts indicating that they have much larger effective population sizes. Based on the distribution of genetic diversity in the peripheral and central species, it appears most likely that historical isolation and subsequent secondary contact has produced the patterns of genetic diversities detected here. Higher mutation rates mediated by environmental stress on the species margin may have further enhanced genetic diversities in the peripheral species. Here, I can only speculate on the processes generating the very high genetic diversities in the species sampled at the species margin. The genetic patterns uncovered by this investigation, however, may form the foundation for further investigations examining the genetic consequences of species' borders in coral reef fishes.

Chapter 7: General Discussion

General summary

This thesis demonstrates the complex nature of the factors that determine the population genetic structure of coral reef fishes on the GBR. By examining a low dispersal species in detail I demonstrated that population genetic structure may be scale dependent and vary between local and regional spatial scales. I also revealed the capacity of a low dispersal species to display metapopulation dynamics at local scales. The frequency of local extinctions varied geographically among regions and increased towards the margin of this species' range. Using the length of the pelagic larval duration as a proxy for dispersal potential, I could only predict population genetic structure in eight pomacentrid species, that varied little in their biological and ecological attributes, when a directly developing species was included in the analysis. Population genetic structure, therefore, did not appear to be related to this life-history trait among species from this reef-fish family. Position in the species range did not appear to influence the level of genetic structure and levels of genetic differentiation were similar among species sampled towards the periphery of their ranges compared to those sampled in the centre of their ranges. Genetic diversities were not reduced in peripheral populations compared to central populations, however, genetic diversities were much greater in peripheral species overall compared to central species. In all, these results suggest that the population genetic structure of coral reef fishes may be affected by the spatial scale at which it is examined, it may vary geographically within the species range and be greatly influenced by historical factors that may act in a species-specific manner.

Summary of key findings

The application of population genetic models to the genetic structure of *A. polyacanthus* on the GBR varied among spatial scales, with evidence of isolation-by-distance at the largest spatial scale (among regions) and metapopulation dynamics within regions (Chapter 2). Consequently, these results demonstrate that isolation-by-distance and metapopulation models are not mutually exclusive, but instead can operate within the same species at different spatial scales. Genetic structures conforming to the isolation-by-distance model have been commonly identified across large spatial scales in many species (e.g., Palumbi et al. 1997; Planes and Fauvelot 2002; Bay et al. 2004). It is unclear, however, whether such species display significant genetic structure at local scales, because the sampling regimes of most population genetic studies of coral reef fishes have not included any local-scale sampling (but see Doherty et al. 1995). The effect of scale was also evident in the demographic history analyses of *A. polyacanthus* (Chapter 3). In the southern region of the GBR, half the sampled populations contained a genetic signal of a recent demographic bottleneck and this was evident at the regional level, with the southern region displaying lower genetic diversities, a more recent demographic expansion, and a higher growth rate. Consequently, an accurate appraisal of the population genetic structure of coral reef fishes require an understanding of both local and regional scale patterns.

I identified an extensive capacity of a coral reef fish with low dispersal to display complex genetic structure that conformed to predictions based on metapopulation theory largely developed and tested in terrestrial systems (Chapter 2 and 3). Populations of *A. polyacanthus* displayed a genetic structure consistent with low migration (Chapter 2), propagule-pool colonisation (Chapter 2), and periodic local extinctions (Chapter 3). This represents the first comprehensive example of metapopulation dynamics in a coral reef fish and is one of only a handful of investigations examining the genetic consequences of migration, extinction and re-colonisation in a single study (reviewed by Giles and Goudet 1997). It is at present unclear whether other coral reef species, in particular those with pelagic larvae, also display metapopulation dynamics. Investigations of the population genetic structure of coral reef fishes have either not sampled populations at local scales, or when local scale genetic structure has been examined, metapopulation theory has not used to interpret the patterns, even when strong genetic structure was found (e.g., Nelson et al. 2000; Planes et al. 2001; but see Planes et al. 1996).

The length of the pelagic larval phase has commonly been used to predict population genetic structure of coral reef fishes (e.g., Waples 1989; Doherty et al. 1995; Riginos and Victor 2001), however, most previous relationships have included a range of distantly related species and consequently, we have a limited understanding of how this relationship applies within the taxonomic level of families. Estimates of PLD typically show little variation at a particular time and place, but may vary greatly among sampling times, locations with regions, and regions (Chapter 4). It is likely that such variation in PLDs may affect the population genetic structure of species, however, this has not previously been incorporated into examinations of the relationship between PLD and genetic structure. I identified a relationship between PLD and population genetic structure based on mtDNA and nucDNA markers, however, this relationship was dependent on the inclusion of a directly developing and highly genetically structured species (Chapter 4). The relationship between PLD and population genetic structure varied depending on the estimate of PLD and molecular marker used. Genetic structure based on the mtDNA could be predicted from mean, minimum and maximum PLD, whereas the genetic structure based on nucDNA (both the ISSRs and allozymes from Doherty et al. 1995) could only be predicted from my estimate (Chapter 4) of minimum PLD. These results suggest that the relationship between PLD and population genetic structure may depend on the molecular markers used, the estimate of PLD and its variation, and the species included in analyses.

Theory suggests that species borders may evolve in response to a decline in demographic processes from the centre to the periphery of geographic ranges or through changing metapopulation dynamics towards the species' range margin (Lennon et al. 1997; Holt and Keitt 2000). Empirical support for this theory, however, remains equivocal. The population genetic structure of *A. polyacanthus* appeared to support the predictions of this theory (Chapter 3). Population expansions were more recent, population growth rates were higher and genetic diversities were lower in half of the populations in the southern region located close to the species' margin of this species. This indicates that extinction rates in local populations may increase towards the margin of the species' range. Although this design did not allow a separation of the population genetic species' border effects from any potential geographical effects, these results indicate that metapopulation effects may play an important role in determining the geographical border in this low dispersal species (Chapter 3). The predicted species border effects were not evident in species with greater dispersal potential (Chapter 6). All species included in this analysis displayed a population genetic structure consistent with moderate gene flow between sampling locations and genetic diversities were not reduced in peripheral populations. It is likely that moderate levels of gene flow may have prevented the predicted decay of genetic diversity in the peripheral populations (Vucetich and Waite 2003). Genetic diversities were much greater in the peripheral species, and higher than most comparable estimates from other reef fishes (e.g., Grant and Bowen 1998; Fauvelot et al. 2003). This result indicates that processes are operating on the periphery of these species' ranges are complex and are not easily explained within the existing theoretical framework.

The distribution of genetic variation within and among populations can be greatly affected by historical processes (Benzie 1999; Avise 2000). All the species examined in this thesis appear to have been substantially affected by historical effects, however, these effects differed greatly among species. Most species contained a signal of demographic bottleneck, most likely associated with the initial colonisation of the GBR (Chapter 3, 5 and 6). This result supports the suggestion that species in habitats affected by Pleistocene sea level fluctuations may contain a signal of demographic expansion (Fauvelot et al. 2003). I identified extensive intraspecific variation in the timing of demographic bottlenecks and population growth rates in *A. polyacanthus* among regions and reefs within regions of the GBR (Chapter 3). Reductions in size and/or local extinctions appeared to be frequent in this species and one fifth of sampled populations contained a genetic signal of recent expansion, a high population growth rate and reduced genetic diversities (Chapter 3). This suggests that coral reef fish metapopulations may be more greatly affected by local extinctions than previously thought (e.g., Planes et al. 1996; Planes 2002). Species sampled towards the periphery of their ranges also appeared to be affected by historical processes, however, in these species such processes increased rather than reduced genetic diversity (Chapter 6). The distribution of genetic variation in at least two of the peripheral species indicated secondary contact among historically differentiated lineages, suggesting either hybridisation between different species, or that historical gene flow on the species periphery was restricted compared to gene flow among population in species that were sampled in the centre of their ranges.

Overall conclusions and future directions

Studies of the population genetic structure of coral reef fishes are accumulating at a rapid rate and, concomitantly, so is our understanding of the processes that determine the population genetic structure in such systems. By examining one species in detail in this thesis I demonstrated an extensive capacity of a low dispersal species to display complex spatial genetic structure that conformed to predictions from metapopulation theory. There is, however, a poor understanding of how this theory may apply to other coral reef species with greater dispersal potential. A good understanding of the spatial genetic structure of coral reef fishes is critically important to their effective management and conservation (Palumbi et al. 2003; Palumbi 2004). The use of highly sensitive molecular markers, such as microsatellites or ISSRs, coupled with nested sampling designs incorporating local and regional spatial scales may facilitate the resolution of this issue.

The application of a comparative framework has revealed many insights into the processes that determine the population genetic structure of coral reef fishes (e.g., Doherty et al. 1995; Riginos and Victor 2001; Fauvelot et al. 2003; Rocha et al. 2005; Chapter 5 and 6). Further insights into the evolution of population genetic structure of coral reef fishes are likely to be gained from studies that not only compare patterns among species, but also apply phylogenetically controlled analyses to such data. The application of comparative designs will allow relationships between, for example, PLD and population genetic structure, to be compared among species with different biological and ecological attributes such as spawning strategy, level of ecological specialisation, and geographical range size among others, while controlling for the role of phylogeny. Such analyses have the potential to increase our understanding of this important relationship further.

This study used ISSRs, a genetic fingerprinting technique, for the first time to examine the population genetic structure of coral reef fishes. The application of this technique increased the resolution of this study and allowed a comparison between mtDNA and nucDNA markers rarely undertaken in population level analyses. While mtDNA genes suitable for population level analyses can be readily amplified from universal primers, nuclear population level markers (such as microsatellites and SNPs) generally require prior sequence information that may not be easily obtained for a large number of species. ISSRs may be useful in comparative investigations because they allow highly variable and repeatable nuclear genetic fingerprints to be obtained from a range of distantly related species using universal primers. Despite of analytical restrictions because of their dominant nature, ISSRs may therefore provide an alternative nuclear marker in studies where the number of species or the lack of sequence information make the development of co-dominant markers impracticable.

Lastly, this thesis reports of substantial differences in the population genetic structure in a single species and generally among species sampled towards the periphery of their ranges. In the species with pelagic larvae, however, the population genetic structure associated with range edges were contrary to theoretical expectations with genetic diversities being much greater in these species compared to most other coral reef fishes for which comparable diversity estimates exist. The use of more sensitive molecular markers, such as microsatellites, and a sampling strategy that includes multiple populations sampled towards the species margin, may reveal any potential border effects that were not evident from the analyses presented in Chapter 6. Such an approach would allow a more comprehensive evaluation of the application of species border theory to species with moderate dispersal levels. Furthermore, it would be interesting to examine if the patterns reported here apply more generally among species, among borders (latitudinal and longitudinal) and among reef systems. Such investigations will facilitate a better understanding of the processes that determine the extent of species' ranges in coral reef fishes, in particular, and evolutionary dynamics in tropical marine systems in general.

Literature cited

Aars J, Ims RA (2000) Population dynamics and genetic consequences of spatial density-dependent dispersal in patchy populations. American Naturalist 155: 252 - 265

Andrews JC, Clegg S (1989) Coral Sea circulation and transport deduced from modal information models. Deep-Sea Research 36: 957 - 974

Arndt A, Smith MJ (1998) Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Molecular Ecology 7: 1053 - 1064

Avise JC (2000) Phylogeography. The history and formation of species. Harvard University Press, Cambridge, Massachusetts

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590 - 1605

Ayre DJ, Hughes TP (2004) Climate change, genotypic diversity and gene flow in reef-building corals. Ecology Letters 7: 273-278

Balloux F, Brunner H, Lugon-Moulin N, Hausser J, Goudet J (2000) Microsatellites can be misleading: An empirical and simulation study. Evolution 54: 1414-1422

Balloux F, Goudet J (2002) Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. Molecular Ecology 11: 771-783

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000) Biogeography: a marine Wallace's line? Nature 406: 692 - 693

Bay LK, Choat JH, van Herwerden L, Robertson DR (2004) High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? Marine Biology 144: 757 - 767

Beamish RJ, Fournier DA (1981) A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Science 38: 982 - 983

Beerli P, Felsenstein J (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152: 763-773

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Science USA 98: 4563-4568

Belyaev DK, Borodin PM (1982) The influence of stress on variation and its role in evolution. Biologisches Zentralblatt 100: 705-714

Benzie JAH (1999) Genetic structure of coral reef organisms: ghost of dispersal past (1). American Zoologist 39: 131-145

Bernardi G (2000) Barriers to gene flow in *Embiotoca jacksoni*, a marine fish lacking a pelagic larval stage. Evolution 54: 226-237

Bernardi G, Holbrook SJ, Schmitt RJ (2001) Gene flow at three spatial scales in a coral reef fish, the three-spot dascyllus, *Dascyllus trimaculatus*. Marine Biology 138: 457 - 465

Bernardi G, Holbrook SJ, Schmitt RJ, Crane NL, Edward D (2002) Species boundaries, populations and colour morphs in the coral reef three-spot damselfish (*Dascyllus trimaculatus*) species complex. Proceedings of the Royal Society of London B 269: 599 - 605

Bernardi G, Holbrook SJ, Schmitt RJ, Crane NL (2003) Genetic evidence for two distinct clades in a French Polynesian population of the coral reef three-spot damselfish *Dascyllus trimaculatus*. Marine Biology 143: 485-490

Bernardi G, Vagelli A (2004) Population structure in Banggai cardinalfish, *Pterapogon kauderni*, a coral reef species lacking a pelagic larval phase. Marine Biology 145: 803-810

Betancourt JL, Schluser WS, Mitton JB, Anderson RS (1991) Fossil and genetic history of a pinyon pine (*Pinus edilus*) isolate. Ecology 72: 1685-1697

Birky CW, Fuerst P, Maruyama T (1989) Organelle gene diversity under migration, mutation and drift - equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121: 3613 - 3627

Blundell GM, Ben-David M, Groves P, Bowyer RT, Geffen E (2002) Characteristics of sex-biased dispersal and gene flow in coastal river otters: implications for natural recolonisation of extirpated populations. Molecular Ecology 11: 289 - 303

Bohonak AJ (1999) Dispersal, gene flow, and population structure. Quarterly Review of Biology 74: 21 - 45

Bonhomme F, Planes S (2000) Some evolutionary arguments about what maintains the pelagic interval in reef fishes. Environmental Biology of Fishes 59: 365 - 383

Bornet B, Branchard M (2001) Nonanchored inter simple sequence repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter 19: 209-215

Bowen BW, Clark AM, Abreu-Grobois FA, Chaves A, Reichart HA, Ferl RJ (1997) Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) as inferred from mitochondrial DNA sequences. Genetica 101: 179-189

Briggs JC (1974) Marine Zoogeography. McGraw-Hill Book Company, new York

Briggs JC (2000) Centrifugal speciation and centres of origin. Journal of Biogeography 27: 1183-1188

Briggs JC (2004) A marine center of origin: Reality and conservation. In: Lomolino MV, Heaney LR (eds) Frontiers of biogeography. New directions in the geography of nature. Sinauer Associates, Inc., Sunderland, pp 255-269

Brothers EB, Thresher RE (1985) Pelagic duration, dispersal, and the distribution of Indo-Pacific coral-reef fishes. In: Reaka ML (ed) The ecology of coral reefs. NOAA's Undersea Research Program, Washington, pp 53 - 70

Brothers EB, Williams DM, Sale PF (1983) Length of larval life in twelve families of fishes at "One Tree Lagoon" Great Barrier Reef, Australia. Marine Biology 76: 319 - 324

Brown JH (1984) On the relationship between abundance and distribution of species. American Naturalist 124: 255-279

Brown WLJ (1957) Centrifugal speciation. Quarterly Review of Biology 32: 247-277

Cabot E, Beckenbach AT (1989) Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Computer Applications in Bioscience 5: 233 - 243

Caley MJ, Hixon MA, Hughes TP, Jones GP, Menge BA (1996) Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics 27: 477-500

Church JA (1987) East Australian Currents adjacent to the Great Barrier Reef. Australian Journal of Marine and Freshwater Research 38: 671 - 683

Clarke A, Saether B, Roskaft E (1997) Sex biases in avian dispersal: a reappraisal. Oikos 79: 429 - 348

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001-2014

Cowen RK (1991) Variation in the planktonic larval duration of the temperate wrasse *Semicossyphus pulcher*. Marine Ecology Progress Series 69: 9 - 15

Cowen RK, Sponaugle S (1997) Relationships between early life history traits and recruitment among coral reef fishes. In: Christopher CR, Trippel EA (eds) Early life history and recruitment in fish populations. Chapman and Hall, London, pp 423 - 450

Crow JF, Aoki K (1984) Group selection for a polygenic behavioral trait - estimating the degree of population subdivision. Proceedings of the National Academy of Science USA 81: 6073-6077

Darwin C (1859) On the origin of species by means of natural selection. John Murray, London

Davies PJ (1989) Evolution of the Great Barrier Reef - reductionist dream or expansionist vision. Proceedings of the 6th International Coral Reef Symposium 1: 9 - 17

Dobson F (1982) Competition for mates and predominantly male dispersal in mammals. Animal Behaviour 30: 1183 - 1192

Doherty PJ, Mather P, Planes S (1994) *Acanthochromic polyacanthus*, a fish lacking larval dispersal, has genetically differentiated populations at local and regional scales on the Great Barrier Reef. Marine Biology 121: 11 - 21

Doherty PJ, Planer S, Mather P (1995) Gene flow and larval duration in seven species of fish from the Great Barrier Reef. Ecology 76: 2373-2391

Dudgeon CL, Gust N, Blair D (2000) No apparent genetic basis to demographic differences in scarid fishes across the continental shelf of the Great Barrier Reef. Marine Biology 137: 1059 - 1066

Durka W (1999) Genetic diversity in peripheral and subcentral populations of *Corrigiola litoralis* L. (Illecebraceae). Heredity 83: 476-484

Dybdahl MF (1994) Extinction, recolonization, and the genetic structure of tidepool copepod populations. Evolutionary Ecology 8: 113-124

Estoup A, Cornuet J-M (1999) Microsatellite evolution: inferences from population data. In: Goldstein DB, Schlotterer C (eds) Microsatellites. Evolution and Applications. Oxford University Press, Oxford, pp 49 - 65

Excoffier L, Smouse PE, Quattro JE (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479 - 491

Fauvelot C, Bernardi G, Planes S (2003) Reductions in the mitochondrial DNA diversity of coral reef fish provide evidence of population bottlenecks resulting from Holocene sea-level change. Evolution 57: 1571-1583

Fauvelot C, Planes S (2002) Understanding origins of present-day genetic structure in marine fish: biologically or historically driven patterns? Marine Biology 141: 773-788

Fishelson L (1998) Behaviour, socio-ecology and sexuality in damselfishes (Pomacentridae). Italian Journal of Zoology 65: 387-398

Fisher R (2005) Swimming speeds of larval coral reef fishes: impacts on self-recruitment and dispersal. Marine Ecology Progress Series 285: 223 - 232

Fraser DJ, Lippe C, Bernatchez L (2004) Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). Molecular Ecology 13: 67 - 80

Gapare WA, Aitken SN (2005) Strong spatial genetic structure in peripheral but not core populations of Sitka spruce [*Picea sitchensis* (Bong.) Carr.]. Molecular Ecology 14: 2659-2667

Gapare WJ, Aitken SN, Ritland CE (2005) Genetic diversity of core and peripheral Sitka spruce [*Picea sitchensis* (Bong.) Carr.] populations: implications for conservation of widespread species. Biological Conservation 123: 113-123

Garner TWJ, Pearman PB, Angelone S (2004) Genetic diversity across a vertebrate species' range: a test of the central-peripheral hypothesis. Molecular Ecology 13: 1047-1053

Gaston KJ (2003) The structure and dynamics of geographical ranges. Oxford University Press, Oxford

Geertjes GJ, Postema J, Kamping A, van Delden W, Videler JJ, van de Zande L (2004) Allozymes and RAPDs detect little genetic population substructuring in the Caribbean stoplight parrotfish *Sparisoma viride*. Marine Ecology Progress Series 279: 225-235

Giles BE, Goudet J (1997) A case study of genetic structure in a plant metapopulation. In: Hanski IA, Gilpin ME (eds) Metapopulation biology. Ecology, genetics and evolution. Academic Press, San Diego, pp 429 - 454

Gilpin ME (1991) The genetic effective size of a metapopulation. In: Gilpin ME, Hanski IA (eds) Metapopulation dynamics: Empirical and theoretical considerations. Academic Press, London

Godwin JR, Thomas P (1993) Sex change and steroid profiles in the protandrous anemonefish *Amphiprion melanopus* (Pomacentridae, Teleostei). General and Comparative Endocrinology 91: 144-157

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139: 463 - 471

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/software/fstat.htlm.

Grant MC, Antonovics J (1978) Biology of ecologically marginal populations of *Anthoxanthum odoratum*. I. Phenetics and dynamics. Evolution 32: 822-838

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Heredity 89: 415 - 426

Greenwood P (1980) Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28: 1140 - 1162

Grosberg RK, Cunningham CW (2001) Genetic structure in the sea. From populations to communities. In: Bertness MD, Gaines S, Hay ME (eds) Marine community ecology. Sinauer Associates, Sunderland, pp 61-84

Guo QF, Taper M, Schoenberger M, Brandle J (2005) Spatial-temporal population dynamics across species range: from centre to margin. Oikos 108: 47-57

Gust N (2004) Variation in the population biology of protogynous coral reef fishes over tens of kilometres. Canadian Journal of Fisheries and Aquatic Sciences 61: 205-218

Gust N, Choat JH, McCormick M (2001) Spatial variability in reef fish distribution, abundance, size and biomass: a multi-scale analysis. Marine Ecology Progress series 214: 237-251

Hanski IA (1991) Metapopulation Ecology. Oxford University Press, Oxford, pp 313

Hanski IA, Gilpin ME (1997) Metapopulation biology. Ecology, genetics and evolution. Academic Press, San Diego, pp 512

Hanski IA, Gyllenberg M (1993) Two general metapopulation models and the core-satellite species hypothesis. American Naturalist 142: 17 - 41

Hanski IA, Simberloff D (1997) The metapopulation approach, its history, conceptual domain, and adaptation to conservation. In: Hanski IA, Gilpin ME (eds) Metapopulation biology. Ecology, genetics and evolution. Academic Press, San Diego, pp 5 - 26

Harrison S (1991) Local extinction in a metapopulation context: An empirical evaluation. In: Gilpin ME, Hanski IA (eds) Metapopulation dynamics: Empirical and theoretical investigations. Academic Press, London, pp 73 - 88

Harrison S, Hastings A (1996) Genetic and evolutionary consequences of metapopulation structure. Trends in Ecology and Evolution 11: 180-183

Hartl DL, Clark AG (1997) Principles of population genetics. Sinauer Associates, Inc., Sunderland

Hedgecock D (1986) Is gene flow from larval dispersal important in the adaptation and evolution of marine invertebrates? Bulletin of Marine Science 39: 550 - 564

Hedrick, PW (1999) Highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313 – 318

Hedrick, PW (2005) A standardised genetic differentiation measure. Evolution 59: 1633 - 1638

Hellberg ME (1996) Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50: 1167 - 1175

Hellberg ME, Burton RS, Neigel JE, Palumbi SR (2002) Genetic assesment of connectivity among marine populations. Bulletin of Marine Science 70: 273 - 290

Hoffman EA, Blouin MS (2004) Historical data refute recent range contraction as cause of low genetic diversity in isolated frog populations. Molecular Ecology 13: 271-276

Hoffman EA, Kolm N, Berglund A, Arguello JR, Jones AG (2005) Genetic structure in the coral-reef-associated Banggai cardinalfish, *Pterapogon kauderni*. Molecular Ecology 14: 1367-1375

Hoffmann AA, Parsons PP (1991) Evolutionary genetics and environmental stress. Oxford University Press, Oxford

Holt RD (1987) Population dynamics and evolutionary processes: the manifold roles of habitat selection. Evolutionary Ecology 1: 331 - 347

Holt RD, Keitt TH (2000) Alternative causes for range limits: a metapopulation perspective. Ecology Letters 3: 41-47

Holt RD, Keitt TH (2005) Species' borders: a unifying theme in ecology. Oikos 108: 3-6

Holt RD, Keitt TH, Lewis MA, Maurer BA, Taper ML (2005) Theoretical models of species' borders: single species approaches. Oikos 108: 18-27

Hopley D, Thom BG (1983) Australian Sea levels in the last 15000 years: An introductory review. In: Hopley D (ed) Australian Sea levels in the last 15000 years: A review. Department of Geography, James Cook University, Townsville, pp 29 - 36

Huelsenbeck JP, Ronquist F (2001) MR. BAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755

Hunt A (1993) Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Patriella clacar* and *P. exigua.* Marine Ecology Progress Series 92: 179 - 186

Hutchings JA, Gerber L (2002) Sex-biased dispersal in a salmonid fish. Proceedings of the Royal Society of London Series B 269: 2487-2493

Jain SK, Rai KN, Singh RS (1981) Population biology of Avena XI. Variation in peripheral isolates of *A. barbata*. Genetica 56: 213-215

Jones GP, Milicich MJ, Emslie MJ, Lunow C (1999) Self-recruitment in a coral reef fish population. Nature 402: 802 - 804

Kat PW (1982) The relationship between heterozygosity for enzyme loci and developmental homeostasis in peripheral populations of aquatic bivalves (Unionidae). American Naturalist 119: 824-832

Kavanagh KD (2000) Larval brooding in the marine damselfish *Acanthochromis polyacanthus* (Pomacentridae) is correlated with highly divergent morphology, ontogeny and life-history traits. Bulletin of Marine Science 66: 321 - 337

Kerkis J (1975) Some problems of spontaneous and induced mutagenesis in mammals and man. Mutation Research 29: 271-277

Kerrigan BA (1996) Temporal patterns in size and condition at settlement in two tropical reef fishes (Pomacentridae: *Pomacentrus amboinensis* and *P. nagasakiensis*). Marine Ecology Progress Series 135: 27-41

Kerrigan BA (1997) Variability in larval development of the tropical fish *Pomacentrus ambionensis* (Pomacentridae): the parental legacy. Marine Biology 127: 395 - 402

Kimura M (1955) Solution of a process of random genetic drift with a continous model. Proceedings of the National Academy of Science USA 41: 144 - 150

Kimura M, Crow JF (1963) On the maximum avoidance of inbreeding. Genetics Research 4: 399 - 415

Kimura M, Otha T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. Proceedings of the National Academy of Science 75: 2868 - 2872

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49: 561 - 576

Knowles LL (2004) The burgeoning field of statistical phylogeography. Journal of Evolutionary Biology 17: 1-10

Knowles LL, Maddison WP (2002) Statistical phylogeography. Molecular Ecology 11: 2623-2635

Knowlton N (1993) Sibling species in the sea. Annual Review of Ecology and Systematics 24: 189 - 216

Kritzer JP, Sale PF (2004) Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish and Fisheries 5: 131-140

Kuhner MK, Yamato J, Felsenstein J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149: 429-434

Kuiter RH (1993) Coastal fishes of south-eastern Australia. University of Hawaii Press, Honolulu

Lande R, Engen S, Saether BE (1998) Extinction times in finite metapopulation models with stochastic local dynamics. Oikos 83: 383-389

Larcombe P (2001) Holocene Great Barrier Reef: sedimentary controls and implications for environmental management. In: Gostin VA (ed) Gondwana to greenhouse. Australian environmental geoscience. Southwood Press, Sydney, pp 281 - 294

Leberg PL (2002) Estimating allelic richness: Effects of sample size and bottlenecks. Molecular Ecology 11: 2445-2449

Lecomte F, Grant WS, Dodson JJ, Rodriguez-Sanchez R, Bowen BW (2004) Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Molecular Ecology 13: 2169-2182

Lee W, Conroy J, Howell W, Kocher TD (1995) Structure and evolution of teleost mitochondrial control regions. Journal of Molecular Evolution 41: 54 - 66

Leis JM (1991) The pelagic stage of reef fishes: The larval biology of coral reef fishes. In: Sale PF (ed) The ecology of fishes on coral reefs. Academic Press, San Diego, pp 183 - 230

Leis JM (2002) Pacific coral-reef fishes: the implications of behaviour and ecology of larvae for biodiversity and conservation, and a reassessment of the open population paradigm. Environmental Biology of Fishes 65: 199-208

Leis JM, Carson-Ewart BM (2000) The larvae of Indo-Pacific coastal fishes. An identification guide to marine fish larvae. Fauna Malesiana Handbooks 2, Brill, Leiden

Lennon JJ, Turner JRG, Connell D (1997) A metapopulation model of species boundaries. Oikos 78: 486-502

Levins R (1970) Extinction. In: Gerstenhaber M (ed) Some mathematical problems in biology. American Mathematical Society, Providence, pp 75 - 107

Lindgren D (1972) The temperature influence on the spontaneous mutation rate. Hereditas 70: 165-178

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. Molecular Ecology 3: 91-99

MacPhee DG (1984) Indications that mutagenesis in *Salmonella* may be subject to catabolite repression. Mutation Research 151: 35-41

Mayr E (1963) Animal species and evolution. Belknap Press, Cambridge

McCauley DE (1991) Genetic consequences of local population extinction and recolonization. Trends in Ecology and Evolution 6: 5-8

McCauley RA, Ballard HE (2002) Inferring nativity and biogeographic affinities of central and marginal populations of *Froelichia floridana* (Amaranthaceae) from Inter-Simple Sequence Repeat (ISSR) markers. Journal of the Torrey Botanical Society 129: 311-325

McCormick MI (1994) Variability in the age and size at settlement of the tropical goatfish *Upeneus tragula* (Mullidae) in the northern Great Barrier Reef lagoon. Marine Eology Progress Series 103: 1 - 15

McCormick MI (1998a) Condition and growth of reef fish at settlement: Is it important? Australian Journal of Ecology 23: 258-264

McCormick MI (1998b) Behaviourally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. Ecology 79: 1873 - 1883

McCormick MI (1999) Delayed metamorphosis of a tropical reef fish (*Acanthurus triostegus*); a field experiment. Marine Ecology Progress Series 176: 25 - 38

McCormick MI, Hoey AS (2004) Larval growth history determines juvenile growth and survival in a tropical marine fish. Oikos 106: 225 - 242

McCormick MI, Molony BW (1992) Effects of feeding history on the growth characteristics of a reef fish at settlement. Marine Biology 114: 165-173

McCormick MI, Molony BW (1995) Influence of water temperature during the larval stage on size, age and body condition of a tropical reef fish at settlement. Marine Ecology Progress Series 118: 59 - 68

McMillan WO, Palumbi SR (1997) Rapid rate of control region evolution in Pacific butterflyfishes (Chaetodontidae). Journal of Molecular Evolution 45: 473 - 484

Meekan MG, Carleton J, McKinnon A, Flynn K, Furnas M (2003) What determines the growth of tropical reef fish larval in the plankton: food or temperature? Marine Ecology Progress Series 256: 193 - 204

Messmer V, van Herwerden L, Munday PL, Jones GP (2005) Colour morphs in *Pseudochromis fuscus* (Family Pseudochromidae): genetic and morphological studies. Coral Reefs 24: 392-402.

Miller-Sims V, Atema J, Kingsford MJ, Gerlach G (2005) Characterization and isolation of DNA microsatellite primers in the spiny damselfish *Acanthochromis polyacanthus*. Molecular Ecology Notes 5: 841-843

Mitton JB, Lewis WMJ (1989) Relationships between genetic variability and life-history features of bony fishes. Evolution 43: 1712-1723

Mossman CA, Waser PM (1999) Genetic detection of sex-biased dispersal. Molecular Ecology 8: 1063-1067

Munday PL, van Herwerden L, Dudgeon CL (2004) Evidence for sympatric speciation by host shift in the sea. Current Biology 14: 1498 - 1504

Murdoch J (1995) Variability in the early life history of coral reef fishes. MSc thesis. Marine Biology and Aquaculture, James Cook University. Townsville

Murdoch J, Doherty PJ (1997) Caution is required in back-calculating larval growth rates from presettlement fish collected with lighttraps. Proceedings 8th International Coral Reef Symposium 2: 1149-1154

Muss A, Robertson DR, Stepien CA, Wirtz P, Bowen BW (2001) Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. Evolution 55: 561 - 572

Nei M (1987) Molecular Evolutionary Genetics. Colombia University Press, New York

Nei M (1995) Genetic support for the out-of-Africa theory of human evolution. Proceedings of the National Academy of Science USA 92: 6720 - 6722

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution 29: 1 - 10

Nei MN, Graur D (1984) Extent of protein polymorphism and the neutral mutation theory. Evolutionary Biology 17: 73-118

Neigel JE (1997) A comparison of alternative strategies for estimating gene flow from genetic markers. Annual Review of Ecology and Systematics 28: 105-128

Neigel JE (2002) Is F_{ST} obsolete? Conservation Genetics 3: 167-173

Nelson JS, Hoddell RJ, Chou LM, Chan WK, Phang VEP (2000) Phylogeographic structure of the false clownfish, Amphiprion ocellaris, explained by sea level changes on the Sunda shelf. Marine Biology 137: 727 - 736

Nevo E (1978) Genetic variation in natural populations: patterns and theory. Theoretical Population Biology 13: 121-177

Nylander JAA (2004) MrModeltest V2. Program distributed by the author. Evolutionary Biology Centre. Uppsala University

Olivieri I, Couvet D, Gouyon PH (1990) The Genetics of Transient Populations - Research at the Metapopulation Level. Trends in Ecology and Evolution 5: 207-210

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Annual Review of Ecology and Systematics 25: 547-572

Palumbi SR (2004) Marine reserves and ocean neighborhoods: The spatial scale of marine populations and their management. Annual Review of Environment and Resources 29: 31 - 68

Palumbi SR, Gaines SD, Leslie H, Warner RR (2003) New wave: high-tech tools to help marine reserve research. Frontiers in Ecology and the Environment 1: 73 - 79

Palumbi SR, Grabowsky G, Duda TF, Geyer L, Tachino N (1997) Speciation and population genetic structure in tropical Pacific sea urchins. Evolution 51: 1506 - 1517

Pannell JR (2003) Coalescence in a metapopulation with recurrent local extinction and recolonisation. Evolution 57: 949 - 961

Pannell JR, Charlesworth B (1999) Neutral genetic diversity in a metapopulation with recurrent local extinction and recolonisation. Evolution 53: 664 - 676

Pannell JR, Charlesworth B (2000) Effects of metapopulation processes on measures of genetic diversity. Philosophical Transactions of the Royal Society of London B 355: 1851 - 1864

Pannella G (1971) Fish otoliths: Daily growth layers and periodical patterns. Science 173: 1124 - 1127

Parsons PA (1987) Evolutionary rates under evolutionary stress. Evolutionary Biology 21: 311-347

Parsons PA (1991) Evolutionary rates: stress and species boundaries. Annual Review of Ecology and Systematics 22

Paulay G (1990) Effects of late Cenozoic sea-level fluctuations on the bivalve faunas of tropical oceanic islands. Paleobiology 16: 415-434

Peakall R, Smouse PE (2001) GenALEx V5: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia. http://www.anu.au/BoZo/GenALEx/

Pedersen AA, Loeschcke V (2001) Conservation genetics of peripheral populations of the mygalomorph spider *Atypus affinis* (Atypidae) in northern Europe. Molecular Ecology 10: 1133-1142

Pérez-Lezaun AF, Calafell AF, Mateu E, Comas D, Ruiz-Pacheco R, Bertranpetit J (1997) Microsatellite variation and the differentiation of modern humans. Human Genetics 99: 1 - 7

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90: 502-503

Pitcher CR (1988) Validation of a technique for reconstructing daily pattens in the recruitment of coral reef damselfish. Coral Reefs 7: 105-111

Planes S (1998) Genetic diversity and dispersal capabilities in marine fish. Evolutionary Biology 30: 253-298

Planes S (2002) Biogeography and larval dispersal inferred from population genetic analysis. In: Sale PF (ed) Coral Reef Fishes. Dynamics and diversity in complex ecosystems. Academic Press, London, pp 201 - 220

Planes S, Doherty PJ, Bernardi G (2001) Strong genetic divergence among populations of a marine fish with limited dispersal, *Acanthochromis polyacanthus*, within the Great Barrier Reef and the Coral Sea. Evolution 55: 2263-2273

Planes S, Fauvelot C (2002) Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. Evolution 56: 378-399

Planes S, Galzin R, Bonhomme F (1996) A genetic metapopulation model for reef fishes in oceanic islands: the case of the surgeonfish, *Acanthurus triostegus*. Journal of Evolutionary Biology 9: 103 - 117

Planes S, Parroni M, Chauvet C (1998) Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. Marine Biology 130: 361 - 368

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817 - 818

Pulliam HR (1988) Sources, sinks and population regulation. American Naturalist 132: 652 - 661

Quenouille B, Bermingham E, Planes S (2004) Molecular systematics of the damselfishes (Teleosti: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution 31: 66-88

Radtke R, Kinzie RI, Shafer D (2001) Temporal and spatial variation in length of larval life and size at settlement of the Hawaiian amphidromous goby *Lentipes concolor*. Journal of Fish Biology 59: 928 - 938

Rand DM (2001) The units of selection on mitochondrial DNA. Annual Review of Ecology and Systematics 32: 415-448

Randall JE, Allen GR, Steene RC (1997) Fishes of the Great Barrier Reef and Coral Sea. Crawford House Publishing, Bathurst

Raymond M, Rousset F (1995) Genepop (Version-1.2) - population-genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249

Reynolds J, Weir BS, Cockerham CC (1983) Estimation of the co-ancestry coefficient - basis for a short-term genetic distance. Genetics 105: 767-779

Riginos C, Victor BC (2001) Larval spatial distribution and other early life-history characteristics predict genetic differentiation in eastern Pacific blennoid fishes. Proceedings of the Royal Society of London B 268: 1931 - 1936

Robertson DR, Grove JS, McCosker JE (2004) Tropical panpacific shore fishes. Pacific Science 58: 507-516

Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preference, larval dispersal, and comparative phylogeny of three Atlantic surgeonfishes (Teleostei: Acanthuridae). Molecular Ecology 11

Rocha LA, Robertson DR, Roman J, Bowen BW (2005) Ecological speciation in tropical reef fishes. Proceedings of the Royal Society of London B 272: 573 - 579

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145: 1219 - 1228

Russ G (1984) Distribution and abundance of herbivorous grazing fishes in the central Great Barrier Reef. I. Levels of variability across the entire continental shelf. Marine Ecology Progress Series 20: 23-34

Sale PF (1991) Reef fish communities: open nonequilibrial systems. In: Sale PF (ed) The ecology of fishes on coral reefs. Academic Press Inc., San Diego, pp 564-598

Sale PF, Doherty PJ, Douglas WA (1980) Juvenile recruitment strategies and the coexistence of territorial pomacentrid fishes. Bulletin of Marine Science 30: 147 - 158

Sambrook J, Russell D (2001) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Schnabel A, Hamrich JL (1990) Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminossae). American Journal of Botany 77: 1060-1069

Schneider S, Roessli D, Excoffier L (2000) Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva

Searcy SP, Sponaugle S (2000) Variable larval growth in a coral reef fish. Marine Ecology Progress Series 206: 213 - 226

Selander RK, Kaufman DW (1973) Genetic variability and strategies of adaptation in animals. Proceedings of the National Academy of Science 70: 1875-1877

Shima JS, Findlay AM (2002) Pelagic larval growth rate impacts benthic settlement and survival of a temperate reef fish. Marine Ecology Progress Series 235: 303 - 309

Shulman MJ (1998) What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes? Australian Journal of Ecology 23: 216 - 225

Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of caribbean reef fishes. Evolution 49: 897 - 910

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. Theoretical Population Biology 12: 253 - 262

Slatkin M (1985) Gene flow in natural populations. Annual Review of Ecology and Systematics 16: 393 - 430

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236: 787-792

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. Evolution 471: 264 - 279

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129: 555 - 562

Smith PJ, Fujio Y (1982) Genetic variation in marine teleosts: high variability in habitat specialists and low variability in habitat generalists. Marine Biology 69: 7-20

Smouse PE, Long JC, Sokal RR (1986) Multiple Regression and Correlation Extensions of the Mantel Test of Matrix Correspondence. Systematic Zoology 35: 627-632

Sokal RR, Rohlf FJ (1995) Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Company, New York

Soulé M (1972) Phenetics of natural populations. III. Variation in insular populations of a lizard. American Naturalist 106: 429-446

Soulé M (1976) Allozyme variation: Its determination in space and time. In: Ayala FJ (ed) Molecular Evolution. Sinauer Associates, Sunderland MA, pp 60-77

Sponaugle S, Cowen RK (1994) Larval durations and recruitment patterns of two Caribbean gobies (Gobiidae): contrasting early life histories in demersal spawners. Marine Biology 120: 133 - 143

Sponaugle S, Cowen RK (1997) Early life history traits and recruitment patterns of Caribbean wrasses (Labridae). Ecological Monographs 67: 177 - 202

Sponaugle S, Cowen RK, Shanks A, Morgan SG, Leis JM, Pineda J, Boehlert GW, Kingsford MJ, Lindeman KC, Grimes C, Munro JL (2002) Predicting self-recruitment in marine populations: biophysical correlates and mechanisms. Bulletin of Marine Science 70: 341 - 375

Sponaugle S, Pinkard D (2004) Impact of variable pelagic environments on natural larval growth and recruitment of the reef fish *Thallosoma bifasciatum*. Journal of Fish Biology 64: 34 - 54

Stacey PB, Johnson VA, Taper ML (1997) Migration within metapopulations: The impact upon local population dynamics. In: Hanski IA, Gilpin ME (eds) Metapopulation biology. Ecology, genetics and evolution. Academic Press, San Diego, pp 267 - 291

Stacey PB, Taper M (1992) Environmental variation and the persistence of small populations. Ecological Applications 2: 18 - 29

Stobutzki IC (1998) Interspecific variation in sustained swimming ability of late pelagic stage reef fish from two families (Pomacentridae and Chaetodontidae). Coral Reefs 17: 111 - 119

Swearer S, Caselle J, Lea D, Warner R (1999) Larval retention and recruitment in an island population of a coral-reef fish. Nature 402: 799 - 802

Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR (2002) Evidence of self-recruitment in demersal marine populations. Bulletin of Marine Science 70: 251 - 271

Swofford DL (1998) Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauers Associates, Sunderland, Massachusetts

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. Genetics 105: 437 - 460

Tajima F (1993) Measurement of DNA polymorphism. In: Takahata N, Clark AG (eds) Mechanisms of molecular evolution. Introduction to molecular paleopopulation biology. Japan Scientific Press, Sinauer Associates, Inc., Tokyo, pp 37 - 59

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144: 389 - 399

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512 - 526

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larval in a Caribbean reef fish. Science 299: 107 - 109

Thorrold SR, Milicich MJ (1990) Comparison of larval duration and pre- and post-settlement growth in two species of damselfish, *Chromis atripectoralis* and *Pomacentrus coelestis* (Pisces: Pomacentridae), from the Great Barrier Reef. Marine Biology 105: 375 - 384

Thresher RE (1985) Distribution, abundance, and reproductive success in the coral reef fish *Acanthochromis polyacanthus*. Ecology 66: 1139 - 1150

Thresher RE, Brothers EB (1985) Reproductive ecology and biogeography of Indo-west Pacific angelfishes (Pisces: Pomacanthidae). Evolution 39: 878 - 887

Thresher RE, Brothers EB (1989) Evidence of intra- and inter-oceanic regional differences in the early life history of reef-associated fishes. Marine Ecology Progress Series 57: 187 - 205

Thresher RE, Colin PL, Bell LJ (1989) Planktonic duration, distribution and population structure of Western and Central Pacific damselfishes (Pomacentridae). Copeia 1989: 420 - 434

Tigerstedt PMA (1973) Studies on isozyme variation in marginal and central populations of *Picea abies*. Hereditas 75: 47-60

Valone TJ, Brown JH (1995) Effects of competition, colonisation and extinction on rodent species diversity. Science 267: 880 - 883

van Herwerden L, Doherty PJ (2006) Contrasting genetic structures across two hybrid zones of a tropical reef fish, *Acanthochromis polyacanthus* (Bleeker 1855). Journal of Evolutionary Biology 19: 239-252

van Rossum F, Vekemans X, Meerts P, Gratia E, Lefebre C (1997) Allozyme variation in relation to ecotypic differentiation and population size in marginal populations of *Silene nutans*. Heredity 78: 552-560

Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Molecular Ecology 11: 139-151

Vermeij GJ (1987) The dispersal barriers in the tropical Pacific: implications for molluscan speciation and extinction. Evolution 41: 1046-1058

Victor BC (1982) Daily otolith increments and recruitment in two coral-reef wrasses *Thallosoma bifasciatum* and *Halichoeres bivittatus*. Marine Biology 71: 203 - 208

Victor BC (1986a) Duration of the planktonic larval stage of one hundred species of Pacific and Atlantic wrasses (family Labridae). Marine Biology 90: 317 - 326

Victor BC (1986b) Delayed metamorphosis with reduced larval growth in a coral reef fish (*Thallosoma bifasciatum*). Canadian Journal of Fisheries and Aquatic Science 43: 1208 - 1213

Victor BC (1991) Settlement strategies and biogeography of reef fishes. In: Sale PF (ed) The ecology of fishes on coral reefs. Academic Press, San Diego, pp 231 - 260

Victor BC, Wellington GM (2000) Endemism and the pelagic larval duration of reef fishes in the eastern Pacific Ocean. Marine Ecology Progress Series 205: 241 - 248

Vucetich JA, Waite TA (2003) Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape conservation genetics. Conservation Genetics 4: 639-645

Wade MJ, McCauley DE (1988) Extinction and recolonization - their effects on the genetic differentiation of local populations. Evolution 42: 995-1005

Walker GC (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiological Reviews 48: 60-93

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41: 385 - 400

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Heredity 89: 438 - 450

Weir BS, Cockerham CC (1984) Estimating *F*- statistics for the analysis of population structure. Evolution 38: 1358 - 1370

Weiss GH, Kimura M (1964) A mathematical analysis of the stepping-stone model of genetic correlation. Journal of Applied Probability 2: 129 - 149

Wellington GM, Victor BC (1989) Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). Marine Biology 101: 557 - 567

Wellington GM, Victor BC (1992) Regional differences in duration of the planktonic larval stage of reef fishes in the eastern Pacific Ocean. Marine Biology 113: 491 - 498

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction - genetic correlations within founding groups. Evolution 44: 1717-1724

Wilson DT, McCormick MI (1999) Microstructure of settlement-marks in the otoliths of tropical reef fishes. Marine Biology 134: 29 - 41

Wilson DT, McCormick MI (1997) Spatial and temporal validation of settlement-marks in the otoliths of tropical reef fishes. Marine Ecology Progress Series 153: 259-271

Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. International review of cytology 141: 173 - 216

Wright S (1931) Evolution in Mendelian populations. Genetics 16: 97 - 159

Wright S (1943) Isolation by distance. Genetics 28: 114 - 138

Zapata FA, Herron PA (2002) Pelagic larval duration and geographic distribution of tropical eastern Pacific snappers (Pisces: Lutjanidae). Marine Ecology Progress Series 230: 295 - 300

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification Genomics 20: 176-183

Appendix 1

Reciprocal migration rates ($4N_eM$) among reefs in the northern, central and southern regions estimated for the microsatellites reduced by 1/3 and 1/6. The thickness of the arrows indicate the migration rates and the colour indicates statistical difference between reciprocal migration rates (black = 95% confidence intervals of estimates did not overlap; grey = 95% confidence intervals of estimates overlapped).