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The Importance of Fine-Scale Environmental Heterogeneity in Determining Levels of Genotypic Diversity and Local Adaptation

A thesis submitted in fulfilment of the requirements for the award of the degree

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by

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The intertidal sea anemone *Actinia tenebrosa*. Photograph by A.M Martin

Certification

I, Craig D. H. Sherman, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Craig Sherman
13 January 2005

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Abstract

Fine-scale environmental heterogeneity is predicted to be important in determining variation in genotypic diversity and in selection for important life history traits in natural populations. For example, theory suggests that organisms with complex life histories that involve both sexual and asexual modes of reproduction use sex to produce genotypically diverse and widely dispersed propagules for the colonisation of distant or unstable habitats, but rely on asexual reproduction to restock or maintain populations within their parental habitat. Such organisms should also have great potential for site-specific adaptation as multiple generations may compete within relatively static conditions. Surprisingly, little is still known about the importance of fine-scale genotypic variation and the degree of local adaptation within populations of clonal marine organisms.

In this study, I used two brooding corals (*Pocillopora damicornis* and *Seriatopora hystrix*) and one brooding sea anemone (*Actinia tenebrosa*), to test for evidence of fine-scale adaptation and the effects of environmental heterogeneity on variation in genotypic diversity. Using a combination of genetic and experimental techniques I assessed: i) if reproductive mode varies with environmental heterogeneity across habitats, ii) how genotypic diversity varies over fine spatial scales (centimetres and meters), and iii) if different clonal genotypes show evidence of fine-scale adaptation to specific habitats.

My data on the population genetics and mode of reproduction for the corals *P. damicornis* and *S. hystrix* did not support theoretical predictions. Brooded larvae from *P. damicornis* colonies collected in five reef habitats were all produced asexually. In contrast, brooded larvae of *Seriatopora hystrix* were sexually produced, with up to three sires contributing to some broods (r_p (±SE) = 0.32 ± 0.43), and almost half (46%) of the larvae resulting from self-fertilisation (mean outcrossing rates were t_m (±SE) = 0.54 ± 0.22). The population genetic structure of *S. hystrix* from One Tree Island matched that expected from the mating system; i.e. a high level of genetic subdivision due to restricted dispersal of gametes, and consistent heterozygote deficits within populations associated with inbreeding. However, populations of *P. damicornis* showed unexpectedly high levels of genotypic diversity and appear to be maintained by sexual reproduction; G_o/G_e ranged from 69 to 100% of that

expected for random mating within 14 sites across six habitats. Interestingly, at three sites in two habitats G_o/G_e ranged from 35 to 53%. Two of these sites were recently bleached, suggesting that asexual recruitment may be favoured after disturbance, although disturbance alone is probably insufficient to explain this species' continued investment in clonal reproduction.

Using a combination of variable microsatellite and allozyme markers, I assessed the genetic origin of brooded juveniles from adult *Actinia tenebrosa* collected from boulder and rock pool habitats to determine if the mode of reproduction varied with environment. Brooded juveniles displayed identical multi-locus genotypes to that of the brood parent, irrespective of habitat type or location. However, I found that the level of genotypic diversity varied widely among 19 *A. tenebrosa* populations across 2500km of its geographic range along the east coast of Australia. Some populations showing high levels of clonality while others displayed the level of genotypic diversity expected for sexual reproduction.

For *A. tenebrosa*, my results indicate that the importance of sexual and asexual reproduction may indeed vary among habitats with different levels of heterogeneity confirming predictions from evolutionary theory. Fine-scale genetic surveys ($<1m^2$) on the distribution of clones of *A. tenebrosa* revealed that clonal diversity was greater on individual boulders (71%) compared to rock pools (23%). However, samples collected over larger spatial scales ($25m^2$) revealed little difference in genotypic diversity between boulder (80%) and rock pool habitats (70%). Clones had limited distributions, although some could be spread throughout an entire habitat. With the exception of a single clone, I found no overlap of genotypes between boulder and rock pool habitats on the same rocky shore. This distinct segregation of genotypes to habitats within the same rocky shore may result either from highly limited dispersal of asexual propagules and/or fine-scale selection for certain genotypes in particular habitats.

To test for evidence of local adaptation to fine-scale environmental variation in different habitats, I reciprocally transplanted *A. tenebrosa* both within and between habitats. I found no evidence of adaptation of clones within habitats, with transplanted anemones performing

equally well to native anemones in terms of survivorship ($F_{1,\ 0.01}$ = 11.79, P = 0.075), proportion of adults brooding juveniles ($F_{1,\ 0.01}$ = 0.40, P = 0.592), mean number of juveniles/site ($F_{1,\ 2281}$ = 0.801, P = 0.068), mean number of juveniles/brood ($F_{1,\ 12.2}$ = 1.238, P = 0.382), and growth ($F_{1,\ 0.03}$ = 0.007, P = 0.942). However, between-habitat transplants provided evidence that clones of *A. tenebrosa* are locally adapted at the habitat scale. Native anemones consistently out-performed foreign anemones transplanted from the adjacent habitat (survivorship $F_{2,\ 0.298}$ = 9.58, P < 0.001; proportion adults brooding $F_{2,\ 0.139}$ = 3.12, P = 0.05; mean number of juveniles/site $F_{2,\ 14039}$ = 3.90, P = 0.028; growth $F_{2,}$ = 4.77, P = 0.014).

In summary, the results from this study show little evidence that reproductive mode varies predictably among habitats for any of the three species tested. Furthermore, there appears to be a mismatch between the population genetic structure and the reproductive output for two of the three species. Level of genotypic diversity was shown to vary over different spatial scales, and with habitat to some degree, both in *P. damicornis* and *A. tenebrosa*, and transplant experiments provide evidence of fine-scale adaptation to specific habitats for *A. tenebrosa*. These results suggest that for some species, such as the brooding sea anemone *Actinia tenebrosa*, the importance of sexual and asexual reproduction may indeed vary among habitats with different environmental heterogeneity in the manner predicted by evolutionary theory.

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Chapter 1 General Introduction

The last century has seen major advances in our understanding of the ecological and evolutionary importance of genotypic variation in populations and the manner in which natural selection influences the evolution of life history characteristics. This has been particularly aided by a greater understanding of the genetic basis for inheritance of traits and the development of molecular techniques that offer powerful tools for determining mating systems and quantifying levels of genotypic variation within and between populations. However, it has become increasingly clear that the selection of important life history traits may be occurring at much smaller spatial scales than has previously been realised, especially in heterogenous environments where fine-scale temporal and spatial variation result in a number of microhabitats that differ in their selective regimes (Koehn *et al.* 1980; Yamada 1989; Johannesson *et al.* 1993; Linhart and Grant 1996; Rolan-Alvarez *et al.* 1997; Riginos and Cunningham 2005). Theoretical models predict that the likely importance of selection, mediated through competition or fine-scale environmental variation, and the level of habitat stability and heterogeneity, will determine the genotypic and phenotypic composition of populations (Weismann 1889; Hamilton 1980; Bell 1982; Michod and Levin 1988; Ladle 1992).

Organisms with the capacity both for sexual and asexual reproduction provide an ideal system with which to test for the effects of fine-scale environmental variation on the distribution of genotypic diversity, and the potential for local adaptation. Theoretical predictions suggest that levels of genotypic diversity in populations of organisms with the potential both for sexual and asexual reproduction should increase predictably with increasing levels of environmental heterogeneity, while selection, mediated through inter-clonal competition and fine-scale environmental variation, will result in locally adapted clones (Williams 1975; Maynard Smith 1978; Bell 1982; Michod and Levin 1988; Ladle 1992; Vrijenhoek 1998).

1.1 Predicted Roles of Sexual and Asexual Propagules

A number of theoretical models have been proposed to explain the circumstances under which sexual and asexual reproduction will be most adaptive (Williams 1975; Maynard

Smith 1978; Bell 1982; Michod and Levin 1988). At the simplest level these models predict that organisms that can reproduce both sexually and asexually are expected to be favoured by selection if they use sexual reproduction to generate diverse and relatively widely dispersed propagules, and asexual reproduction for recruitment within the parental habitat patch (Williams 1975; Maynard Smith 1978; Bell 1982). At equilibrium populations should consist of a small number of the fittest genotypes with the life history characteristics of those clones, and hence the population, dependent upon the regime of selection.

This dichotomy of roles of sexual and asexual propagules can clearly be seen in a number of plant, freshwater and marine taxa. In plants, many species reproduce sexually by seed, and asexually by some form of vegetative propagation (e.g., stolons, rhizomes, bulbs, buds or fragments). Seeds often have structures that aid and ensure their dispersal outside of the parental habitat patch, while vegetative propagules have much more limited dispersal abilities resulting in the local retention of such propagules (e.g. strawberries). Likewise, the life cycles of many marine and freshwater invertebrate species often involves asexual reproduction, punctuated by episodes of sexual reproduction (Hughes and Cancino 1985; Hughes 1989; Harrison and Wallace 1990; Shick 1991). Asexual reproduction can occur by a variety of mechanisms that include adult fission, pedal laceration, budding, cyclical parthenogenesis, the brooding of asexual larvae and fragmentation (Ottaway and Kirby 1975; Black and Johnson 1979; Shick *et al.* 1979; Sebens 1982; Stoddart 1983; Johnson and Threlfall 1987; Hughes and Cancino 1985; Hebert *et al.* 1988; Hughes 1989), while sexual reproduction can occur both via gamete release followed by external fertilisation and development (Babcock *et al.* 1986; Shick 1991; Marshall *et al.* 2004) and the internal brooding of larvae (Ayre and Resing 1986).

For organisms with the capacity both for sexual and asexual reproduction levels of genotypic diversity can vary widely both within and among populations, and numerous studies have reported populations that contain one or a few common and widely spread genotypes: i.e. plants and grasses (Ellstrand and Roose 1987; Widen *et al.* 1994; Lehmann 1997); aquatic plants (Eckert et al. 2003); cladocerans (Hebert and Crease 1980; Carvalho

1994; Chaplin and Ayre 1997); sea anemones (Shick and Lamb 1977; Black and Johnson 1979; Shick *et al.* 1979; Ayre 1984b; Hoffmann 1986; Shaw 1991; Billingham and Ayre 1996); scleractinian corals (Neigel and Avise 1983; Stoddart 1984a; Ayre and Willis 1988; Hunter 1993; McFadden 1997); and gorgonian corals (Lasker 1990; Coffroth and Lasker 1998b; Chen *et al.* 2002). In contrast, other studies have shown that local populations may contain high levels of genotypic diversity, often approaching that expected for solely sexual reproduction: i.e. plants (Ziegenhagen *et al.* 2003; Arnaud-Haond *et al.* 2005; Ruggiero *et al.* 2005); cladocerans (Hebert 1974; Hebert and Ward 1976); fish (Angus 1980; Vrijenhoek 1998); sea anemones (Sebens and Thorne 1985; Shaw 1991); and scleractinian corals (Benzie *et al.* 1995; Ayre and Hughes 2000; Miller and Ayre 2004).

A number of ecological models have been proposed that emphasise the importance of temporal and spatial variation in determining levels of genotypic diversity within populations. Temporal variation may select for different clones under different environmental conditions, while habitat heterogeneity may allow different clones to succeed in a variety of microhabitats (i.e. niche separation within a heterogeneous environment) (Jackson 1985; Sebens and Thorne 1985). Models such as Williams (1975) Aphid-Rotifer and Strawberry-Coral models, present two such models under which sexually and asexually produced propagules are adapted to different ecological roles.

In the Aphid-Rotifer model, the ecological roles of sexual and asexual propagules relates to changing environmental conditions (temporal variation), which causes a shift from one mode of reproduction to another. This is characteristic of many freshwater cyclical parthenogens (i.e. *Daphnia spp.*) that use sexual reproduction to produce genotypically diverse diapausing (dormant) eggs during periods of harsh environmental conditions (Carvalho and Wolf 1989). These eggs then hatch following the return of favourable and stable conditions and intense inter-genotypic competition results in the selection for genotypes that are competitively superior. These surviving genotypes then use asexual reproduction to proliferate, such that each pond becomes dominated by a small number of highly replicated clones (Hebert *et al.* 1988) (for a similar example in aphids, see Rispe *et al.* 1998).

The Strawberry-Coral model differs from the Aphid-Rotifer Model in that the short-term costs of sex may be overcome if sexual and asexual propagules differ in their dispersal abilities in heterogeneous environments. The model predicts that for sessile organisms (such as strawberries and corals), asexual reproduction will be used for the production of locally dispersed clones. The combined effects of asexual reproduction and localised dispersal means that exceptionally fit genomes can be inherited intact and propagules will be retained within the parental habitat to which they are locally adapted. However, when a clone reaches the limits of this favourable habitat patch, either due to the presence of competing clones or encountering unfavourable environmental conditions, sexual reproduction should be the favoured tactic for the colonisation of areas outside the parental habitat patch. This is because the production of genetically diverse progeny increases the probability of producing an individual that may be fitter in a new habitat patch.

Clear predictions can be made about the genotypic structure of populations for organisms that use sexual and asexual reproduction in the manner predicted by the Strawberry-Coral model. Generally, asexual reproduction will be responsible for the structuring of populations over fine-spatial scales (centimetres to meters) while sexual reproduction will be responsible for patterns over larger scales (kilometres). As such one would expect that localised asexual reproduction would result in the formation of clonal aggregations, the extent of which will be determined by a combination of inter-clonal competition and localised selection. In contrast, if sexual reproduction occurs under conditions of random mating and free recombination, then within each breeding population, clonal genotype frequencies should conform to single locus Hardy-Weinberg equilibria, and should approach multi-locus linkage equilibrium.

However, under the Strawberry-Coral model, the level of genotypic diversity within each population may also be dependent on the level of environmental heterogeneity and the frequency and history of disturbance (Sebens and Thorne 1985). Habitats that show higher levels of heterogeneity, or that experience intermediate levels of disturbance, are more likely to display higher levels of genotypic diversity in comparison to less heterogeneous

and more stable habitats (Ellstrand and Roose 1987; Hunter 1993; Lehmann 1997). This is because heterogeneous environments are likely to offer a greater number of microhabitats that allows greater survivorship of sexually generated colonists due to increased opportunity for niche separation among competing clones (Jackson 1985), while periodic episodes of disturbance will open up new recruitment space (Connell 1978; Connell 1997). This means that the relative importance of sexual reproduction (and hence levels of genotypic diversity) should vary predictably among habitats with varying levels of environmental heterogeneity.

Surprisingly, little is still known about the importance of fine-scale (centimetres to meters) genotypic variation, how this varies with levels of environmental heterogeneity, and the degree of local adaptation within populations of clonal invertebrates. Additionally, for many organisms that reproduce both sexually and asexually, the likely importance of selection, mediated through inter-clonal competition or fine-scale environmental variation, is believed to be a major determinant of the genotypic and phenotypic composition of populations (Williams 1975), yet the scale of selection and levels of local adaptation remain unclear for the majority of clonal organisms. The main focus of this thesis is therefore to examine the relative importance of sexual and asexual reproduction in determining how fine-scale genotypic diversity varies with environmental heterogeneity, and the scale over which selection is acting. Studying the effects of fine-scale environmental variation and levels of habitat heterogeneity in organisms with the capacity both for sexual and asexual reproduction offers the best opportunity to understand the scale at which selection may operate. This is not only important for organisms with mixed reproductive strategies but are likely to be equally important in understanding the distribution of genetic variation and level of adaptation for a large number of sessile marine organisms with localised dispersal of sexually produced propagules.

1.2 The Study Species

In this study I will use two tropical, brooding corals (*Pocillopora damicornis* and *Seriatopora hystrix*) and one temperate, brooding sea anemone (*Actinia tenebrosa*), to test

how levels of habitat heterogeneity influences the fine-scale distribution of genotypic diversity and the scale at which selection may be operating on such species. Previous genetic, histological and ecological studies of these three species indicate that their life histories appear to fit some of the predictions of the Strawberry-Coral Model.

Genetic studies of Australian populations of *A. tenebrosa*, indicate that populations appear to be founded by widely dispersed and genotypically diverse (i.e. sexual) recruits, while asexually brooded juveniles represent the main source of recruits into established populations (Ayre 1983b; 1984b; Ayre *et al.* 1991a). Clones are believed to be exposed to prolonged periods of site-specific selection, resulting in only the fittest and locally adapted clones surviving and dominating within each population. Evidence for local adaptation of clones comes from a number of reciprocal transplant experiments where evidence of local adaptation has been shown over scales of 2-4 km and between different geographic regions separated by hundreds of kilometres (Ayre 1985; 1995). In these studies Ayre (1985; 1995) found that native clones were consistently fitter (as judged by asexual fecundity) than imported clones. However, these previous studies have been limited somewhat by their ability to identify the scale of local adaptation and levels of genotypic diversity within populations, and how this varies with environmental heterogeneity. This is in part due to the lack of highly variable genetic markers for this species, which allows accurate determination of the levels of fine-scale genotypic diversity and the identification of the extent and distribution of clones. If fine-scale environmental heterogeneity is important in determining the distribution and levels of genotypic diversity within populations of *A. tenebrosa*, this suggests that local adaptation may be occurring over much finer scales than has previously been tested.

For the brooding coral *Pocillopora damicornis*, in Western Australia and Hawaii the reproductive biology and population structure appears to support the predicted roles of sexual and asexual reproduction within a single life history (Stoddart 1983; 1984a; 1988). Asexual reproduction occurs via the brooding of larvae, while sexual reproduction has been inferred from histological studies that indicate broadcast spawning of gametes (Stoddart 1983; Stoddart and Black 1985; Ward 1992). Populations are dominated by a small

number of clones, while levels of genotypic diversity over larger scales indicates that populations are founded, at least initially, by widespread dispersal of sexually produced colonists (Stoddart 1984a; 1984b; 1988). In contrast to this, populations on the Great Barrier Reef, and the isolated Lord Howe Island Reef, show much higher levels of genotypic diversity and little evidence of clonal recruitment into local populations (Ayre *et al.* 1997b; Miller and Ayre 2004). This is surprising as a recent study has shown that the brooded larvae of *P. damicornis*, collected from the reef crest and reef flat habitats, are asexually produced (Ayre and Miller 2004). However, it remains unclear if such asexual larvae are important for local recruitment in the vast majority of reef habitats in which *P. damicornis* can be found, especially the more stable and benign lagoon habitats where it is predicted that asexual reproduction is more likely to be favoured (Williams 1975; Bell 1982).

The brooding coral, *S. hystrix* inhabits a similar range of habitats to *P. damicornis* and these two species can often be found side-by-side. Previous genetic studies have shown that levels of genotypic diversity within populations of *S. hystrix* on the Great Barrier Reef, are comparable to that seen in *P. damicornis* (Ayre and Hughes 2000). However, genetic studies on a single brood indicate, that in contrast to *P. damicornis*, *S. hystrix* broods are sexually produced (Ayre and Resing 1986). While a more comprehensive examination of the mating system of this species is needed, this species will allow an important comparison with *P. damicornis* as both appear to occupy similar habitats and display similar levels of genotypic diversity, yet use apparently different reproductive tactics. This comparison will therefore enable the elucidation of central processes that generate and maintain genotypic variation within these species.

1.3 Aims

Using these three model species, this study aims to specifically address the following questions:

1) Does the mode of reproduction vary among habitats with differing level of environmental heterogeneity?

2) How do levels of genotypic diversity vary over fine-spatial scales (centimetres and meters), and among habitats with differing level of environmental heterogeneity?

3) Do clones from different habitats show evidence of fine-scale adaptation to specific habitats?

1.4 Thesis Outline

This study will be the most comprehensive attempt to examine the underlying ecological and evolutionary processes determining the fine-scale genotypic structure and the importance of selection within a range of anthozoan species with sexual and asexual life histories.

The data chapters of this thesis have been written as separate publishable units, and as such there is some overlap between the introductions of some chapters.

In chapters 2 and 3, I present the results of the genotypic surveys of *P. damicornis* and *S. hystrix* from a variety of reef habitats at the One Tree Island Reef, Great Barrier Reef, Australia. I assess the mating system of *P .damicornis* across a variety of habitats and calculate outcrossing rates for broods of *S. hystrix* from one habitat.

In chapter 4, in order to assess if the mode of reproduction can vary among habitats with different levels of heterogeneity, I investigate the mating system of *A. tenebrosa* in both boulder and rock pool habitats using a combination of microsatellite and allozyme markers.

In chapter 5, I assess the distribution of clones and fine-scale genotypic diversity of *A. tenebrosa* within and between rock pool and boulder habitats. The spatial extent of clones is determined over several spatial scales (i.e. individual rock pools and boulders (centimetres); within a habitat (10s' of metres) and between habitats (100s' of metres)).

In chapter 6, using information gained from the fine-scale surveys of clones within and between rock pool and boulder habitats, I test for evidence that clones of *A. tenebrosa* are

locally adapted to their habitat of origin using reciprocal transplants both within and between habitats.

In chapter 7, I present the results of a large-scale genetic survey of *A. tenebrosa* populations along the east coast of Australia and Tasmania. This allows comparisons of the levels of genotypic diversity and gene flow among local populations and regions.

In chapter 8, I bring together the results from each chapter. I discuss some of the major findings from each chapter and some of their theoretical implications for current understanding of ecological and evolutionary theory, and outline some exciting areas of future research within this field.

Chapter 2 Asexual Reproduction does not Produce Clonal Populations within a Range of Reef Habitats for the Brooding Coral *Pocillopora damicornis* on the Great Barrier Reef, Australia

This chapter has been slightly modified from a paper published by *Coral Reefs*.

Sherman C.D.H., Ayre D.J., Miller K.J. (2005) Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia, Coral Reefs, DOI 10.1007/s00338-005-0053-x

2.1 Introduction

Corals display a diverse range of reproductive patterns, with many showing the capacity both for sexual reproduction (via internal fertilisation or broadcast spawning), and asexual reproduction (via fission, fragmentation and the asexual production of brooded larvae) (Harrison and Wallace 1990). Theoretical predictions of the relative roles of sexual and asexual reproduction suggest that organisms with mixed life history strategies use sexual reproduction to produce genotypically diverse and widely dispersed propagules, thus enabling colonisation of distant or unstable habitats. In contrast, asexual reproduction will be used to restock or maintain populations within the parental habitat patch (Williams 1975; Maynard Smith 1978; Bell 1982).

The effects of habitat stability and levels of disturbance on sexual and asexual reproduction may, however, be complex. For example, many sessile marine invertebrates, such as sponges and branching corals, fragment and disperse under the action of storms and strong waves (Tunnicliffe 1981; Highsmith 1982; Wulff 1985; Lasker 1990; Wulff 1991); while, in contrast, many freshwater zooplankton species reproduce sexually in response to physically stressful or changing environmental conditions (Hebert and Ward 1976; Hebert 1978; Hebert *et al.* 1988; Hughes 1989). Simulations of genotypic diversity and population structure, in response to disturbances, predict that maximum genotypic diversity will occur

in populations subjected to intermediate levels of disturbance (Sebens and Thorne 1985), however, the relationship between habitat stability and the levels of genotypic diversity may be more complex.

For the brooding coral *Pocillopora damicornis*, in Western Australia and Hawaii, the reproductive biology and population structure appears to support the predicted roles of sexual and asexual reproduction within a single life history. The colonies are asexually viviparous and local populations are highly clonal and genetically distinct (Stoddart 1983, 1984a); although population genetic and histological evidence implies that broadcast spawning may be used for longer distance colonisation (Stoddart and Black 1985; Stoddart 1988; Ward 1992). In contrast, on Australia's Great Barrier Reef (GBR), the populations of *P. damicornis* typically show high levels of genotypic diversity and low levels of genetic differentiation that are consistent with predominantly sexual reproduction and recruitment (Benzie *et al.* 1995; Ayre *et al.* 1997b; Ayre and Miller 2004). A recent study, however, has shown that for at least one location on the southern GBR, the brooded larvae of *P. damicornis* are produced asexually (Ayre and Miller 2004); Hence the reproductive effort and the genotypic diversity of local populations appears to be mismatched.

A number of hypotheses can be proposed to explain the observed disparity between population genetic structure of *P. damicornis* and the prevalence of asexual reproduction in this species. Firstly, habitats with differing physical and biological characteristics may give rise to differences in the genetic composition of populations, due to varying contributions of sexual and asexual reproduction, and the selection of locally adapted genotypes within habitats (Williams 1975; Potts 1984; Jackson 1986). Resolution of this paradox may, therefore, lie in the expansion of studies to determine whether reproductive modes and the relative contributions of sexual and asexual vary among different reef habitats. Secondly, asexual reproduction may be an adaptation that allows the exploitation of newly available substrata after a disturbance event. The availability of suitable substratum after a disturbance event may allow for the rapid re-colonisation of these areas by the localised recruitment of asexually generated larvae from surviving colonies. Hence, populations that have recently been impacted or which have suffered population decline, may show high

levels of clonality among new recruits. Thirdly, the presence of cryptic or sibling species within *P. damicornis* may have masked the ability to detect asexual recruitment in previous genetic studies. *P. damicornis* shows high levels of morphological variability and several authors have reported local and regional variation in morphological, life history and physiological characters (Richmond and Jokiel 1984; Knowlton 1993; Takabayashi and Hoegh-Guldberg 1995; Ayre and Hughes 2000). Populations of *P. damicornis* on the GBR may actually comprise two or more taxonomic groups that have different reproductive strategies, with only the asexually brooding taxa releasing larvae during the experimental collections of Stoddart (1983) and Ayre and Miller (2004). The earlier examinations of the genotypes of broods by Stoddart (1983) and Ayre and Miller (2004) inevitably focused only on those colonies that released planulae, and ignored other sympatric adults. Genotypic surveys are therefore needed to determine whether brooding *P. damicornis* colonies are genetically distinct from non-brooding colonies and, hence, represent different taxonomic groups.

To test these hypotheses I use d a combination of genetic and histological data to assess the population structure and mode of reproduction of *P. damicornis* from the southern GBR. My aims were to: (1) determine the genetic structure and, hence, the contribution of sexual and asexual reproduction to recruitment within populations of *P. damicornis* from six different reef habitats at One Tree Island, on the southern GBR, (2) determine the genotypic diversity of recruits after a major disturbance event (3) determine whether the mode of production of brooded larvae varies among different reef habitats, and, (4) test for the presence of cryptic species that might have different reproductive modes.

2.2 Materials and Methods

2.2.1 Study Site and Sample Collection

I sampled populations of *P. damicornis* at One Tree Island Reef (23° 30′ S; 152° 06′ E) on the southern Great Barrier Reef, Australia (Figure 2.1). I collected samples from two to three sites, within each of six reef habitats, during November/December 2001-2003. Four habitats were within the lagoon (reef flat, lagoon wall at 5 m depth, patch reefs at 2 m depth, and micro-atolls at 2 m depth) and the other two habitats included samples from the upper and lower reef slope (upper reef slope at 2-6 m depth and the lower reef slope at 7-11 m depth) (Figure 2.1). Separation between habitats varied from 200-1,000 m, except for reef slope habitats where equivalently numbered upper and lower reef slope sites were separated by only 10-15m (Figure 2.1). I selected these habitats a priori because they represented a range of habitats in which *P. damicornis* is found and differed in their physical and biological characteristics to that of the reef crest (Done 1982). I purposely avoided the reef crest habitat as it has been the focus of most other studies of *P. damicornis* on the GBR. The two micro-atoll sites that I sampled showed evidence of a disturbance, due to a major coral bleaching event that effected this area in 1998 (Baird and Marshall 1998; Booth and Beretta 2002). The effects of this disturbance were clearly patchy, with Booth and Beretta (2002) reporting a significant decrease in the coral cover (primarily of the *Pocilloporid* species which dominate the lagoon) in two of four lagoon sites, but negligible change in live coral cover in two other lagoon sites.

I made collections of 2-5 cm branch fragments from 39 to 50 coral colonies from each site (25-50 m^2) within the six reef habitats (except the micro-atoll sites, where I collected 94 to 100 specimens) (Table 2.1). The collections consisted of a haphazard selection of available colonies within each site (sites separated by 50-100 m), which included both small colonies (<7 cm colony diameter) and larger colonies (>8 cm colony diameter). To avoid collection of clonal fragments, I sampled only those colonies firmly attached to the reef matrix and which displayed a symmetrical growth form consistent with the growth from a settled larva. I placed each fragment in an individual zip-lock bag two-thirds filled with seawater, for transport back to the laboratory where they were immediately frozen in liquid nitrogen.

The samples were subsequently stored at –80°C prior to genotyping, using allozyme electrophoresis.

Figure 2.1 Map of One Tree Island Reef showing approximate locations of *Pocillopora damicornis* collection sites. Insert shows location of One Tree Island Reef along the Great Barrier Reef, Australia.

2.2.2 Electrophoresis

Allozyme electrophoresis was carried out on horizontal starch gels (12% w/v) using a tris citrate (TC8), tris-EDTA-borate (TEB) or tris-maleate (TM) buffer (buffers 5, 6 and 9 respectively of Selander et al. 1971). I determined the genotypes of *P. damicornis* samples collected from each of the five reef habitats for eight allozyme loci that had previously been found to be variable at sites within the GBR (Ayre et al. 1997). I assayed glucosephosphate isomerase (*Gpi,* E.C. 5.3.1.9), malate dehydrogenase (*Mdh1&2*, E.C. 1.1.1.37), and mannose phosphate isomerase (*Mpi*, E.C. 5.3.1.8) on buffer TC8; hexokinase (*Hk1&2*, E.C. 2.7.1.1) and phosphoglucomutase (*Pgm2*, E.C. 5.4.2.2) on buffer TM; and leucyl proline peptidase (*Lpp*, E.C. 3.4.11), and leucyl glycylglycyl peptidase (*Lgg1&2*, E.C. 3.4.11) on

buffer TEB. I detected between two and six alleles at each locus, and described alleles numerically in order of decreasing electrophoretic mobility.

2.2.3 Larval Collections

To assess whether brooded larvae were produced sexually or asexually across different reef habitats, I collected larvae from adult colonies of *P. damicornis* during the main summer planulation period (Tanner 1996) in November/December 2002 and 2003. Four to 15 colonies were collected from each of the six reef habitats and held in individual aquaria on wet tables for 6-8 days, using a flow-through seawater system. I collected larvae using overflow traps lined with 200µm plankton mesh. I successfully collected larvae from a total of 12 colonies taken from five of the six reef habitats (no larvae were released from micro-atoll colonies during the experimental collections). I used allozyme electrophoresis to compare the genotypes of adult colonies and their broods to determine whether larvae were produced sexually or asexually. Methods were as described above, although larvae were scored for only a subset of the loci previously described, and an additional locus, leucyl tryosine peptidase (*Ltp*, E.C. 3.4.11), was scored for some larvae. Larvae were genotyped within 24hr of release as per Ayre and Miller (2004).

2.2.4 Testing for Cryptic Species

Colonies that were held in aquaria during the main planulation period, and which didn't release larvae were examined histologically for the presence of larvae. For each colony I decalcified a middle branch fragment for 24hr in 10% hydrochloric acid and then viewed these under a stereomicroscope, examining the body cavity and mesenteries for the presence of larvae. To determine whether non-brooding colonies represented a different or more complex taxonomic group to those colonies that did release larvae, tissue samples of all colonies used for larvae collections were taken and the 8-locus allozyme genotypes determined as described above. I then compared the genotypes of brooding and non-brooding groups of colonies using principal coordinate analysis, which may reveal the presence of genetic groups that relate to the presence of cryptic species. However, the absence of brooded larvae in some colonies does not exclude the possibility that these

colonies may have already released brooded larvae, or may not have began their brooding cycle.

2.2.5 Statistical Analyses

2.2.5.1 Fine-Scale Population Structure and Levels of Genetic Subdivision

I expressed levels of genetic and genotypic variation within sites as the mean number of alleles per locus (N_a), effective number of alleles per locus (N_e) and expected heterozygosity (H_E) calculated using GENEPOP V3.4 (Raymond and Rousset 1995). I tested for differences in the level of genetic variation among habitats using a single-factor ANOVA for each of the three measures. To ensure that my larger sample sizes from the two micro-atoll sites did not influence diversity measures, I randomly selected only 50 individuals from each of these two sites in testing for heterogeneity of genetic diversity among habitats.

In order to determine whether each locus assorted independently I tested each pair-wise combination of loci for linkage disequilibrium (Weir 1979) for each site using GENEPOP V3.4. Consistent associations may invalidate them as independent measures of genotypic diversity and population structure, because they could reflect the physical linkage of loci. From a total of 450 pair-wise tests, only 22 significant inter-locus associations were detected ($P < 0.05$), however, only eight of these associations (all for micro-atoll site 2) remained significant after application of a sequential Bonferroni correction (Rice 1989).

I then quantified levels of population subdivision using an hierarchical analysis of standardised genetic variance (F) statistics (Wright 1969) to partition genetic variation within and among habitats. Subscripts were used to denote the source of variation: F_{SH}, variation among sites within each habitat; F_{HT}, total variation among habitats; and F_{ST}, total variation among all sites. I calculated these parameters using the formulations of Weir & Cockerham's (1984) (i.e. standardised genetic variance = θ) using the program TFPGA, which executes numerical re-sampling (jackknifing) to provide an estimate of variance across loci. Values for F should range from zero (i.e. no variation among sites due to complete panmixia) to a theoretical maximum of one (i.e. populations fixed for alternative

alleles). Values of F were judged to be statistically significant when zero lay outside the 95% confidence interval of the mean. Nei's (1978) unbiased genetic distance (D) was used to examine the genetic relationship among sites and habitats and UPGMA analysis and trees drawn using the program TFPGA (Miller 1997b). The robustness of each node was evaluated by bootstrapping allele frequencies 100 times. Because the level of allelic differentiation of habitats may be partially confounded by the effects of varying degrees of geographic separation I tested for correlation between matrices of pairwise $F_{ST} / (1 - F_{ST})$ values and the geographical distances (meters) between sites using a Mantel test (10 000 permutation, GENEPOP V3.4).

2.2.5.2 Assessing Relative Contributions of Sexual and Asexual Reproduction

In order to assess the relative effect of sexual and asexual reproduction on the genetic composition of populations, I first calculated the magnitude and direction of departures from Hardy-Weinberg equilibrium for each locus within each site. Departures were expressed as Wright's (1978) fixation index, F_{IS} where positive and negative values represent deficits or excess of heterozygotes respectively. For those loci that were sufficiently variable (i.e. frequency of the most common allele <95%, Hedrick (2000)) I employed chi-square tests to assess if the observed numbers of heterozygotes were significantly different to those expected under Hardy-Weinberg equilibrium using the genetics program GENEPOP V3.4. To reduce the chance of type I errors I applied a sequential Bonferroni correction.

Secondly, for each site I compared the number of colonies sampled (N) to the number of unique multi-locus genotypes (N_g) detected. Because the allozyme loci examined represent only a small proportion of the genome, colonies with identical 8-locus genotypes may still not be clone mates. Therefore, the ratio of N_g/N provides a maximum estimate of the contribution of asexual reproduction to localized recruitment. I then compared the ratio of observed multi-locus genotypic diversity (G_o) to that expected under conditions of sexual reproduction with free recombination (G_e), as described by (Stoddart and Taylor 1988). Departures of G_o/G_e from unity should reflect the combined effects of departures from single-locus Hardy-Weinberg equilibria and of multi-locus linkage disequilibria. Such

departures are a predicted consequence of asexual reproduction (but may also result from other factors such as population subdivision). In contrast, a population with high levels of sexually derived recruitment will display a G_o/G_e ratio close to unity. I tested for significance departures from unity by determining if G_o lay outside the 95% confidence interval of G_e (Stoddart and Taylor 1988). To reduce the chance of type I errors I applied a sequential Bonferroni correction.

2.2.5.3 Assessing Mode of Reproduction

To assess whether brooded larvae are produced sexually or asexually I compared the genotypes of larvae to that of their brood parent. Outcrossing is likely to result in the presence of non-maternal alleles, while selfing would result in an increase in homozygous offspring in comparison to a heterozygous parent. If an adult colony were heterozygous at a particular locus, I would then expect, on average, 50% of the brood to be identically heterozygous to the parent if larvae were generated via sexual reproduction (the value expected for self-fertilisation or exclusive mating with either another identically heterozygous individual(s) or an individual homozygous for one of the two parental alleles). I therefore calculated the maximum probability that sets of 'n' brooded larvae could be identically heterozygous at one or more loci (as a result of sexual reproduction) as the product of the single locus probabilities, where for any given locus: $P = 0.5^n$. This assumes independent assortment and free recombination (Black and Johnson 1979)

2.3 Results

2.3.1 Genetic Variation Among Sites and Habitats

I detected consistently high levels of allelic diversity in my collections of *P. damicornis* from all sites and habitats (Table 2.1). Overall, the average number of alleles per locus across all sites was 3.75 ± 1.49 (SD), with an average of 1.71 ± 0.57 effective alleles per locus and a mean expected heterozygosity of 0.33 ± 0.21. However, although differences among habitats were relatively slight, coral populations from the reef slope had significantly higher numbers of effective alleles ($F_{4,12}$ = 10.81, P = 0.006) and greater levels of expected heterozygosity ($F_{4,12}$ = 9.74, P = 0.001) than coral populations in the lagoon (Table 2.1). I used levels of single locus heterozygosity to test for departures from random mating at each site. Of 99 single locus tests across eight loci, I found 30 cases of departures from expected values, with all except one representing heterozygous deficits. However, only five of these remained significant after a sequential Bonferroni correction of significance levels and all were heterozygote deficits (Table 2.2). The large and consistent heterozygous deficits detected cannot be explained by simple asexual reproduction, as this should generate similar levels of both heterozygous excesses and deficits, and are more likely to reflect breeding between closely related individuals or Wahlund effects.

2.3.2 Population Subdivision

The hierarchical analysis of *F*-statistics revealed significant levels of population subdivision among all sites (P < 0.05, Table 2.3). The mean F_{ST} (± SD) across eight loci was 0.06 ± 0.01, with most of this variation (83%) due to variation among habitats (F_{HT} = 0.05 ± 0.02). I detected little genetic differentiation among sites within each habitat (F_{SH} ranging from 0.001 to 0.02, Table 2.3). The UPGMA cluster analysis, based on Nei's (1978) unbiased genetic distance (*D*), clearly indicated that the majority of genetic differentiation observed between habitats was due to differences between the lagoon and reef slope populations (Figure 2.3). Sites sampled from a habitat showed a high similarity and generally clustered together (i.e. sites in the same habitat were more similar than sites from other habitats). However, my analysis using a Mantel test did reveal some evidence of significant isolation by distance among all sites (P < 0.001), although this relationship explains only 35% of the total variation.

Table 2.1 Allele frequencies for colonies of *Pocillopora damicornis* collected from three to five sites within each of six reef habitats from the One Tree Island reef on the Great Barrier Reef.

		Habitat																
		Lagoon wall			Patch reef			Micro-atoll		Reef flat			Upper slope			Lower slope		
Locus	Allele	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Gpi1	1	-	-	-	-	-	-	-	0.03	0.04	0.02	0.01	0.03	0.07	0.08	-	0.01	0.01
	2	0.03	0.03	0.04	0.12	0.06	0.11	0.03	0.03	0.05	0.12	0.08	0.04	0.04	0.04	0.07	0.04	0.03
	3	0.19	0.18	0.17	0.34	0.34	0.23	0.19	0.17	0.32	0.22	0.35	0.14	0.19	0.11	0.11	0.29	0.11
	4	0.77	0.79	0.77	0.52	0.60	0.66	0.76	0.76	0.53	0.59	0.49	0.76	0.53	0.70	0.81	0.62	0.82
	5	-	-	-	0.02	-	-	0.02	0.01	0.06	0.05	0.06	0.03	0.13	0.07	0.01	0.04	0.03
	6	0.01	-	0.02	-	-	-	-	0.01	-	-	-	-	0.03	-	-	-	-
Hk1	1	0.47	0.45	0.46	0.39	0.44	0.54	0.47	0.49	0.35	0.45	0.33	0.55	0.72	0.68	0.65	0.79	0.71
	2	0.47	0.54	0.54	0.61	0.56	0.46	0.53	0.51	0.65	0.55	0.67	0.43	0.28	0.32	0.33	0.21	0.28
	3	0.06	0.01	-	-	-	-	-	-	-	-	-	0.02	-	-	0.02	-	0.01
Hk2	1	0.13	0.18	0.06	0.09	0.13	0.15	0.15	0.18	0.03	0.08	0.05	0.21	0.17	0.18	0.15	0.23	0.16
	2	0.87	0.82	0.94	0.91	0.87	0.85	0.85	0.82	0.97	0.92	0.95	0.79	0.83	0.81	0.85	0.77	0.84
	3	-	-	-	-	-	-	0.00	0.01	-	-	-	-	-	0.01	-	-	-
Lggp2	1	-	-	-	0.02	0.01	0.01	0.02	0.10	-	0.02	0.05	0.12	0.09	0.22	0.13	0.05	0.12
	2	0.07	0.03	0.01	0.11	0.09	0.05	0.04	0.02	0.07	0.13	0.13	0.09	0.26	0.22	0.23	0.31	0.19
	3	0.28	0.25	0.28	0.27	0.15	0.22	0.23	0.44	0.20	0.22	0.16	0.32	0.18	0.18	0.28	0.22	0.16
	4	0.65	0.72	0.71	0.60	0.74	0.71	0.70	0.45	0.73	0.63	0.67	0.47	0.47	0.39	0.36	0.42	0.53
	5	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-
Lpp	1	0.98	0.99	0.98	0.98	0.97	0.97	0.97	0.99	1.00	1.00	1.00	0.99	0.93	0.96	0.89	1.00	0.95
	2	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.01	-	-	-	0.01	0.07	0.04	0.11	-	0.05
Mdh1	1	0.98	1.00	0.99	0.99	0.99	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2	0.02	-	0.01	0.01	0.01	0.01	-	0.01	-	-	-	-	-	-	-	-	-
Mpi	1	-	-	-	-	-	0.09	0.06	-	0.01	-	-	-	-	-	0.01	-	-
	2	0.12	0.09	0.02	0.01	0.01	0.07	0.05	0.04	0.05	0.01	0.03	0.05	0.25	0.37	0.15	0.18	0.30
	3	0.53	0.52	0.69	0.39	0.60	0.46	0.28	0.39	0.38	0.36	0.32	0.69	0.54	0.44	0.63	0.63	0.52
	4	0.35	0.39	0.29	0.56	0.39	0.38	0.60	0.56	0.56	0.63	0.65	0.26	0.21	0.17	0.20	0.18	0.18
	5	-	-	-	0.04	-	-	-	0.01	-	-	-	-	-	0.02	-	-	-
Pgm2	1	0.09	0.09	0.05	0.07	0.01	-	-	0.01	0.12	0.04	0.10	0.12	0.07	0.08	0.13	0.27	0.08
	2	0.08	0.10	0.08	0.09	0.09	0.15	0.05	0.05	0.07	0.12	0.07	0.11	0.14	0.13	0.23	0.17	0.23
	3	0.82	0.81	0.87	0.84	0.89	0.83	0.95	0.94	0.79	0.84	0.81	0.73	0.76	0.74	0.61	0.55	0.68
	4	0.01	-	-	-	0.01	0.02	-	0.01	0.02	-	0.01	0.03	0.03	0.05	0.02	0.01	0.01
n/site		49	50	50	50	49	47	100	94	50	50	48	49	49	50	49	39	47
H_E		0.33	0.31	0.27	0.29	0.31	0.33	0.33	0.33	0.31	0.32	0.31	0.35	0.39	0.39	0.38	0.38	0.35
H_O		0.30	0.30	0.24	0.30	0.29	0.30	0.22	0.30	0.30	0.32	0.29	0.34	0.40	0.35	0.34	0.31	0.28

Table 2.2 Wrights fixation index (F_{IS}) and significant departures from levels of heterozygosity expected under Hardy-Weinberg equilibrium for *Pocillopora damicornis* collected from three to five sites within each of six reef habitats at the One Tree Island reef, Great Barrier Reef. Significant departures determined after the application of a sequential Bonferroni correction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

	Lagoon wall			Patch reef			Micro-atoll		Reef flat			Upper slope			Lower slope		
Locus	site 1	site 2	site 3	site 1	site 2	site 3	site 1	site 2	site 1	site 2	site 3	site 1	site 2	site 3	site 1	site 2	site 3
Gpi	0.303	0.134	0.318	0.042	-0.009	0.115	0.152	0.107**	-0.041	-0.048	-0.091	-0.096	-0.111	0.226	0.028	-0.194	0.067
Hk1	-0.129	-0.216	-0.037	-0.125	0.223	-0.061	0.322	-0.113	0.087	-0.243	-0.209	-0.386	-0.166	-0.277	-0.215	0.226	-0.168
Hk2	0.035	0.062	-0.054	-0.089	0.035	0.338	0.064	0.006	-0.021	-0.077	0.376	-0.020	0.227	0.047	-0.171	0.290	0.296
Lggp2	0.099	-0.042	1.000	0.288	0.022	0.283	0.320***	0.041	-0.080	0.191	0.238	0.294	0.039	0.194	0.171*	0.414	0.346**
Lpp	-0.011	-	-0.010	1.000	0.662	-0.02	1.000	1.000	-	-	-	-	0.241	-0.032	0.698	-	0.376
Mdh1	-0.011	-	-	-	-	-	-	1.000	-	-	-	-	-	-	-	-	-
Mpi	0.275	0.272***	0.326	0.146	-0.205	0.033	0.208	-0.016	0.083	0.081	0.225	0.195	0.032	0.104	0.132	0.076	0.343
Pgm2	0.049	-0.033	-0.100	0.369	0.307	0.127	0.167	0.145	0.112	0.076	0.173	0.159	-0.103	0.026	0.270	0.280	0.183

Table 2.3 Hierarchical analysis of standardized genetic variation (calculated as Weir and Cockerham's θ) showing F_{ST} (total variation among all sites), F_{HT} (variation among habitats) and F_{SH} (variation between sites within a habitat) for collections of *Pocillopora damicornis* at the One Tree Island Reef, Great Barrier Reef

Locus	F_{ST}	F_{HT}	F_{SH}					
			Lagoon wall	Patch reef	Micro-atoll	Reef flat	Upper slope	Lower slope
Gpi	0.039	0.016	0.001	0.007	-0.005	0.010	0.024	0.041
Hk1	0.070	0.064	0.004	0.014	-0.005	0.011	0.021	0.015
Hk2	0.018	0.017	0.022	-0.003	-0.002	0.008	0.002	0.008
Lggp2	0.053	0.030	0.004	0.009	0.089	0.007	0.019	0.016
Lpp	0.019	0.009	0.001	-0.014	-0.001	0.000	0.016	0.040
Mdh1	-0.002	0.003	0.007	-0.010	0.001	0.000	0.000	0.000
Mpi	0.104	0.079	0.018	0.034	0.012	0.005	0.050	0.012
Pgm2	0.057	0.051	0.004	0.001	-0.006	0.007	0.002	0.019
Mean (±SD)	0.062 (0.013)	0.046 (0.012)	0.001 (0.005)	0.013 (0.006)	0.023 (0.022)	0.002 (0.002)	0.021 (0.012)	0.014 (0.006)
95% CI	0.040 - 0.084	0.024 - 0.0649	-0.007 - 0.011	0.002 - 0.023	-0.004 - 0.056	-0.003 - 0.006	0.001 - 0.041	0.006 - 0.027

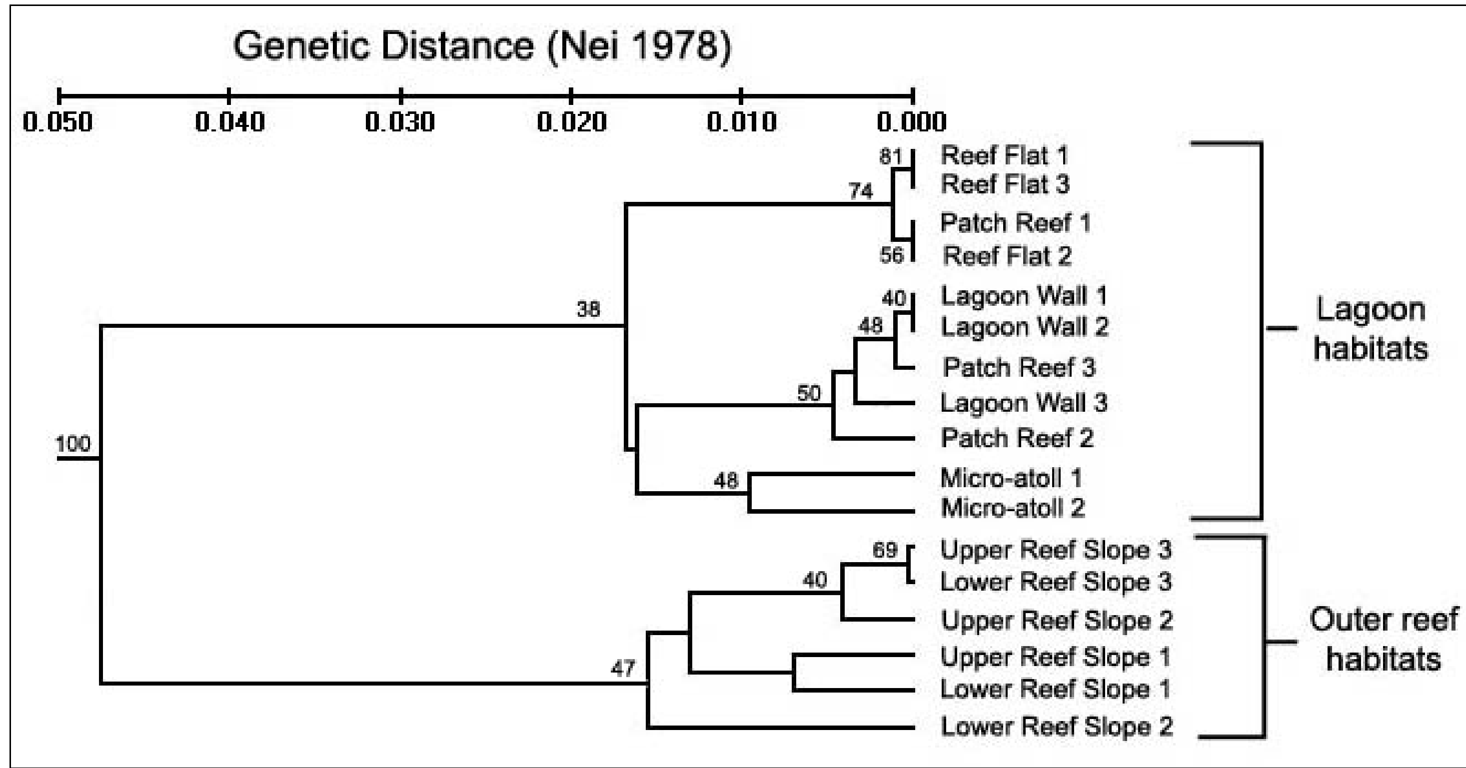

Figure 2.2 Dendrogram showing the genetic relationship between 17 collections of *Pocillopora damicornis* made within lagoon and outer reef habitats at One Tree Island on the southern Great Barrier Reef, Australia. Nei's genetic distance (1978) was calculated based on data for eight enzyme encoding loci and clustering determined using UPGMA. Bootstrapped values over 35% (based on 100 randomisations) are shown next to corresponding nodes.

2.3.3 The Relative Importance of Sexual and Asexual Reproduction Among Habitats

The genotypic composition of sites revealed that the relative importance of sexual and asexual recruitment varied across habitats. I detected high levels of genotypic diversity within collections of *P. damicornis* from the lagoon wall, reef flat, lower reef slope, upper reef slope, and for two sites within the patch reef habitat, consistent with sexually derived recruitment (Table 2.4). For these sites the number of unique genotypes (N_g) detected, compared to the number of colonies sampled (N) was consistently high with N_g/N ranging from 0.68 to 0.92 (Table 2.4). Some replicate genotypes were found within these sites, but most were represented by only two or three individuals and no single clone was numerically dominant. Within these sites I detected 69 to 100% (G_o/G_e = 0.69 to 1.00) of the genotypic diversity expected for sexual reproduction (Table 2.4). The separate analysis of large and small (and potentially younger) colonies, from each site, revealed no increased levels of clonality with either group, with G_o/G_e ranging from 0.74 to 1.12 for larger colonies and 0.83 to 1.09 for smaller colonies (data not shown).

In contrast, within the two micro-atoll sites and patch reef site 2, I detected low levels of genotypic richness (N_g/N = 0.54 to 0.65), with one genotype represented by 18 individuals in micro-atoll 2. Collections from both micro-atoll sites and patch reef site 2 showed only 35 to 53% of the genotypic diversity expected for sexual reproduction (G_o/G_e = 0.35 to 0.53, Table 2.4). This suggests that, within these three sites, asexual recruitment may be more important for maintaining populations. The separate analysis of larger and smaller colonies showed higher levels of genotypic diversity, with 39 to 68% of the diversity expected for sexual reproduction detected among larger colonies and 57 to 72% of the diversity expected for sexual reproduction detected for collections of smaller colonies (data not shown).

Table 2.4 Comparison of the observed and expected multi-locus genotypic diversity within collections of *Pocillopora damicornis* made from three to five sites within each of six reef habitats from the One Tree Island Reef on the Great Barrier Reef. *N*, number of individual colonies N_g, number of unique multi-locus genotypes, G_o: observed multi-locus genotypic diversity, G_e: expected multi-locus genotypic diversity for random mating. Significant levels of G_o / G_e from panmixis are following sequential Bonferroni correction for simultaneous tests. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

	N	N_g	N_g:N	G_o	G_e (SD)	G_o/G_e	*P*
Lagoon Wall							
Site 1	49	45	0.92	42.12	42.52 (4.75)	0.99	>0.05
Site 2	50	43	0.86	39.06	40.28 (5.30)	0.97	>0.05
Site 3	50	34	0.68	22.73	30.90 (5.59)	0.74	>0.05
Patch Reef							
Site 1	50	40	0.80	33.78	43.85 (4.52)	0.77	>0.05
Site 2	49	32	0.65	14.38	36.91 (5.44)	0.39	<0.01**
Site 3	47	43	0.91	40.16	41.69 (4.34)	0.96	>0.05
Micro-atoll							
Site 1	100	55	0.55	32.68	61.52 (6.17)	0.53	<0.001***
Site 2	94	51	0.54	19.94	56.31 (5.96)	0.35	<0.001***
Reef Flat							
Site 1	50	41	0.82	36.76	41.90 (5.07)	0.88	>0.05
Site 2	50	46	0.92	43.10	42.90 (4.77)	1.01	>0.05
Site 3	48	38	0.79	28.80	41.46 (4.68)	0.69	>0.05
Upper Slope							
Site 1	49	44	0.90	39.36	45.66 (3.57)	0.86	>0.05
Site 2	49	44	0.90	39.36	47.75 (2.21)	0.82	<0.01**
Site 3	50	44	0.88	39.06	46.63 (2.33)	0.84	<0.01**
Lower Slope							
Site 1	49	42	0.86	36.94	47.00 (2.90)	0.79	<0.05*
Site 2	39	33	0.85	31.04	38.03 (2.08)	0.82	<0.05*
Site 3	47	41	0.87	33.98	43.81 (3.32)	0.78	>0.05

2.3.4 Variation in Mode of Production of Brooded Larvae Among Habitats

In total 23 colonies released larvae, but only 12 released enough larvae (> four) to produce a powerful test for evidence of asexual reproduction. Despite the high levels of allelic diversity that I detected within samples of *P. damicornis* at the One Tree Island Reef (Table 2.1), I found that all larval genotypes from colonies collected from five reef habitats were electrophoretically identical to their brood parent (262 larvae from 12 adult colonies, Table 2.5). Six of the 12 broods produced larvae (110 larvae in total) that displayed genotypes that were identically heterozygous to the brood parent for at least one locus, the probability of this occurring due to sexual reproduction is extremely small ($P = 7.7 \times 10^{-34}$). Moreover I detected 28 larvae that were identically heterozygous to the brood parent for three loci,

again an extremely unlikely occurrence due to sexual reproduction ($P = 5.17 \times 10^{-26}$). None of the larvae that I genotyped displayed non-maternal alleles.

Table 2.5 Multi-locus genotypes of *Pocillopora damicornis* colonies and their brooded larvae collected over two consecutive years from the One Tree Island Reef, Great Barrier Reef

	Habitat	*Gpi*	*Hk1*	*Mdh1*	*Ltp*	*Lggp1*	*Lggp2*	*Lpp*
Nov. 2002								
Colony 4	Patch Reef	BC	AA	AA	BB	-	-	-
Larvae (n=53)		BC (31)	AA (22)	AA (10)	BB (22)	-	-	-
Colony 8	Patch Reef	CC	AA	AA	BB	-	-	-
Larvae (n=40)		CC (20)	AA (20)	AA (20)	BB (20)	-	-	-
Colony 11	Upper Slope	BB	AA	AA	BB			
Larvae (n=26)		BB (13)	AA (13)	AA (13)	BB (13)	-	-	-
Colony 16	Patch Reef	BB	BB	AA	BB	-	-	-
Larvae (n=14)		BB (6)	BB (8)	AA (6)	BB (8)	-	-	-
Colony 17	Patch Reef	CC	AB	AA	AA	-	-	-
Larvae (n=17)		CC (6)	AB (11)	AA (6)	AA (11)	-	-	-
Colony 18	Patch Reef	BB	-	AA	-	-	-	-
Larvae (n=4)		BB (4)	-	AA (4)	-	-	-	-
Nov. 2003								
Colony 1	Lagoon Wall	BB	BB	AA	-	-	-	-
Larvae (n=4)		BB (4)	BB (4)	AA (4)	-	-	-	-
Colony 5	Reef Flat	BD	BB	AA	-	AA	AB	AA
Larvae (n=35)		BD (35)	BB (35)	AA (35)	-	AA (20)	AB (20)	AA (20)
Colony 19	Reef Flat	AD	AB	AA	-	AA	AC	AA
Larvae (n=5)		AD (5)	AB (5)	AA (5)	-	AA (5)	AC (5)	AA (5)
Colony 20	Reef Flat	BB	AA	AA	-	AA	CC	AA
Larvae (n=45)		BB (45)	AA (45)	AA (45)	-	AA (45)	CC (45)	AA (45)
Colony 28	Lower Slope	BB	BB	AA	-	AA	AC	AA
Larvae (n=5)		BB (5)	BB (5)	AA (5)	-	AA (5)	AC (5)	AA (5)
Colony 30	Lagoon Wall	BC	AB	AA	-	AA	BC	AA
Larvae (n=23)		BC (23)	AB (23)	AA (23)	-	AA (23)	BC (23)	AA (23)

2.3.5 Tests for the Presence of Cryptic Species

The microscopic examination of colonies, used for the collection of brooded larvae, revealed that of the 42 colonies that did not release larvae, four of these were brooding larvae within their polyps, but had not released their broods during the experimental period. Based on these results, 27 colonies were identified as brooding and 38 colonies as non-brooding. A principal coordinates analysis, based on a genetic distance matrix, revealed no obvious structuring within my One Tree Island collection (Figure 2.4) and, although the

analysis was relatively weak (the first two principle components accounted for only 41% of the variation), it was clear that the set of non-brooding colonies did not contain any distinct genetic groupings that could potentially represent a cryptic species.

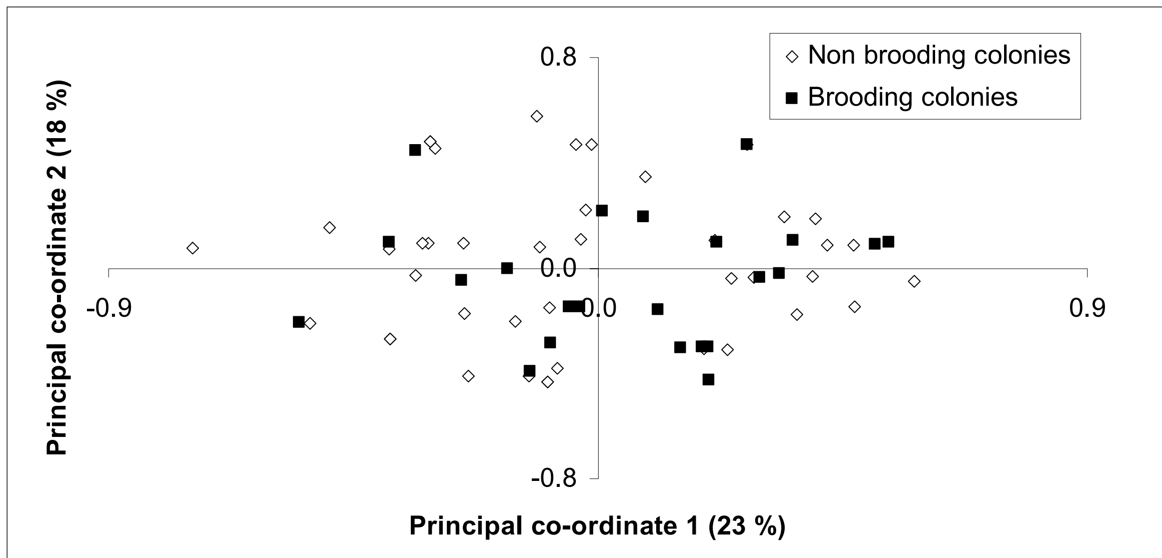

Figure 2.3 Principal coordinates analysis based on a genetic distance matrix (Nei 1978) for individual brooding and non-brooding colonies of *Pocillopora damicornis* collected from the One Tree Island Reef on the southern Great Barrier Reef, Australia.

2.4 Discussion

My data for populations of *P. damicornis* from a wide variety of reef habitats, confirm and extend the scale of the apparent mismatch between reproductive mode and localised recruitment, reported by Ayre and Miller (2004) for GBR populations of *P. damicornis*, but also indicate that clonal recruitment may be favoured within recently disturbed sites. In contrast to my expectations, I found that populations of *P. damicorni*, from 14 of 17 sites, displayed high levels of genotypic diversity, consistent with recruitment from sexual reproduction. However, my electrophoretic analysis of the genotypes of brooded larvae, from a range of habitats, suggested that all broods surveyed in this study were produced asexually. Interestingly, I detected significantly lower levels of genotypic diversity at three sites within the reef lagoon, two of which were known to have undergone severe disturbance in the last six years. The apparent mismatch between the reproductive mode and recruitment could not be explained by the presence of cryptic species at One Tree Island (Ayre *et al.* 1991b; Knowlton *et al.* 1992; Miller and Benzie 1997). Rather, I found that a principal coordinates analysis plot, based on a genetic distance matrix, showed that all colonies tested (including brooding and non-brooding colonies) formed a single genetic cluster.

2.4.1 Variation in Genotypic Diversity Across Habitats

I had anticipated that the apparent paradox implied by Ayre and Miller's (2004) finding, that reef flat and reef crest colonies of *P. damicornis* produce clonal broods, while reef crest populations are typically highly diverse (Benzie *et al.* 1995; Ayre *et al.* 1997b; Ayre and Miller 2004), might be resolved if local populations in other habitats displayed high levels of clonality. I reasoned that the persistence of asexual viviparity on reef crests could, therefore, be maintained despite apparently high levels of sexual recruitment if asexual reproduction were favoured elsewhere, and that the reef crests were effectively a genetic sink (Ayre and Miller 2004). However, my data provide no such simple explanation, since I found that asexual recruitment contributes little to maintaining local populations of *P. damicornis* within each of six other reef habitats. Indeed, sites from most of the sheltered lagoon habitats (lagoon wall, patch reefs, and reef flat) showed similar levels of genotypic diversity to sites on the highly speciose and heterogeneous reef slopes. The high levels of

genotypic diversity within these sites were comparable not only to those from earlier studies of *P. damicornis* on the GBR, but were also similar to levels reported for exclusively sexually brooding corals (e.g. Ayre and Dufty 1994; Ayre and Hughes 2000), including two other Pocilloporids.

The hypothesis that asexual larvae disperse over greater distances than the spatial scales sampled in this and earlier studies, may provide an explanation for apparent lack of clonal structure in these populations. Competency and energetic studies of brooded larvae of *P. damicornis* from Hawaii and Japan suggest that brooded larvae may, indeed be capable of remaining in the plankton for extended periods (up to 100 days), and therefore have the potential to be widely dispersed (Richmond 1987; Isomura and Nishihira 2001; Harii *et al.* 2002). It is clear from this study that the asexually produced larvae of *P. damicornis*, in populations on the GBR, are rarely successful in recruiting close to their brood parent. This means that either asexual propagules are used for widespread dispersal or remain unused and do not recruit in the majority of reef habitats (although disturbed habitats may provide an exception to this). While it is not possible to reject the hypothesis that the genotypic diversity of local populations of *P. damicornis* is maintained by the widespread dispersal of asexually generated larvae, it is difficult to understand the selective process that would favour the evolution and maintenance of a life history that used both sexual and asexual modes of reproduction for the widespread dispersal of propagules. For organisms with both sexually and asexually generated propagules, theory predicts that asexual reproduction will be used to restock the parental habitat and sexual reproduction will be used for widespread dispersal or colonisation of more heterogeneous habitats (Williams 1975). This dichotomy of roles is well supported from studies in a number of taxa (Ayre 1984b; Hoffmann 1986; Hebert *et al.* 1988; Darling *et al.* 2004). Direct comparisons of the relative dispersal capabilities and relative fitness of sexual *versus* asexual larvae, are clearly needed to understand under what conditions either mode of reproduction is likely to be important. Interesting, despite *P. damicornis* being one (if not the most) intensively studied coral, there have been no direct observations of broadcast spawning or the capture of sexual larvae for direct comparisons with asexual larvae. Additionally, more intensive and extensive sampling of populations is needed to identify the potential scale of dispersal of

asexually produced larvae. *P. damicornis* can occur at very high densities and may require the sampling of a large proportion of a population (i.e. thousands of individuals), including both adults and recent recruits, to determine the scale of dispersal and recruitment of asexually generated larvae.

2.4.2 Low Levels of Genotypic Diversity Within Disturbed Habitats

While asexual reproduction may be more commonly associated with the absence of disturbance or the predictability of the parental habitat (Williams 1975), I expect that disturbances, such as bleaching or cyclonic storms, would have a simplifying effect on normally speciose coral habitats that might favour localised asexual recruitment for a period of years. During such periods, coral densities and species richness are reduced (Hughes *et al.* 1992; Connell 1997; Hughes and Connell 1999) and, hence, the potential for both interspecific and inter-clonal competition is also reduced (Connell *et al.* 1997). This could facilitate the re-colonisation of this space through recruitment of clonal larvae produced by a few surviving adults. My results support this hypothesis, since I detected high levels of asexual recruitment in three lagoon sites, two of which are known to have experienced recent (in the last six years) disturbance events. I detected only 35-53% of the genotypic diversity expected for sexual reproduction, with free recombination, within these sites. A major bleaching event in 1998 had resulted in large mortalities of adult corals (including *P. damicornis*) within a number of lagoonal sites at the One Tree Island Reef (Booth and Beretta 2002). A study by Benzie et al. (1995), prior to the bleaching event, had found high levels of genotypic diversity and little evidence of asexual recruitment within the micro-atoll habitats, including one of the sites sampled in this study. Benzie et al. (1995) found that micro-atoll two (micro-atoll one in this study) showed high levels of genotypic diversity (G_o:G_e = 0.93) and few replicated genotypes (N:N_g = 0.87), while I detected considerably lower levels of genotypic diversity (G_o:G_e = 0.53) and unique multi-locus genotypes (N:N_g = 0.55). This decrease in genotypic diversity, within the micro-atoll site, may be explained by re-colonisation of this space by asexual produced larvae produced by surviving adults after the bleaching event. All the genotypes of the smaller colonies sampled within these sites were represented by at least one of the adult colonies, thus supporting the hypothesis that the larvae originated from adult colonies within these sites.

Localised recruitment, by even a small proportion of asexually brooded larvae, could lead to reduced diversity in an expanding population. However, the continued recruitment of larvae from other areas may, subsequently, lead to an increase in diversity over time. Additionally, micro-atoll sites typically form enclosed ponds at low tide, which is likely to facilitate the retention of asexually generated larvae. The structure and local hydrodynamic regimes of habitats may, therefore, also play an important role in determining the level of clonality within a habitat and may also explain the higher levels of clonality observed in Western Australian populations, which typically consist of small embayments that may facilitate the local retention of larvae (Stoddart 1984). Therefore, the lack of localised recruitment of asexual larvae in other habitats may, in large part, be due to a combination of longer periods of time since the disturbance event and differences in local hydrodynamic regimes that do not favour the retention of asexually produced larvae within the parental habitat.

2.4.4 Genetic Variation and Subdivision

My analysis of genetic variation found significant but modest levels of genetic differentiation among all sites ($F_{ST} = 0.06$). However, closer inspection implies that there is very little differentiation among sets of neighbouring sites within habitats. My hierarchical analysis of F_{ST} suggested that the majority of the variation present was due to differences between habitats, rather than between sites within habitats. However, I recognise that the relatively larger level of variation, which I report among habitats rather than among sites (0.045 vs 0.028 – 0.001), does include both the effects of variation among habitats and some effect of isolation by distance. The occurrence of isolation by distance implies that individuals within neighbouring sites are more closely related than sets from distant sites; nevertheless, this should not reduce the value of comparisons of genotypic diversity or reproductive modes across habitats, as these characteristics can vary over finer spatial scales (e.g. variation among patch reefs, Table 2.4).

Taken together, my data indicates that clonal larvae of *P. damicornis* do not contribute significantly to maintaining local populations in the majority of reef habitats; although my data do suggest that asexual recruitment may be favoured after a disturbance event,

presumably when competition is reduced. The extent of clonal recruitment in a wider variety of disturbed habitats warrants further investigation to determine if this type of disturbance is frequent enough to drive the persistence of this asexual mode of reproduction. Comparisons of genotypes of brooded larvae to that of their brood parent is also needed to clarify the mode of production of brooded larvae, in locations such as Japan, where a sexual origin of larvae has been inferred form histological studies (Permata *et al.* 2000). Finally, until comparative studies of dispersal and the relative fitness of sexual *versus* asexual larvae, under different environmental conditions, has been carried out, it will be difficult to understand under which conditions either mode of reproduction would be favoured.

Chapter 3 Intermediate Levels of Selfing in the Hermaphroditic Brooding Coral, *Seriatopora hystrix*, on the Great Barrier Reef

Preamble to Chapter 3

Seriatopora hystrix is arguably (along with *Styllophora pistillata*) the closest ecological equivalent to *Pocillopora damicornis* on Australia's GBR, and could equally have formed the basis for a detailed test of the generality of life history models. Like *P. damicornis*, *S. hystrix* forms small to moderate size branching colonies that are relatively abundant in reef habitats ranging from the reef slope and crest to lagoons, and it is a hermaphroditic brooder. However, *S. hystrix* has been the target of more intensive population genetic studies than *P. damicornis* (Resing and Ayre 1985; Ayre and Dufty 1994; Ayre and Hughes 2000; 2004). These studies demonstrate high levels of genetic subdivision at fine and coarse scales but with little variation in population structure or allele frequencies across habitats within reefs. Many aspects of the population structure of *S. hystrix*, including apparent dependency on sexually derived recruits, are similar to that which I have described for *P. damicornis* (chapter 2). However, the only genetic comparison of a *S. hystrix* adult and its brood revealed that brooded larvae of *S. hystrix* appear to be generated sexually through outcrossing (Ayre and Resing 1986), which contrasts to *P. damicornis* that broods asexually generated larvae (Chapter 2 and Ayre and Miller 2004).

In this chapter I provide the first rigorous estimates of mating system parameters based on direct comparison of a group of adults and their broods, and indirect estimates based on the adult genotypes within each of several sites. I have also compared population structure and levels of genotypic diversity in *S. hystrix* from two reef habitats with that found in *P. damicornis*. Such comparative approaches are useful in elucidating patterns and processes structuring coral populations and the general applicability of life history models.

3.1 Introduction

For organisms that produce eggs and sperm simultaneously within the same individual (i.e. simultaneous hermaphrodites) there is potential for self-fertilisation. While reproduction through exclusive self-fertilisation may be rare for the majority of hermaphroditic plants and animals, mating systems where a proportion of progeny are outcrossed and the remaining are selfed, appear to be an important reproductive strategy in some groups (reviewed in Jarne and Charlesworth 1993). Despite the potential for lowered fitness of self relative to outcrossed progeny due to inbreeding depression (Grosberg 1987; Hunter and Hughes 1993; Hoare and Hughes 2001), the capacity to self-fertilise many be selected for as a mechanism of reproductive assurance when potential mates are scarce (Henry *et al.* 2005), or as a mechanism of purging deleterious recessive alleles (Lande *et al.* 1994). Current understanding of the evolutionary and ecological significance of self-fertilisation *versus* outcrossing is mainly derived from investigation of terrestrial plant species (reviewed in Jarne *et al.* 1996; Linhart and Grant 1996; Hedrick and Kalinowski 2000), however, the importance of such mating systems has rarely been investigated for sessile marine invertebrates (Knowlton and Jackson 1993).

Scleractinian corals display a diverse range of reproductive strategies that include: both sexual and asexual modes; hermaphroditism and gonochorism; and broadcast spawning and internal brooding (Harrison and Wallace 1990). Despite the vast majority of scleractinian corals having the potential to self-fertilise (more than 68% of species are hermaphroditic, Harrison and Wallace; 1990), surprisingly little is known about natural rates of self-fertilisation within this group. The most common reproductive strategy in scleractinian corals involves a combination of simultaneous hermaphroditism and synchronised spawning events, in which colonies release large amounts of gametes into the water column where fertilisation occurs (Harrison *et al.* 1984; Babcock *et al.* 1986). For such broadcast spawning species, outcrossing appears to be the most common mode of reproduction (Carlon 1999). This is supported by fertilisation trials of 23 broadcasting species that have revealed generally low levels of self-compatibility (Heyward and Babcock 1986; Knowlton *et al.* 1997; Miller and Babcock 1997; Szmant *et al.* 1997; Willis *et al.* 1997), although

high levels of self-fertilisation have been reported for the broadcast spawner *Goniastrea favulus* (Heyward and Babcock 1986; Stoddart *et al.* 1988).

For brooding coral species, colonies must rely on the dispersal of sperm from one colony to another for outcrossing. While some plants have evolved associations with pollinators to assist in the transfer pollen from one individual to another, corals lack specific sperm vectors to facilitate outcrossing and fertilisation success. The use of pheromones and other chemo-tactic attractants may be used by some marine taxa to increase fertilisation success (e.g. Coll *et al.* 1995), and some marine invertebrates are known to filter and store allosperm (Hughes *et al.* 2002; Pemberton *et al.* 2004). However, limited sperm motility, rapid dilution in the water column, and relatively short lifespan of sperm, means that fertilisation in the marine environment is likely to be restricted to colonies/individuals in close proximity (Babcock *et al.* 1994; Levitan and Petersen 1995; Coffroth and Lasker 1998a). Therefore, despite the potential for decreased fitness due to inbreeding through self-fertilisation, the ability of ensuring fertilisation in the absence of potential mates or under sperm limited conditions, means that self-fertilisation may be an important reproductive tactic for many coral species.

In brooding corals, determining natural selfing rates is potentially much easier than for broadcast spawners as direct genetic comparisons of adults and their broods can be made. While the mode of production of broods (i.e. outcrossed, selfed or asexual) has been tested genetically for just eight coral species (Ayre and Resing 1986; Brazeau *et al.* 1998; Hellberg and Taylor 2002; Ayre and Miller 2004; Ayre and Miller in press), only one of these studies has made estimates of natural outcrossing rates both from adult and progeny array analysis (Ayre and Miller in press). Such studies are important, because not only do they determine if a species reproduces by outcrossing (it takes only one other coral colony to ensure outcrossing), but also whether outcrossing is due to multiple individuals (i.e. the number of fathers that sire a brood), and whether this is occurring among closely related individuals (i.e. biparental inbreeding).

The brooding coral *Seriatopora hystrix* is widely distributed throughout the Indo-Pacific and is found within most reef habitats (Veron 1986). It has a delicate branching morphology that may be susceptible to fragmentation, although genetic studies indicate that asexual reproduction via fragmentation makes little contribution to local populations (Ayre and Dufty 1994; Ayre and Hughes 2000). The most conspicuous mode of reproduction in *S. hystrix* is the release of internally brooded larvae which are competent to settle within hours of release (Atoda 1951). A genetic study carried out on a brood from a single colony showed that some larvae display non-maternal alleles, while others displayed only maternal alleles, indicating that broods could potentially be produced by outcrossed sexual reproduction or a mix of outcrossing and self-fertilisation (Ayre and Resing 1986). Population genetic data, based on allozyme markers, have revealed large and consistent heterozygote deficits within populations of *S. hystrix* from the Great Barrier Reef, Australia (Ayre and Dufty 1994; Ayre and Hughes 2000). These heterozygote deficits may be interpreted as evidence for extensive inbreeding, although Ayre and Duffty (1994) estimated that almost 64% of observed heterozygote deficits might be explained by a Wahlund effect. A Wahlund effect results from the inadvertent sampling of multiple genetic subpopulations with differing allele frequencies, which results in lower-than-expected heterozygosity (Wahlund 1928; Hedrick 2000). However, without direct confirmation on the mode of production of brooded larvae, the importance of self-fertilisation and inbreeding within populations of *S. hystrix* remains unclear.

In this study I use co-dominant allozyme markers to determine the mode of production of brooded larvae of *S. hystrix* for colonies collected from One Tree Island, on the southern Great Barrier Reef, Australia. Estimates of the levels of outcrossing, and the potential number of sires contributing to each brood, were made using information from progeny arrays and surrounding adult genotype frequencies. Additionally, I carried out fine-scale genetic surveys to assess if the adult population structure was consistent with the observed mating system. The mating system and population structure of *S. hystrix* is then contrasted with that determined for the brooding coral *P. damicornis* (Chapter 2).

3.2 Methods

3.2.1 Adult and Larval Collections

I made collections of adult *S. hystrix* from three sites (~25m^2) in each of two reef habitats, the reef slope (7-11m depth) and reef flat, during November/December 2002 at the One Tree Island Reef (23° 30′ S; 152° 06′ E) on the southern Great Barrier Reef. Sites within each habitat were separated by a minimum of 50m, while the two habitats were separated by approximately 2km. Each collection consisted of 47-50 fragments (~2cm long) taken haphazardly from available colonies, and representing most adult colonies within each site. Samples were frozen in liquid nitrogen prior to transportation back to the laboratory, where they were stored at -80°C until needed for electrophoresis.

For larval collections, I removed 15 adult colonies (> 8cm diameter) from the reef flat (site 3) and transported them to the laboratory in seawater. Colonies were held in separate aquaria with flow through seawater pumped directly from the reef lagoon through a sand filter. A control aquarium, which did not contain a coral colony, was used to determine if water pumped from the reef lagoon contained coral larvae that may confound my results. I detected no coral larvae within the control aquarium during the experimental period. Six of the 15 colonies released larvae (up to 85 larvae/colony) over an 11-day period. I collected larvae either in overflow traps lined with plankton mesh (200µm pore diameter), or siphoned them off the bottom of each aquarium. Genotypes of larvae and the brood parent were determined by allozyme electrophoresis within 24 hrs of larval release.

3.2.2 Electrophoresis

Tissue extracts and electrophoresis methods were the same as those described by Ayre and Dufty (1994). Electrophoresis was carried out on horizontal starch gels (12% w/v) using a tris citrate (TC8), tris-EDTA-borate (TEB) or tris-maleate (TM) buffer modified from Selander *et al.* (1971). A total of eight enzyme loci were consistently resolvable. These included: glucosephosphate isomerase (*Gpi,* E.C. 5.3.1.9); malate dehydrogenase (*Mdh1&2*, E.C. 1.1.1.37), assayed on TC8; hexokinase (*Hk*, E.C. 2.7.1.1); phosphoglucomutase (*Pgm2*, E.C. 5.4.2.2), assayed on TM; leucyl-proline peptidase (*Lpp*, E.C. 3.4.11); and leucyl-glycylglycyl peptidase (*Lggp1&2*, E.C. 3.4.11), assayed on TEB.

Between three and six alleles were detected at each locus, and alleles were described numerically in order of decreasing electrophoretic mobility. Due to their small size (~400µm diameter, Baird and Babcock 2000), larvae could only be assayed on a single buffer system (TC8) and, therefore, were only scored for *Gpi*, *Mdh*2 and *Hk* to maximise the number of loci scored. *Mdh1* was invariable in the site from which broods were collected and, therefore, was excluded from progeny array analysis.

3.2.3 Statistical Analysis

3.2.3.1 Mating System Parameters

I calculated outcrossing rates and other mating system parameters using the multi-locus mating program *MLTR* (Ritland 2002). The program uses maximum likelihood analysis to provide minimal variance estimates of mating system parameters under a mixed model. The mixed mating model assumes that a proportion of the matings represent selfing while the remaining proportion represent outcrosses which have occurred at random within the population.

Larval and maternal genotypes, and those of neighbouring adult colonies from the reef flat (site 3) were used to calculate the following mating system parameters: 1) single (t_s) and multi-locus (t_m) outcrossing rates (where $t = 1$ for complete outcrossing and $t = 0$ for complete self-fertilisation); 2) the rate of biparental inbreeding ($t_m - t_s$) which gives a measure of the degree of mating among close relatives; and 3) the correlation of outcrossed paternity within progeny (i.e. the likelihood that a randomly chosen pair of progeny from a single mother share the same father, r_p). This measure can also be translated into an estimate of the effective number of sperm donors per brood; ($1/r_p$). These mating system parameters were calculated using Newton-Raphson iteration, and 1000 bootstrap replicates were performed to obtain standard errors (SE) for each parameter. If the mating system is at equilibrium, direct estimates of the inbreeding coefficient can be calculated from outcrossing estimates using the relationship $f_e = (1 - t_m) / (1 + t_m)$ (Hedrick 2000).

3.2.3.2 Population Structure and Genotypic Diversity

To ensure that each locus behaved independently, I tested each pairwise combination of loci for linkage disequilibrium for each site (168 tests) using the program GENEPOP V3.4 (Raymond and Rousset 1995). To assess the effects of inbreeding on population structure, I calculated the magnitude and direction of departures from Hardy-Weinberg equilibria within each site for each locus. Departures were expressed as a fixation index, F_{IS} (Wright 1978), where positive and negative values represent deficits or excesses of heterozygotes respectively. For those loci that were sufficiently variable (i.e. frequency of the most common allele <95%, Hedrick 2000), I used chi-square to determine if the observed numbers of heterozygotes were significantly different to those expected under Hardy-Weinberg equilibrium using the program GENEPOP V3.4 (Raymond and Rousset 1995) and applied a sequential Bonferroni correction to reduce the chance of type I errors (Rice 1989).

I assessed levels of genotypic diversity within each site by first comparing the number of colonies sampled (N) to the number of unique multi-locus genotypes (N_g) detected. I then compared the ratio of observed multi-locus genotypic diversity (G_o) to that expected under conditions of sexual reproduction with free recombination (G_e), as described by Stoddart and Taylor (1988). Departures of G_o/G_e from unity should reflect the combined effects of departures from single-locus Hardy-Weinberg equilibrium, and of multi-locus linkage disequilibrium. Such departures may result from factors such as inbreeding, population subdivision and asexual reproduction. In contrast, a population with complete random mating will display a G_o/G_e ratio of close to unity. The statistical significance of differences between G_o and G_e was then assessed by determining if G_o lay outside the 95% confidence interval of G_e (Stoddart and Taylor 1988). To reduce the chance of type I errors a sequential Bonferroni correction was then applied.

I used an hierarchical analysis of standardised genetic variance (F) statistics (Wright 1969) to partition genetic variation among sites, and between habitats. Subscripts were used to denote the source of variation: F_{ST}, total variation among all sites; F_{SH}, variation among sites within habitats; and F_{HT}, total variation between habitats. Parameters were calculated

using the formulations of Weir and Cockerham (1984) using the program TFPGA (Miller 1997b), which executes numerical resampling to provide estimates of variances across loci (bootstrapping). Values of F were judged to be statistically significant when zero lay outside the 95% confidence interval of the mean.

3.3 Results

3.3.1 Mating System

The brooded larvae of *S. hystrix* (237 larvae from six adult colonies) all showed the presence of at least one maternal allele at each locus, as expected for Mendelian inheritance, and the presence of non-maternal alleles in some larvae indicates that broods are produced sexually. Estimates of the mean multi-locus outcrossing rate were intermediate (t_m = 0.54 ± 0.22 SE), indicating a moderate level of self-fertilisation within these broods. However, standard deviations were relatively large, and individual colony outcrossing rates varied from <1% to 96% indicating large variability between individual broods. The mean single-locus estimates of outcrossing were slightly lower than multi-locus estimates (t_s = 0.49 ± 0.21 SE). Consequently, estimates of biparental inbreeding were relatively low, but were significantly different from zero (t_m - t_s = 0.06 ± 0.03 SE), suggesting that at least some outcrossing events occur between closely related individuals. The estimation of correlated paternity (i.e. the likelihood that a randomly chosen pair of progeny from a single mother share the same father) was highly variable and did not differ from zero (r_p = 0.32 ± 0.43 SE), indicating that multiple sires contribute to each brood. This measure can also be translated into an estimate of the effective number of sperm donors for the outcrossed larvae ($1/r_p$), which suggested two to four fathers contributed to each brood. *Gpi* was the most variable of the three loci used to genotype broods, and therefore made the largest contribution to estimates of outcrossing within progeny arrays. When *Gpi* was excluded from the analysis, estimates of mean multi-locus outcrossing rates dropped to only 0.16 ± 0.14 indicating that *Gpi* accounts for approximately 75% of the detectable outcrosses.

3.3.2 Allelic Variation and Population Subdivision

3.3.2.1 Allelic Variation

Within each of the six sites, *S. hystrix* showed similar levels of allelic diversity, with three to six alleles detected at each locus (mean ± SE = 4.25 ± 1.04), although I did detect a slightly greater number of rare alleles within reef slope sites (Table 3.1). Tests for linkage disequilibria revealed 65 significant interlocus associations in a total of 168 pairwise tests ($P < 0.05$), however, this seems unlikely to reflect physical linkage between loci as there

was no consistent pattern of linkage between loci, and none of these associations remained significant after application of a sequential Bonferroni correction.

Table 3.1 Allele frequencies for *Seriatopora hystrix* colonies collected from three sites (25m^2) within each of two habitats from the One Tree Island Reef on the Great Barrier Reef, Australia.

Locus	Allele	Reef flat			Reef slope		
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Gpi	1	-	-	-	0.09	0.02	-
	2	0.33	0.48	0.40	0.31	0.11	0.28
	3	0.19	0.18	0.19	0.17	0.27	0.14
	4	0.48	0.34	0.41	0.44	0.61	0.58
Hk	1	-	0.01	-	0.01	-	0.01
	2	0.63	0.70	0.52	0.48	0.79	0.62
	3	0.18	0.10	0.24	0.29	0.14	0.14
	4	0.18	0.19	0.22	0.17	0.06	0.14
	5	0.01	-	0.02	0.05	0.01	0.09
Mdh1	1	-	-	-	0.02	0.02	0.03
	2	1.00	0.96	0.98	0.93	0.98	0.97
	3	-	0.04	0.02	0.05	-	-
Mdh2	1	-	-	-	-	0.01	-
	2	0.01	0.05	0.07	0.04	0.06	0.05
	3	0.67	0.65	0.65	0.52	0.80	0.59
	4	0.32	0.30	0.28	0.44	0.11	0.36
	5	-	-	0.00	-	0.02	-
Pgm2	1	-	-	0.02	-	-	-
	2	0.06	0.09	0.16	0.27	0.04	0.25
	3	0.94	0.92	0.82	0.72	0.99	0.75
	4	-	-	-	0.01	-	-
Lggp1	1	0.05	0.06	-	0.21	0.02	0.07
	2	0.40	0.50	0.60	0.41	0.72	0.53
	3	0.30	0.16	0.06	0.20	0.07	0.16
	4	0.22	0.28	0.34	0.13	0.17	0.19
	5	0.02	-	-	-	0.01	0.04
	6	-	-	-	0.04	0.01	-
Lggp2	1	0.03	0.19	0.26	0.10	0.31	0.08
	2	0.90	0.81	0.69	0.75	0.67	0.92
	3	0.06	-	0.05	0.16	0.02	-
Lpp	1	-	-	-	0.03	0.06	-
	2	0.77	0.81	0.76	0.47	0.76	0.57
	3	0.23	0.19	0.24	0.48	0.18	0.43
	4	-	-	-	0.02	-	-

3.3.2.2 Population Structure

Estimates of single-locus heterozygosity for *S. hystrix* rarely matched expectations for Hardy-Weinberg equilibrium, with a total of 36 departures from expected values out of 42 statistically valid single-locus tests across eight loci (Table 3.2). All departures represented heterozygote deficits, 28 of which remained significant after the application of a sequential Bonferroni correction, indicating the probable effects of inbreeding and/or self-fertilisation (Table 3.2). The large and consistent heterozygote deficits detected across all sites and loci cannot be explained by asexual reproduction via fragmentation, as this should generate similar levels both of heterozygote excesses and deficits. If the mating system is at equilibrium, the levels of outcrossing detected for site 3 (t_m = 0.54) can be translated into a direct estimate of the inbreeding coefficient using the relationship $f_e = (1 - t) / (1 + t)$ (Hedrick 2000). This equates to an inbreeding coefficient of f_e = 0.30, which is slightly lower but similar to that estimated indirectly from adult genotypic data for site 3 (F_{IS} = 0.36) (Table 3.2). This means that the majority (83%) of the observed heterozygote deficits detected can be attributed to the mating system, with only a small proportion (17%) explained by other factors such as the Wahlund effect.

3.3.2.3 Genotypic Diversity

High levels of multi-locus genotypic diversity were detected for *S. hystrix* across all sites. The ratio of unique multi-locus genotypes (N_g) to number of colonies sampled (N) was consistently high within all sites with N_g/N ranging from 0.80 to 0.96 (Table 3.4). While I did detect some genotypes represented by up to eight individuals within a site, only two or three individuals represented most of the potentially clonal genotypes detected and no single genotype was numerically dominant within any given site. Within sites, *S. hystrix* showed between 63-83% of the genotypic diversity expected for sexual reproduction (G_o/G_e = 0.63-0.83), (Table 3.4). These high levels of genotypic diversity suggest that predominately sexually derived recruits maintain these populations, although I did detect significant deviations from expectations for random mating for two reef slope sites (Table 3.4). The number of unique genotypes detected within these sites was similar to the other four sites, indicating that these deviations are the result of inbreeding and population subdivision, rather than asexual reproduction via fragmentation.

3.3.2.4 Population Subdivision

Analysis of standardised genetic variance (Wright 1978) revealed significant levels of population subdivision among sites (F_{ST} = 0.041 ± 0.006 SE, 95% CI = 0.030 to 0.056) but little differentiation between habitats (F_{HT} = 0.004 ± 0.009 SE, 95% CI = – 0.013 to 0.020) (Table 3.3). Interestingly, I detected high and significant levels of genetic differentiation between sites from the reef slope habitat (F_{SH} = 0.06 ± 0.009 SE, 95% CI = 0.077 to 0.045), however, levels of genetic differentiation among sites from the reef flat habitat were not significantly different from zero (F_{SH} = 0.013 ± 0.009 SE, 95% CI = 0.030 to –0.004).

Table 3.2 Wright's (1978) fixation index (F_{IS}) and significant departures from levels of heterozygosity expected under Hardy-Weinberg equilibrium for *Seriatopora hystrix* from three sites (25m^2) within each of two reef habitats from the One Tree Island Reef, Great Barrier Reef, Australia. Significant departures determined after the application of a sequential Bonferroni correction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3.3 Hierarchical analysis of standardized genetic variation calculated using the formula of Weir and Cockerham (1984) for *Seriatopora hystrix* from three sites ($25m^2$) within each of two reef habitats from the One Tree Island Reef, Great Barrier Reef, Australia.

Locus	F_{IS}	F_{ST}	F_{HT}	F_{SH}	
				Reef Flat	Lower Slope
Gpi	0.140	0.034	0.028	0.011	0.029
Hk1	0.300	0.027	-0.008	0.016	0.045
Mdh1	0.544	0.006	0.001	0.014	0.015
Mdh2	0.554	0.026	-0.017	0.002	0.068
Pgm1	0.604	0.061	0.001	0.022	0.071
Lgg1	0.410	0.038	-0.002	0.031	0.042
Lggp2	0.761	0.060	-0.031	0.053	0.075
Lpp	0.835	0.063	0.040	0.003	0.065
Mean (±SE)	0.460 (0.096)	0.041 (0.007)	0.004 (0.009)	0.013 (0.009)	0.060 (0.009)
95% CI	0.668 to 0.309	0.056 to 0.030	0.020 to – 0.013	0.030 to –0.004	0.077 to 0.045

F_{IS} = variation among individuals (i.e. inbreeding coefficient), F_{ST} = variation among all sites, F_{HT} = variation among habitats, and F_{SH} = variation among sites within a habitat.

Table 3.4 Comparison of the observed and expected multi-locus genotypic diversity for *Seriatopora hystrix* from three sites ($25m^2$) within each of two reef habitats from the One Tree Island Reef, Great Barrier Reef, Australia.

	N	*Ng*	*Ng/N*	G_o	G_e (SD)	G_o/G_e	*P*
Reef flat							
Site 1	47	43	0.91	40.16	44.52 (2.77)	0.90	>0.05
Site 2	48	44	0.92	41.90	44.40 (3.10)	0.94	>0.05
Site 3	50	48	0.96	46.30	48.20 (2.61)	0.96	>0.05
Lower slope							
Site 1	47	43	0.91	38.75	46.72 (1.13)	0.83	<0.001***
Site 2	48	41	0.85	33.88	41.38 (4.55)	0.82	>0.05
Site 3	50	42	0.84	32.89	47.82 (2.87)	0.69	<0.001***

N, number of individual colonies sampled; N_g, number of unique multi-locus genotypes detected, G_o, observed multi-locus genotypic diversity; G_e, multi-locus genotypic diversity expected for random mating. The statistical significance of differences between G_o and G_e was assessed by determining if G_o lay outside the 95% confidence interval of G_e (Stoddart and Taylor 1988) and applying a sequential Bonferroni correction for simultaneous tests. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.4 Discussion

My direct genetic comparisons of *S. hystrix* colonies and their broods, indicates that almost half of the brooded larvae were produced by self-fertilisation and half by outcrossing. On average, three sires contribute to each brood, however, the small but significant levels of biparental inbreeding suggests that some of these outcrossing events may be occurring between closely related individuals. Population genetic surveys also revealed large and consistent heterozygote deficits and significant genetic subdivision among all sites consistent with high levels of inbreeding and the potential limited dispersal of gametes and/or larvae. *S. hystrix* therefore appears to have a mixed mating system characterised by almost equal proportions of self-fertilisation and outcrossing, and larvae which appear to have restricted dispersal resulting in some matings between closely related individuals.

These results confirm and extend Ayre and Resing's (1986) findings that *S. hystrix* broods are sexually produced. Moreover, the levels of biparental inbreeding and selfing detected in this study are consistent with previous reports of large heterozygote deficiencies in populations of *S. hystrix* from two other studies along the GBR (Ayre and Dufty 1994; Ayre and Hughes 2000). Heterozygote deficits are a common feature of many marine populations and are often presented as evidence in support of mating systems that are characterised by high levels of inbreeding and/or selfing (Ayre and Dufty 1994; Viard *et al.* 1996; Edmands and Potts 1997; Ayre and Hughes 2000; Reusch 2001; Ayre and Hughes 2004; Goffredo *et al.* 2004). However, other potential contributions to heterozygote deficits include selection against heterozygotes (e.g. Borsa *et al.* 1992), the presence of null alleles (Foltz 1986; Ayre *et al.* 1997b), and the inadvertent sampling of multiple genetic subpopulations with differing allele frequencies (i.e. the Wahlund effect, Hedrick 2000). In this study, estimates of the inbreeding coefficient from adult allele frequencies within reef flat site 3 (F_{IS} = 0.36), were similar to that calculated directly from outcrossing rates for the same site (f_e = 0.30). This implies that levels of self-fertilisation and biparental inbreeding could by themselves be sufficient to explain most of the heterozygote deficits detected in adult populations, and that the Wahlund effect accounts for little of the observed heterozygote deficits. Overall estimates of inbreeding for all six sites in this study (average

F_{IS} = 0.46), were within the range previously reported by Ayre and Dufty (1994) and Ayre and Hughes (2000) (F_{IS} = 0.23 and 0.51 respectively).

Interestingly, I detected significant levels of subdivision (mean F_{ST} = 0.04, 95% CI 0.056 to 0.030) among all sites, indicating the potential restricted dispersal of larvae and/or sperm. This was consistent among sites on the reef slope where significant differentiation was detected among sites separated by less than 50m, however, I detected no significant differentiation among sites from the reef flat, or between the two habitats, indicating some gene flow among habitats. While these levels of subdivision are an order of magnitude lower compared to those reported by Ayre and Dufty (1994) and Ayre and Hughes (2000) (F_{ST} = 0.04 *cf.* F_{ST} = 0.2), these previous studies had been carried out over much larger spatial scales (i.e. 90-1500 km between sites) than the current study where sites within a habitat were typically separated by less than 50m and habitats were no more than 2 km apart. The scale of sampling in this study is likely to more closely reflect the realised dispersal distances of larvae, and is consistent with Atoda's (Atoda 1951) laboratory observations that the majority of larvae settle within the first day after release with many settling in close aggregations.

The patterns of genotypic diversity and allelic variation I detected among sites are also consistent with sexual reproduction, with intermediate (approximately 50%) levels of selfing and biparental inbreeding. I found no evidence that asexual reproduction contributed significantly to the fine-scale genotypic structure within sites, with the majority (84 - 96%) of adults displaying unique multi-locus genotypes. I detected significant departures from random mating in all three reef slope sites, which are most likely the result of inbreeding and not the effects of asexual reproduction via fragmentation. Additionally, within each site I only detected heterozygote deficits, while asexual reproduction should generate similar levels both of heterozygote excesses and deficits (Table 3.2). Interestingly, levels of genotypic diversity detected for *S. hystrix* are remarkably similar to that detected for *P. damicornis* within the reef flat and lower reef slope habitats (G_e/G_o = 0.79-0.92 and 0.85-0.87 for the reef flat and lower slope respectively, Chapter 2). How such similar levels of genotypic diversity can occur in two species with apparently quite

different mating system remains unclear; however, it does suggest that sexual reproduction within *P. damicornis* remains an important source of genotypic diversity and that inbreeding (including the potential for self-fertilisation) also may be important in maintaining populations of *P. damicornis.*

The levels of self-fertilisation reported here for *S. hystrix* are similar to those reported for the broadcast spawning coral *G. favulus* (50 - 65%) determined from laboratory fertilisations (Heyward and Babcock 1986; Stoddart *et al.* 1988). These levels of selfing are also comparable to that purportedly calculated from randomly amplified polymorphic DNA (RAPD) markers for two Caribbean hermaphroditic brooding coral species, *Favia fragum* (49%) and *Porites astreoides* (34%) (Brazeau *et al.* 1998). However, as Brazeau *et al.* (1998) indicate, estimates from their study need to be interpreted with caution as confidence limits of selfing estimates could not be calculated because of a lack of data on the frequency of informative bands in potential parents in the surrounding adult population, and no objective means of predicting the genotypes expected under different mating systems.

Interestingly, the levels of self-fertilisation detected for *S. hystrix* in this study are in stark contrast to those reported for the only other study of a brooding hermaphroditic coral, *Acropora palifera*, for which outcrossing estimates have been made from progeny arrays (Ayre and Miller, in press). In that study, Ayre and Miller found that broods collected from colonies from two sites (150m^2 and 200m^2), were generated almost exclusively by outcrossing (t_m = 0.92 to 0.96), and that sperm had potentially dispersed over ten's of metres. This suggests that there is large interspecific variation in levels of self-compatibility among brooding species, similar to that previously seen in broadcast spawners (Willis *et al.* 1997). Despite the potential for decreased fitness of offspring and inbreeding depression as a consequence of self-fertilisation, the ability of assuring fertilisation success in the absence of potential mates or under sperm limited conditions means that self-fertilisation is likely to be an important mode of reproduction in some brooding species (Shields 1982; Knowlton and Jackson 1993). However, until a greater number of brooding species have been investigated it remains unclear as to the extent and

importance of self-fertilisation in brooding corals. Additionally, estimates of the relative fitness and survival of selfed vs. outcrossed larvae would be invaluable in determining the likely importance of self-fertilisation within this group.

Although my overall estimates of outcrossing, based both on adult genotype frequencies and progeny array analysis, consistently imply intermediate levels of outcrossing, I detected large inter-colony variation in outcrossing rates (<1% to 96%). This could result from a number of factors, including variation in the distance between nearest neighbours, levels of clonality within a population (and hence the opportunity for matings between rametes belonging to the same genet), variation in levels of self-compatibility among individual colonies, and variation in local hydrodynamic regimes that affect the dispersal and dilution of gametes. Additionally, the mean outcrossing rate was calculated based on six broods from only a single 25m^2 site. Therefore mating system parameters estimated in this study need to be interpreted with some caution as outcrossing rates may vary between populations, habitats and individuals, and more extensive estimates of outcrossing within a great number of sites need to be carried out before more general conclusions can be drawn. Nevertheless overall estimates of outcrossing from this study are consistent with indirect measures of the levels of inbreeding from two previous studies of *S. hystrix* on the GBR (Ayre and Dufty 1994; Ayre and Hughes 2000). The loci used in this study were chosen for their relatively high levels of variability within these populations (Table 3.1), and are likely to have detected the majority of outcrossed events; however, the use of more variable molecular marker (such as microsatellites) may reveal slightly higher levels of outcrossing than detected here. The recent development of highly variable microsatellite markers for a number of brooding (Maier *et al.* 2001; Magalon *et al.* 2004) and broadcast spawning (Miller and Howard 2004) corals, will greatly increase the ability of studies to accurately determine levels of outcrossing and inbreeding.

The close agreement between the population structure and levels of inbreeding within adult populations and estimates of outcrossing, does suggest that self-fertilisation plays an important part in the mating system of *S. hystrix*. Taken together, the relatively high levels of self-fertilisation combined with highly restricted dispersal of sperm and/or larvae are the

likely source the high levels of inbreeding and genetic subdivision reported in populations of *S. hystrix* along the GBR. This supports a growing body of evidence that indicates that self-fertilisation is an important reproductive strategy for many marine and freshwater hermaphroditic species (Bucklin *et al.* 1984; Stoddart *et al.* 1988; Edmands 1995; Cohen 1996; Viard *et al.* 1997; Carlon 1999; Jarne *et al.* 2000; Reusch 2001).

Chapter 4 Mode of Reproduction Does Not Vary with Habitat Heterogeneity in the Sea Anemone *Actinia tenebrosa*

4.1 Introduction

Life history theory predicts that for organisms capable of sexual and asexual modes of reproduction, sexual reproduction will be used to exploit temporary or unstable habitats, while limited dispersal of asexually produced propagules will be used to maintain populations comprised of locally adapted clones within relatively stable parental habitats (Williams 1975; Bell 1982). Strong empirical support for such apparently distinct roles of sexual and asexual reproduction comes from a range of studies that have shown a shift in the mode of reproduction in response to changing or unfavourable environmental conditions. For example, many freshwater zooplankton will reproduce asexually under stable and favourable conditions, but will switch to sexual reproduction when conditions deteriorate (Bell and Wolfe 1985; Hebert *et al.* 1988; Carvalho 1994; O'Connell and Eckert 2001).

The reproductive biology of sea anemones in the genus *Actinia* has attracted a great deal of interest and controversy, particulary regarding the mode of production of brooded juveniles which have been variously argued to be produced sexually or asexually (Chia and Rostron 1970; Ottaway and Kirby 1975; Black and Johnson 1979; Carter and Thorp 1979; Gashout and Ormond 1979; Orr *et al.* 1982; Perrin *et al.* 1999; Yanagi *et al.* 1999). The presence of adult male and female individuals within populations of *A. equina* led early researchers to assume the brooded young were of a sexual origin (e.g. Stephenson 1929; 1935; Chia and Rostron 1970). However, it was observed that despite a large degree of colour polymorphism among adults, the colour of brooded juveniles were almost always the same as that of the parent (Stephenson 1935; Chia and Rostron 1970; Cain 1974). This observation led to a number of hypotheses that attempted to provide an explanation for this discrepancy (Chia and Rostron 1970; Cain 1974; Gashout and Ormond 1979). These included the re-entry of planula larvae from the plankton into adults of their own colour or genotype (Chia and Rostron 1970; Ottaway and Kirby 1975); the altering of the colour of

juveniles by the brood parent during development (Lubbock and Allbut 1981); and the production of juveniles by asexual or pathenogenetic means (Cain 1974; Gashout and Ormond 1979). Cain (1974) was the first to suggest that the brooded juveniles were more likely the result of asexual reproduction, and that brooding adults of *A. equina* were unlikely to be adoptive parents of juveniles that had re-entered from the plankton as suggested by Chia and Rostron (1970).

Empirical support for an asexual origin of brooded juveniles comes from genetic studies using allozyme electrophoresis that showed the brooded juveniles of *A. equina*, and the Australasian congener *A. tenebrosa*, were genetically identical to the brood parent (Ottaway and Kirby 1975; Black and Johnson 1979; Carter and Thorp 1979; Orr *et al.* 1982). Gashout and Ormond (1979) proposed that the brooded juveniles were produced by some form of parthenogenesis. However, the presence of juveniles in the enteron of male and non-reproductive individuals (i.e. those without gonads) is not consistent with a parthenogenetic origin. Subsequently, for the species *A. tenebrosa, A. equina* and *A. bermudensis*, the prevailing view is that the brooded juveniles are produced asexually (Black and Johnson 1979; Orr *et al.* 1982; Monteiro *et al.* 1998; Yanagi *et al.* 1999) via some form of undescribed somatic embryogenesis (i.e. the development of juveniles from somatic tissue).

The presence of functional gonads in *Actinia* species that are morphologically similar to other sexually reproducing species of sea anemones does suggest, however, that sexual reproduction occurs and is likely to be via broadcast spawning of gametes into the water column (Ayre 1988; Carter and Miles 1989). Support for a sexual mode of reproduction in *Actinia* species also comes from several studies that have revealed substantial amounts of genetic variation in populations (Ayre 1983a; Quicke *et al.* 1983; Ayre 1984b; Ayre *et al.* 1991a; Perrin *et al.* 1999; Douek *et al.* 2002). Interestingly though, studies of *A. tenebrosa* recruitment into established populations along the east and west coasts of Australia, have failed to detect any evidence of sexual recruitment over 22 years, suggesting that sexually generated recruitment may be rare (Ayre, unpublished data). This is surprising considering the demonstrated localised adaptation of clones to conditions within a single rocky

headland (Ayre 1985; 1995, see Chapter 6). Local adaptation requires sufficient levels of genotypic diversity within population for selection to act on. Nevertheless, patterns and levels of genotypic diversity detected in the majority of adult populations of *A. tenebrosa* on stable rock platforms, are consistent with the establishment of populations from widely dispersed sexually generated colonists, but which are maintained by localised asexual recruitment (Ayre 1983b; 1984b; Ayre *et al.* 1991a).

Interestingly, on more heterogeneous boulder shores, Ayre (1984b) detected significantly higher levels of genotypic diversity to that seen on rock platforms, suggesting that the relative contributions of sexual and asexual recruitment varies between habitats based on environmental heterogeneity and complexity. This is consistent with evolutionary theory that predicts sexual reproduction will be favoured in more heterogeneous and complex habitats, while asexual reproduction is more likely to be important in more stable and less heterogeneous habitats (Williams 1975; Maynard Smith 1978; Bell 1982). Higher levels of genotypic diversity within boulder habitats may result from increased production of sexual recruits, including the possibility that individuals produce sexually derived broods. However, to date no studies have attempted to determine if mode of reproduction varies with environmental heterogeneity in an *Actinia* species.

The last three decades have seen a dramatic increase in the use of biochemical and molecular markers for the assessment of mating systems and the genetic structure of populations. Co-dominant markers are one of the most powerful methods for inferring mating systems, and have been used successfully to identify both sexual and asexual modes of reproduction in a number of brooding sea anemones (e.g. *A. equinia* (Carter and Thorp 1979; Orr *et al.* 1982) *A. tenebrosa* (Ottaway and Kirby 1975; Black and Johnson 1979) *Epiactis spp.* (Edmands 1995)) and corals (e.g. *Acropora palifera*, *Seriatopora hystrix* (Ayre and Resing 1986, Chapter 3), *Ballanophylia elegans* (Hellberg and Taylor 2002) and *Pocillopora damicornis* (Stoddart 1983; Ayre and Miller 2004, Chapter 2)). However, the use of allozyme markers alone does not provide conclusive evidence of the asexual production of brooded juveniles. This is because of the potential for carryover of maternal enzymes in early larvae as was found by Stoddart *et al.* (1988) and Ayre and Standish

(unpub) for some cnidarian larvae. In those studies it was found that the maternal phenotype continues to be expressed in the planula larva for between 4-17 days after fertilisation. This effect is postulated to result from a residual of maternal enzyme and RNA derived from relatively large eggs. Such an effect may lead to the perception of identical genotypes of mothers and their broods and the erroneous conclusion of asexual reproduction.

The use of co-dominant markers for determining the mating system of an organism will also be dependent on the level of allelic variation at distinct loci. A high level of polymorphism and heterozygosity is needed to allow the detection of segregation of alleles and independent assortment of chromosomes in successive generations (i.e. the consequences of meiosis, see Chapter 3). One potential drawback of allozyme markers is their lower levels of variability and heterozygosity that reduces their sensitivity in comparison to co-dominant DNA markers such as microsatellites (Scribner *et al.* 1994; Tessier *et al.* 1995; although see Barker *et al.* 1997). This is because mating between identically homozygous parents or self-fertilisation can result in offspring that are identical to the brood parent. For example, Edmands (1995) compared allozyme and DNA markers to determine the mating system in a number of sea anemones within the genus *Epiactis.* She found that for *E. lisbethae* allozyme markers revealed that adults and their broods were genetically identical. However, the use of DNA markers revealed that some juveniles had different genotypes to their brood parent and could only have been produced via outcrossed sexual reproduction. DNA based markers such as microsatellites are generally more polymorphic than allozymes with mutation rates 2-4 orders of magnitude greater than allozymes (Amos *et al.* 1996). These types of markers, therefore, have the potential to resolve much of the controversy still surrounding the mode of production of brooded larvae in *Actinia* species.

The recent development of microsatellite markers for the Australasian sea anemone *A. tenebrosa* (Mitchellson and Ayre, unpublished) provides the first opportunity to determine unequivocally the mode of reproduction of brooded juveniles in this species. In this study I assess the genetic origin of brooded juveniles from individuals of *A. tenebrosa* from a wide

geographic area along the southeast coast of mainland Australia and Tasmania at four microsatellite loci. In addition, I also use allozyme electrophoresis to genotype a subset of these adults and broods to enable a comparison between these two different markers. In order to test the theoretical predictions that mating system (i.e. sexual or asexual brooding of juveniles) may vary with environmental heterogeneity, genotypes of broods from adults from both rock platform and more heterogeneous boulder habitats were compared.

4.2 Methods

4.2.1 Habitat Heterogeneity

Among marine habitats, intertidal boulder shores constitute heterogeneous habitats in which spatial and temporal variation in environmental parameters may promote a greater variety of microhabitats relative to more homogenous intertidal habitats (Sousa 1979; McGuinness and Underwood 1986; Davis and Wilce 1987; Fuji and Nomura 1990; Hily and Jean 1997; Rios and Mutschke 1999; Takada 1999). While no quantitative measurements of variation in environmental parameters were made between boulder and rock pool habitats in this study, these two habitats differ in a number of important aspects. Boulder shores are structurally more complex than rock platforms, resulting in greater variation in shading, levels of sedimentation, temperature, and salinity regimes. This results in a large number of microhabitats that vary in a number of key environmental parameters (Le Hir and Hily 2005; McGuinness and Underwood 1986; Takada 1999). However, the term habitat heterogeneity can be interpreted in a multitude of ways by researchers working in different areas of ecology (Kolasa and Rollo 1991). To avoid confusion, the following definitions are provided for some key terms used in this study: a habitat represents a delimited spatial area in which particular species may exist, forming a characteristic community or assemblage of species. Habitat heterogeneity refers to the fine-scale variation in environmental parameters that result in several distinct microhabitats, each of which may support different biological sub-communities (Levin 1992; Tokkeshi 1999; Le Hir and Hily 2005). Thus, in this study, a habitat is said to be more heterogeneous to the extent that it contains a greater variety of microhabitats when compared with another habitat.

4.2.2 Collection of Specimens

I collected samples of *A. tenebrosa* from eight locations within three geographic regions along the east coast of mainland Australia and Tasmania (Figure 4.1). Collections were made from intertidal rock pools on rock platforms, except at Cape Banks and Bass Point, where I collected anemones both from rock pool and boulder habitats. Anemones collected were a haphazard selection of available adults and were carefully prised from the substratum using a spatula and placed into individual zip-lock bags with seawater for transportation back to the laboratory. Brooded juveniles were dissected from the gastric

cavity of the brood parent and snap frozen in liquid nitrogen prior to storage at –80ºC. I sampled a total of 221 juveniles from 37 adults, with the size of broods varying between 3-15 juveniles per brooding adult (Table 4.1). Collections from Cape Banks and Bass Point were made during February 2003, while Victorian and Tasmanian collections were made during February 2004.

4.2.3 DNA Extraction and Amplification

I used a small piece of tissue (27 mm^3) from the pedal disc or body wall of an adult or juvenile for DNA extraction, except in the case of very small juveniles where the entire sample was used. Tissue was ground to a fine powder in liquid nitrogen and DNA was extracted using a CTAB extraction protocol (Doyle and Doyle 1987) modified by the addition of polyvinylpyrrolidone to the extraction buffer at a concentration of 1%. Extracted DNA was suspended in 50µl dH_2O. Thirty-one adults and their broods (a total of 204 juveniles) were genotyped at four microsatellite loci developed specifically for *A. tenebrosa* (*At1*, *At5*, *At21a* and *At38*, Table 4.1). Polymerase Chain Reaction (PCR) was carried out in a total volume of 20µl containing reaction buffer (10mM Tris-HCL, 50mM KCl, 0.1% Triton X-100), 2.5mM $MgCl_2$, 200µM each dNTP, 100µM fluorescent labelled dNTP (Fdctp), 1µg bovine serum albumin (BSA), 1U *Taq* DNA polymerase (Promega), 10pmol of each primer and ~20ng DNA template. PCR amplification were carried out with cycling conditions of 94˚C for 3 minutes, 35 cycles of 94˚C for 30 s, 55˚C for 30 s and 72˚C for 1 min. PCR products were electrophoresed and sized on 5% polyacrylamide gels using a GelScan 2000 machine (Corbett Research) and analyzed using ONE-Dscan software (Scanlytics, CSP, Inc).

Figure 4.1 Locations of collecting sites of brooding adult *Actinia tenebrosa* along the east coast of mainland Australia and Tasmania. NSW = New South Wales, VIC = Victoria, TAS = Tasmania. Collections were made from rock pools, except at Cape Banks and Bass Point, where anemones were collected from rock pool and boulder habitats.

Table 4.1 Primer sequence for the microsatellite loci used in this study. All loci were developed from a genomic library specifically designed for *Actinia. tenebrosa* (Mitchellson and Ayre, unpublished).

4.2.4 Allozyme Electrophoresis

The methodology for allozyme electrophoresis of *A. tenebrosa* samples was the same as previously described by Ayre (1982). A total of four enzymes were assayed and up to five loci were consistently resolvable. All enzymes were electrophoresed on a Tris-Citrate buffer (pH 8.0) and stain recipes followed Harris and Hopkinson (1976). Enzymes assayed included glucosephosphate isomerase (GPI, E.C. 5.3.1.9), 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44); malate dehydrogenase (MDH, E.C. 1.1.1.37); and octopine dehydrogenase (ODH, E.C. 1.5.1.11). Tissue extracts from each adult were electrophoresed alongside those from its brooded juveniles. A total of 18 adults and 86 brooded juveniles were used for allozyme electrophoresis.

4.2.5 Statistical Analysis

I compared levels of genetic variation for microsatellite and allozyme loci as the mean numbers of alleles per locus (N_a), effective number of alleles per locus (N_e) and expected heterozygosity (H_e) calculated using GENEPOP V3.4.

To assess if brooded larvae are sexually or asexually produced I compared the genotypes of brooded larvae to that of the parent colony for the four microsatellite and five allozyme loci. If an adult colony were heterozygous at a particular locus, one would then expect a maximum of 50% of the brood to be identically heterozygous to the parent if juveniles were generated via sexual reproduction (the value expected for self-fertilisation or exclusive mating with either another identically heterozygous individual(s) or an individual homozygous for one of the two parental alleles). I, therefore, calculated the maximum probability that sets of 'n' brooded juveniles could be identically heterozygous at one or more loci (as a result of sexual reproduction) as the product of the single locus probabilities where for any given locus, $P = 0.5^n$. This assumes independent assortment and free recombination (Black and Johnson 1979).

4.3 Results

4.3.1 Genetic Variation among Adult Anemones

Levels of polymorphism at the microsatellite loci within the relatively small (n = 37) but geographically widespread sample of adult *A. tenebrosa* was high in comparison to the allozyme markers (Figure 4.2). For microsatellite loci, *At1* and *At5*, I detected ten and four alleles per locus respectively. The remaining microsatellite loci, *At21* and *At38*, and all five allozyme loci, each displayed two alleles per locus. The mean number of alleles per locus was 4.5 ± (1.9) (SE) for the microsatellite loci compared to only 2 ± (0.0) for the allozyme loci (Figure 4.2). Similarly, I detected almost twice the number of effective alleles per locus (N_e ± SE = 2.32 ± 0.52 *cf.* 1.26 ± 0.04), and more than twice the mean expected heterozygosity (H_E ± SE = 0.49 ± (0.12) *cf.* 0.20 ± (0.04) for microsatellite loci compared to allozyme loci (Figure 4.2).

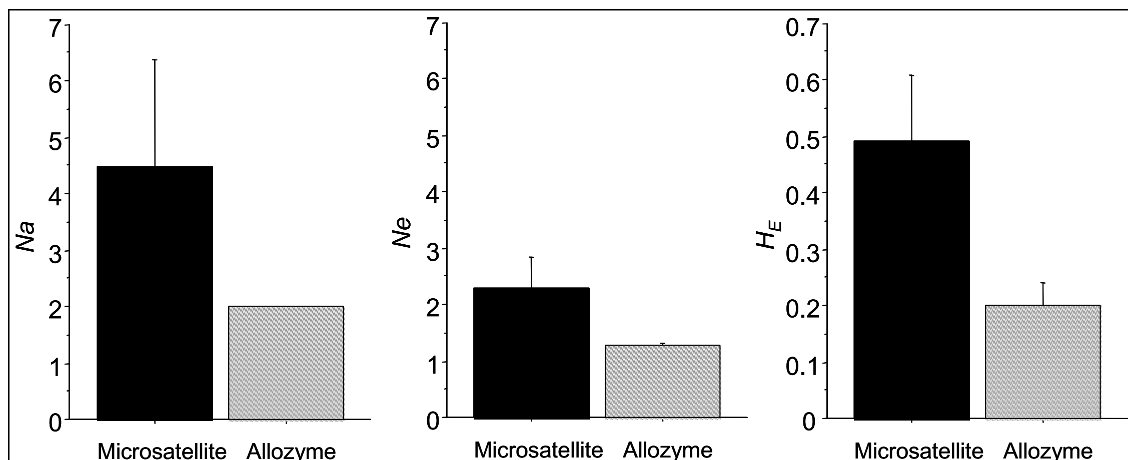

Figure 4.2 Mean (± SE) levels of gene diversity for four microsatellite loci and five allozyme loci for 37 *Actinia tenebrosa* samples collected across eight locations from the east coast of Australia. *Na* = mean number of alleles/locus; *Ne* = effective number of alleles/locus; H_E = mean heterozygosity.

4.3.2 Asexual Production of Brooded Juveniles

Genetic analysis of brooded juveniles revealed that all 37 broods sampled (221 brooded juveniles) displayed identical multi-locus genotypes to that of their brood parent (Appendix 4.1). Results were consistent across microsatellite and allozyme loci. I found that 28 out of these 37 broods (200 brooded juveniles) were identically heterozygous to the brood parent

for at least one locus (e.g. Figure 4.3). The combined probability of this occurring through sexual reproduction (either through self-fertilisation or mating with an identically heterozygous individual) was extremely small ($P = 6.2 \times 10^{-61}$). Moreover I found 10 broods (70 brooded juveniles) to be identically heterozygous to the brood parent for at least three loci, again an extremely unlikely occurrence due to sexual reproduction (combined probability, $P = 8.5 \times 10^{-22}$). The complete lack of genotypic variation within these broods across a number of heterozygous microsatellite and allozyme loci can only be explained by asexual reproduction.

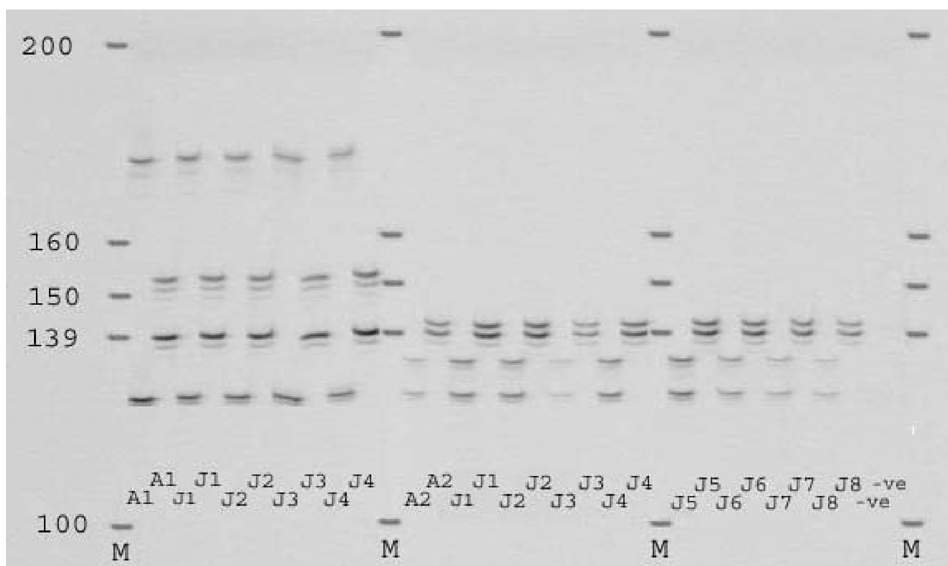

Figure 4.3 PCR amplification products showing clonal genotypes of adult *Actinia tenebrosa* (A1 and A2) and their brooded juveniles (J1,...J 'n') which were heterozygous at two microsatellite loci (*At1* and *At2* in alternate lanes). M = marker lanes, –ve = negative control.

I found no evidence to indicate the production of sexual broods in the boulder habitats at the two locations sampled (Table 4.1). All 11 broods from the boulder shore habitat (80 juveniles) displayed identical multi-locus genotypes to that of the brood parent for both microsatellite and allozyme loci including eight broods that were heterozygous at two or more loci.

Table 4.2 Genotype data of adult *Actinia tenebrosa* and their brooded juveniles collected from eight locations along the east coast of mainland Australia and Tasmania. *N* = number of juveniles genotyped.

			Microsatellite loci								Allozyme loci									
	Location	Habitat	*At1*		*At5*		*At21a*		At38		*Gpi1*		*Gpi2*		*Mdh*		*Odh*		*6pgd*	
Parent	Bass Point	Pools	125	132	141	141	171	171	105	105	1	1	1	1	2	2	2	2	1	1
Juvenile			125	132	141	141	171	171	105	105	1	1	1	1	2	2	2	2	1	1
(*N*)			(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Adult	Bass Point	Pools	132	140	139	154	171	171	105	105	1	1	1	1	1	2	1	2	1	1
Juvenile			132	140	139	154	171	171	105	105	1	1	1	1	1	2	1	2	1	1
(*N*)			(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Adult	Bass Point	Pools	125	125	139	139	171	171	105	105	1	1	1	1	2	2	2	2	1	1
Juvenile			125	125	139	139	171	171	105	105	1	1	1	1	2	2	2	2	1	1
(*N*)			(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Adult	Bass Point	Pools	132	140	139	139	171	173	105	105	1	1	1	1	1	2	2	2	1	1
Juvenile			132	140	139	139	171	173	105	105	1	1	1	1	1	2	2	2	1	1
(*N*)			(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Adult	Bass Point	Pools	125	175	139	139	171	171	105	105	1	1	1	1	2	2	2	2	1	1
Juvenile			125	175	139	139	171	171	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	-	-	-	-	-	-	-	-	-	-
Adult	Bass Point	Boulders	105	132	139	139	171	171	105	105	1	1	1	1	2	2	2	2	1	1
Juvenile			105	132	139	139	171	171	105	105	1	1	1	1	2	2	2	2	1	1
(*N*)			(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Adult	Bass Point	Boulders	125	132	139	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
Juvenile			125	132	139	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	-	-	-	-	-	-	-	-	-	-
Adult	Bass Point	Boulders	105	125	139	152	171	171	104	105	1	1	1	1	2	2	2	2	1	1
Juvenile			105	125	139	152	171	171	104	105	1	1	1	1	2	2	2	2	1	1
(*N*)			(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)
Adult	Bass Point	Boulders	-	-	-	-	-	-	-	-	1	1	1	1	2	2	2	2	1	1
Juvenile			-	-	-	-	-	-	-	-	1	1	1	1	2	2	2	2	1	1
(*N*)			-	-	-	-	-	-	-	-	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Adult	Bass Point	Boulders	-	-	-	-	-	-	-	-	1	1	1	1	1	2	1	1	1	1
Juvenile			-	-	-	-	-	-	-	-	1	1	1	1	1	2	1	1	1	1
(*N*)			-	-	-	-	-	-	-	-	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Adult	Bass Point	Boulders	125	132	139	141	173	173	105	105	-	-	-	-	-	-	-	-	-	-
Juvenile			125	132	139	141	173	173	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	-	-	-	-	-	-	-	-	-	-
Adult	Bass Point	Boulders	105	125	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
Juvenile			105	125	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	-	-	-	-	-	-	-	-	-	-
Adult	Bass Point	Boulders	105	125	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
Juvenile			105	125	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	-	-	-	-	-	-	-	-	-	-
Adult	Cape Banks	Pools	132	132	139	139	173	173	105	105	1	2	1	2	1	2	1	2	1	2
Juvenile			132	132	139	139	173	173	105	105	1	2	1	2	1	2	1	2	1	2
(*N*)			(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Adult	Cape Banks	Pools	105	132	141	154	171	171	105	105	1	1	1	1	2	2	2	2	1	1
Juvenile			105	132	141	154	171	171	105	105	1	1	1	1	2	2	2	2	1	1
(*N*)			(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)
Adult	Cape Banks	Pools	105	125	139	141	171	173	105	105	1	1	1	1	2	2	2	2	1	1
Juvenile			105	125	139	141	171	173	105	105	1	1	1	1	2	2	2	2	1	1
(*N*)			(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)

Table 4.1 continued

Adult	Cape Banks	Boulders	105	132	139	154	171	171	105	105	-	-	-	-	-	-	-	-	-	-
Juvenile			105	132	139	154	171	171	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	-	-	-	-	-	-	-	-	-	-
Adult	Cape Banks	Boulders	125	175	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
Juvenile			125	175	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	-	-	-	-	-	-	-	-	-	-
Adult	Cape Banks	Boulders	118	132	139	139	171	171	104	105	1	1	1	1	2	2	2	2	1	1
Juvenile			118	132	139	139	171	171	104	105	1	1	1	1	2	2	2	2	1	1
(*N*)			(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)
Adult	Waratah Bay	Pools	-	-	-	-	-	-	-	-	1	1	1	1	2	2	2	2	1	1
Juvenile			-	-	-	-	-	-	-	-	1	1	1	1	2	2	2	2	1	1
(*N*)			-	-	-	-	-	-	-	-	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Adult	Waratah Bay	Pools	125	182	139	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
Juvenile			125	182	139	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	-	-	-	-	-	-	-	-	-	-
Adult	Waratah Bay	Pools	125	132	139	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
Juvenile			125	132	139	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	-	-	-	-	-	-	-	-	-	-
Adult	Cape Pat.	Pools	-	-	-	-	-	-	-	-	1	2	1	2	2	2	2	2	1	1
Juvenile			-	-	-	-	-	-	-	-	1	2	1	2	2	2	2	2	1	1
(*N*)			-	-	-	-	-	-	-	-	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Adult	Cape Pat.	Pools	132	175	139	141	171	171	105	105	1	2	1	2	2	2	2	2	1	1
Juvenile			132	175	139	141	171	171	105	105	1	2	1	2	2	2	2	2	1	1
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Adult	Cape Pat.	Pools	132	175	139	141	171	171	105	105	2	2	2	2	2	2	2	2	2	2
Juvenile			132	175	139	141	171	171	105	105	2	2	2	2	2	2	2	2	2	2
(*N*)			(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
Adult	Flinders	Pools	125	162	141	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
Juvenile			125	162	141	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
(*N*)			(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	-	-	-	-	-	-	-	-	-	-
Adult	Flinders	Pools	111	132	141	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
Juvenile			111	132	141	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	-	-	-	-	-	-	-	-	-	-
Adult	Tassie Pen	Pools	132	132	141	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
Juvenile			132	132	141	141	171	171	104	104	-	-	-	-	-	-	-	-	-	-
(*N*)			(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	-	-	-	-	-	-	-	-	-	-
Adult	Tassie Pen	Pools	132	132	139	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
Juvenile			132	132	139	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	-	-	-	-	-	-	-	-	-	-
Adult	Tassie Pen	Pools	125	125	139	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
Juvenile			125	125	139	141	171	171	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	-	-	-	-	-	-	-	-	-	-
Adult	Bicheno	Pools	132	132	139	152	171	171	104	104	-	-	-	-	-	-	-	-	-	-
Juvenile			132	132	139	152	171	171	104	104	-	-	-	-	-	-	-	-	-	-
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	-	-	-	-	-	-	-	-	-	-
Adult	Bicheno	Pools	125	132	141	141	171	171	104	105	-	-	-	-	-	-	-	-	-	-
Juvenile			125	132	141	141	171	171	104	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	-	-	-	-	-	-	-	-	-	-
Adult	Bicheno	Pools	132	132	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
Juvenile			132	132	139	152	171	171	104	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	-	-	-	-	-	-	-	-	-	-

Table 4.1 continued

Adult	Orford	Pools	175	190	139	141	171	171	104	105	-	-	-	-	-	-	-	-	-	-
Juvenile			175	190	139	141	171	171	104	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	-	-	-	-	-	-	-	-	-	-
Adult	Orford	Pools	132	190	139	139	171	171	105	105	-	-	-	-	-	-	-	-	-	-
Juvenile			132	190	139	139	171	171	105	105	-	-	-	-	-	-	-	-	-	-
(*N*)			(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	-	-	-	-	-	-	-	-	-	-

4.4 Discussion

The identical genotypes of juveniles and their brood parent for microsatellite and allozyme loci proves unequivocally that the brooded juveniles of *A. tenebrosa* are asexually produced. This study confirms and extends the work of Black and Johnson (1979) that also showed that the brooded juveniles of *A. tenebrosa* are electrophoretically identical to their brood parent. This result also provides strong support for previous allozyme studies of other *Actinia* species that have been shown to display identical allozyme genotypes between brooded juveniles and their parent (Carter and Thorp 1979; Orr *et al.* 1982; Monteiro *et al.* 1998), and is likely to reflect a widespread reproductive trait within this genus. As yet no species of *Actinia* has been shown conclusively to brood sexually produced larvae, and the prevailing view is that sexual reproduction occurs via broadcast spawning of gametes into the water column where external fertilisation and development occur (Monteiro *et al.* 1997; Perrin *et al.* 1999).

The lack of any sexually produced broods within adults collected from more heterogeneous boulder shores suggests no variation in the mode of production of brooded juveniles between habitats with varying levels of heterogeneity. This means that the higher levels of genotypic diversity detected for these habitats in previous studies (Ayre 1984b; Ayre *et al.* 1991a, Chapter 5) is likely the result of greater survivorship of a wider variety of genotypes possibly associated with the presence of a larger number of microhabitats (i.e. greater number of niches available to competing genotypes). The lack of variation in the mode of production of brooded propagules across habitats with varying levels of environmental heterogeneity is also consistent with recent studies of the brooding coral *P. damicornis* on the Great Barrier Reef (GBR), Australia (Ayre and Miller 2004; Chapter 2). Population genetic studies of *P. damicornis*, have yielded levels of genotypic diversity similar to that expected for predominantly sexual reproduction and comparable to that seen for other exclusively sexually reproducing scleractinian corals (e.g. Ayre and Dufty 1994; Ridgeway *et al.* 2001); therefore, it had previously been assumed that the brooded larvae of *P. damicornis* on the GBR were sexually produced. However, my surveys of brooded larvae, from a range of reef habitats, found that they were always asexually produced. This suggests that at least for some anthozoan species, there appears to be no variation in the

mode of production of brooded juveniles based on environmental heterogeneity, and that other ecological and demographic factors, such as the number of available niches, the competitive ability of established genotypes, and the frequency of sexual recruits from outside populations, are responsible for variation in the levels of genotypic diversity detected among habitats.

Nevertheless, despite the apparent widespread use of asexual reproduction to produce brooded larvae across a large geographical range in *A. tenebrosa*, the high levels of genotypic diversity in some populations, coupled with the presence of gonads, provides strong indirect evidence for an additional sexual mode of reproduction. From an evolutionary perspective, those habitats containing high levels of genotypic diversity may be particularly important, as they may provide a source of genetic diversity during sexual events that helps maintain and preserve genetic variation within this species. However, the role of somatic mutation in generating genotypic diversity within these populations is unclear and remains to be explored (see Chapter 5).

Co-dominant DNA markers such as microsatellites have now become the marker of preference when it comes to assessment of genetic diversity and detecting intra and inter-population variation, and have largely replace traditional nuclear markers such as allozymes. While the microsatellite markers in this study were on average more polymorphic than the allozyme markers, this as mainly due to two loci each of which displayed 10 and 4 alleles respectively. The remaining two microsatellite loci were no more variable than the allozyme markers which each displayed two alleles per locus.

One of the main strengths in using co-dominant DNA microsatellite markers in this study was that it enables me to preclude any possible confounding effects of maternal carryover of enzymes to the brooded juvenile that may have mask the juvenile's true genotype. Additionally, the relatively higher levels of polymorphism and large numbers of heterozygous individuals detected in this study meant that I could conclusively exclude the possibility that these results were due to sexual reproduction between identically

homozygous parents or self-fertilisation, as neither of these could account for the production of uniformly heterozygous broods. I did not test if the microsatellite markers used in this study were transferable to other species of *Actinia*, although it is not uncommon for markers designed for a particular species to readily amplify in other species within that genus. If these markers are indeed transferable, they may prove useful for unambiguously confirming the genetic origin of brooded larvae in other species of *Actinia*. Additionally, the taxonomic status of a number of species within the genus *Actinia* is highly contentious, with reports of a number of undescribed sibling and potentially cryptic species (Quicke *et al.* 1983; Douek *et al.* 2002; Vianna *et al.* 2003; Schama *et al.* 2005). The microsatellite markers used here may provide valuable insight into the taxonomic status of these groups and the potential historical relationship of speciation events.

Taken together, the microsatellite and allozyme data from this study provides the strongest and most conclusive evidence yet that the brooded juveniles of *A. tenebrosa* are asexually produced, and that there is no variation in the mode of production of brooded juveniles between habitats or over the large geographical range on the east coast of Australian.

Chapter 5 Genotypic Diversity and the Distribution of Clones Varies among Habitats, within Populations of the Sea Anemone *Actinia tenebrosa*

5.1 Introduction

Few organisms are exclusively clonal with many combining sexual and asexual reproduction in varying degrees (Ellstrand and Roose 1987; Hughes 1989; Widen *et al.* 1994). Levels of genotypic diversity and the distribution of genotypes within clonal populations will depend not only on the relative production of sexual *versus* asexual offspring, but also the range of ecological conditions under which each type of propagule is successful. Evolutionary theory predicts that asexual reproduction will be most adaptive when used to maintain populations within the parental habitat patch where fitness heritability may be high, while sexual reproduction is best used for dispersal to distant, unpredictable areas (Williams 1975; Bell 1982). Competition among genotypes within a population should then result in the selection for the fittest genotypes, such that each population consists of a few common and locally adapted clones (Williams 1975; Ayre 1985, 1995).

Levels of genotypic diversity and the distribution of clones have been studied using a variety of techniques including colour variation (Billingham and Ayre 1997), tissue grafting (Neigel and Avise 1983; Resing and Ayre 1985; Willis and Ayre 1985), allozyme electrophoresis (Stoddart 1984b; Ayre *et al.* 1991a; Billingham and Ayre 1996; Adjeroud and Tsuchiya 1999), and more recently, DNA markers such as microsatellites and AFLP's (Fuller *et al.* 1999; Chen *et al.* 2002; Darling *et al.* 2004; Duran *et al.* 2004). These and other studies have shown that for some species, populations contain one or a few common and widely spread genotypes: i.e. plants and grasses (Ellstrand and Roose 1987; Widen *et al.* 1994; Lehmann 1997); aquatic plants (Eckert et al. 2003); cladocerans (Hebert and Crease 1980; Carvalho 1994; Chaplin and Ayre 1997); sea anemones (Shick and Lamb 1977; Black and Johnson 1979; Shick *et al.* 1979; Ayre 1984b; Hoffmann 1986; Shaw

1991; Billingham and Ayre 1996); scleractinian corals (Neigel and Avise 1983; Stoddart 1984a; Ayre and Willis 1988; Hunter 1993; McFadden 1997); and gorgonian corals (Lasker 1990; Coffroth and Lasker 1998b; Chen *et al.* 2002). Explanations for high levels of clonality within these populations include the competitive exclusion of all but the fittest genotypes in populations that have been undisturbed for long periods of time, or, the founding of populations by a few genotypes that subsequently exploited local habitat space via asexual proliferation.

However, it is becoming increasingly clear that for many organisms that are capable of reproducing both sexually and asexually, populations can contain high levels of genotypic diversity, often approaching that expected for exclusive sexually reproducing species (Sebens and Thorne 1985; Ayre and Miller 2004; Ruggiero *et al.* 2005). For example, studies have shown that local populations may contain high levels of genotypic diversity with numerous clones coexisting: i.e. plants (Ziegenhagen *et al.* 2003; Arnaud-Haond *et al.* 2005; Ruggiero *et al.* 2005); cladocerans (Hebert 1974; Hebert and Ward 1976); fish (Angus 1980; Vrijenhoek 1998); sea anemones (Sebens and Thorne 1985; Shaw 1991); and scleractinian corals (Benzie *et al.* 1995; Ayre and Hughes 2000; Miller and Ayre 2004). Several mechanisms have been proposed to explain the coexistence of numerous clones within populations (Jackson 1985; Sebens and Thorne 1985), including; the continuous or cyclical production and recruitment of sexual offspring (i.e. aphids and rotifers), extreme similarity among competing genotypes such that they are unable to competitively excluded each other (i.e. clones are ecologically equivalent), temporal variation selecting for different clones under different environmental conditions (i.e. seasonal variation); somatic mutation accumulating within populations over time, and habitat heterogeneity allowing different clones to succeed in a variety of microhabitats (i.e. niche separation within a heterogenous environment).

While the continuous production of sexual recruits and seasonal variation in promoting genotypic diversity within clonal populations has been demonstrated in a number of plants and animals (Ellstrand and Roose 1987; Hebert *et al.* 1988), few studies to date have explored how genotypic diversity varies with environmental heterogeneity, although

several studies have reported large variation in genotypic diversity among populations (Ayre 1984b; Ayre *et al.* 1991a; Hunter 1993; Geedey *et al.* 1996; Lehmann 1997).

One such example is the Australasian sea anemone *Actinia tenebrosa*. This species produces asexually brooded juveniles (Ottaway and Kirby 1975; Black and Johnson 1979; Chapter 4) that have been observed to settle close to their brood parent on release (Ottaway 1979b; Ayre 1983b, 1984b). Patterns and levels of genotypic diversity detected in the majority of populations of *A. tenebrosa* on platform shores are consistent with the establishment of populations from widely dispersed sexually generated colonists with subsequent asexual proliferation of clones (Ayre 1983b, 1984a; Ayre *et al.* 1991a). These studies found that populations on rock platforms are typically dominated by a small number of common genotypes, and that deviate significantly from Hardy-Weinberg expectations. However, surveys of *A. tenebrosa* on more heterogeneous boulder shores have detected higher levels of genotypic diversity to that seen on rock platforms (Ayre 1984b; Ayre *et al.* 1991a), suggesting that genotypic diversity may vary among habitats based on environmental heterogeneity. Similar variation in genotypic diversity with environmental heterogeneity has been reported in other species capable of sexual and asexual reproduction (the coral *Porites compressa* Hunter 1993; the cladoceran *Daphnia pulicaria* Geedey *et al.* 1996; the grass *Calamagrostis epigejos* Lehmann 1997).

Genetic surveys of the broods of *A. tenebrosa* from boulder and rock platform habitats have shown that the higher levels of genotypic diversity seen within the more heterogeneous boulder habitat is not the result of sexual production of broods (Chapter 4). Instead, higher levels of genotypic diversity within this habitat may result from greater survivorship of the initial sexual colonists in a heterogeneous habitat. Boulder habitats are often more physically complex than stable rock platform habitats and quite different biological communities occupy each habitat (Granovitch and Johannesson 2000; Chapman 2002; Motta *et al.* 2003). As such the selective forces within these two habitat types are likely to be different and may result in each habitat containing a distinct set of locally adapted clones. *A. tenebrosa* therefore offers an ideal organism in which to test for variation in the relative importance of habitat heterogeneity on levels of genotypic diversity. However,

when assessing patterns of genotypic diversity in clonal organisms, the scale over which samples are collected needs to be carefully considered. The dispersal of sexual and asexual propagules may be quite different (e.g. Kirschner and Stepanek 1994; McFadden 1997), and as such sampling at different spatial scales is likely to result in different estimates of genotypic diversity. For example, if asexual propagules have highly limited dispersal (i.e. a few centimetres), clones (ramets) may not be detected if sampling is conducted over larger spatial scales (i.e. meters). Understanding of the relative importance of sexual and asexual reproduction to population structure therefore requires sampling at multiple spatial scales. Fine-scale sampling is needed to determine the extent and distribution of clonal aggregations, while large-scale sampling is required to gain an accurate estimate of genotypic diversity within a population.

The importance and role of somatic mutation in generating genotypic diversity within natural populations has been largely overlooked for many clonal taxa. This has been partly due to the difficulty in distinguishing between those genotypes that have arisen from somatic mutation, and those that are the result of sexual reproduction. However, the development and use of more variable DNA markers such as microsatellites, and a better understanding of mutational models for different types of genetic markers (i.e. stepwise or infinite allele models) mean we can now make more accurate predictions about the structure of lineages that have arisen from somatic mutation. Mutation without recombination and segregation should result in genotypes that differ at only one or a few loci (i.e. they have a high genetic similarity). Additionally, mutation without recombination should also result in an excess of heterozygotes as compared with expectations for Hardy-Weinberg equilibrium (although selection for heterozygotes may also contribute to heterozygous excesses), and linkage disequilibrium.

Here I use a combination of microsatellite and allozyme markers to assess levels of genotypic diversity and the distribution of clones of *A. tenebrosa* within habitats with contrasting levels of heterogeneity. I collected samples of *Actinia tenebrosa* over fine (<1.5 meters) and course (5 × 5 meters) spatial scales within rock pool and more heterogeneous boulder habitats. Levels of genotypic diversity and the distribution of

unique multi-locus genotypes were then used to infer the relative importance of habitat heterogeneity in promoting the coexistence of different clones and the relative dispersal distance of clones within and between habitats. In addition, the possible role of somatic mutation to observed levels of genotypic diversity within my samples was assessed.

5.2 Methods

5.2.1 Collection of Samples

5.2.1.1 Fine-Scale Collections

I assessed levels of genotypic diversity and the distribution of clones of *A. tenebrosa* within three rock pools on an intertidal rocky shore at Bellambi and on three boulders at Bass Point, NSW Australia. Detailed maps of the position of all anemones were made prior to their removal. Rock pools ranged in size from 30cm^3 to 825cm^3 and were separated by between three to 30 meters. Individual boulders were approximately 0.5m^3 and were separated by between five and 20 metres. I collected between 54 and 78 individuals from each of the three rock pools at Bellambi and between 25 and 47 individuals from each of the three boulders at Bass Point. A small tissue sample (one or two tentacles) was taken from each individual and immediately frozen in liquid nitrogen prior to DNA extraction and allozyme electrophoresis.

5.2.1.2 Large-Scale Collections

I assessed the levels of genotypic diversity and the distribution of clones within and between two adjacent habitats on the same rocky shore at two locations, Cape Banks and Bass Point, NSW Australia. These two locations were chosen as both supported two discrete and easily identifiable habitats, a rock platform with discrete rock pools and a more heterogenous boulder habitat. Habitats on the same rocky shore were separated by < 500m, and at each location I collected samples of 20 adult anemones from three 5m^2 sites within each of the two habitats. I attempted to minimise the collection of clonemates within these sites by taking only widely spaced individuals. Anemones were levered from the substratum and transported back to the laboratory in separate zip-lock bags filled with seawater and tissue samples were immediately frozen in liquid nitrogen prior to DNA extraction and allozyme electrophoresis.

5.2.2 Microsatellite and Allozyme Genotyping

I genotyped all anemones at four microsatellite loci (*At1*, *At5*, *At21a* and *At38*) and four allozyme loci (*Gpi1, 6Pgd, Mdh* and *Odh*). Methods for genotyping of individuals at both microsatellite and allozyme loci were the same as that described in Chapter 4.

5.2.3 Analysis

5.2.3.1 Power to Identify Clones

I assigned anemones to different clonal genotypes based on genotypic identity at eight polymorphic loci (four microsatellite and four allozyme loci). Those anemones with an identical eight-locus genotype were assumed to represent a single clone. I tested my power to identify different genotypes by calculating the number of unique genotypes identified with increasing locus combinations, and by calculating the probability of identity, P_{ID}, for increasing locus combinations (Waits *et al*. 2001) within each population using the program GenAlex (V6) (Peakall and Smouse 2005). This identification estimator calculates the probability that two individuals drawn at random from a population will have the same genotype at multiple loci and is used to access the statistical confidence for individual identification. $P_{(ID)}$ was calculated for each locus using adult allele frequencies in the population and then multiplied across loci to give an overall P_{ID} (Waits *et al*. 2001).

$P(_{ID}) = \Sigma pi + \Sigma\Sigma\ (2pi\ pj)$

where pi and pj are the frequencies of the ith and jth alleles and $i \neq j$ (Paetkau and Strobeck 1994).

5.2.3.2 Assessing Genotypic Diversity

For samples collected at each spatial scale I used several methods to assess genotypic diversity and the distribution of clones. Firstly, I compared the number of individuals sampled (N) to the number of unique multi-locus genotypes detected (N_g). Because only a small part of the genome can be sampled, individuals with identical multi-locus genotypes may still be non-clonemates. Therefore, the ratio of N_g/N provides a maximum estimate of the contribution of asexual reproduction to localised recruitment. I then compared the ratio of observed multi-locus genotypic diversity (G_o) to that expected under conditions of sexual reproduction with free recombination (G_e), as described by (Stoddart and Taylor 1988). Departures of G_o/G_e from unity are a predicted consequence of asexual reproduction (but may also result from other factors such as population subdivision). In contrast, a population with high levels of sexually derived recruitment will display a G_o/G_e ratio of

close to unity. I tested for significance departures from unity by determining if G_o lay outside the 95% confidence interval of G_e (Stoddart and Taylor 1988). To reduce the chance of type I errors a sequential Bonferroni correction was applied to correct for the large number of similar tests.

5.2.3.3 Sexual Reproduction versus Somatic Mutation as a Source of Genotypic Diversity

In order to gain an initial estimate of the potential for somatic mutation to contribute to observed levels of genotypic diversity within populations of *A. tenebrosa*, I carried out pairwise comparisons of all unique genotypes to identify those individuals within a population that differed at one or a few loci. I then compared those individuals within each population that differed by only a single allele at one of the four microsatellite loci. Microsatellite loci are expected to experience higher rates of mutation compared with allozyme loci (Amos *et al.* 1996; Estoup *et al.* 1998; Ellegren 2004). A single stepwise mutation model was assumed and only those individuals that differed by a single stepwise mutation at one of the four-microsatellite loci were considered to have been derived through mutation from a common clonal ancestor.

In any given sample, if a large proportion of genotypes are derived from somatic mutation from a common clonal lineage, this should result in linkage disequilibrium and departures from single-locus Hardy-Weinberg expectations. In contrast, if observed levels of genotypic diversity are the result of mainly sexual reproduction, then pooled clonal genotype frequencies (i.e. replica genotypes removed from analysis) should approximate single-locus Hardy-Weinberg expectations and loci should be in linkage equilibrium. Departures from Hardy-Weinberg expectations were expressed as Wright's (1978) fixation index, F_{IS}, where positive and negative values represent deficits or excess of heterozygotes respectively and were calculated using the genetics program GENEPOP V3.4 (Raymond and Rousset 1995). In order to assess whether each locus assorted independently I tested each pair-wise combination of loci for linkage disequilibria (Weir 1979). To reduce the chance of type I errors a sequential Bonferroni correction was applied to correct for the large number of similar tests.

5.3 Results

5.3.1 Identification of Genotypes

I detected a total of 27 alleles across the eight loci in a total sample of 521 individuals collected from three populations. The most informative loci were the two microsatellite loci *At1* and *At5*, with each displaying up to six alleles per locus, while the remaining loci each displayed two to three alleles per locus, with an overall average of 3.25 (S.E. 0.64) alleles per locus. The number of unique genotypes identified was almost twice that for the four microsatellite loci than that identified by the four allozyme loci (Figure 5.1). However, the combined use of microsatellite and allozyme loci allowed the highest degree of discrimination among genotypes (Figure 5.1). The asymptotic relationship observed between the number of loci and genotypic diversity (Figure 5.2) indicates that this combination of loci reveals most of the genotypic diversity within these populations and that the use of additional loci is unlikely to further increase the ability to resolve additional genotypes.

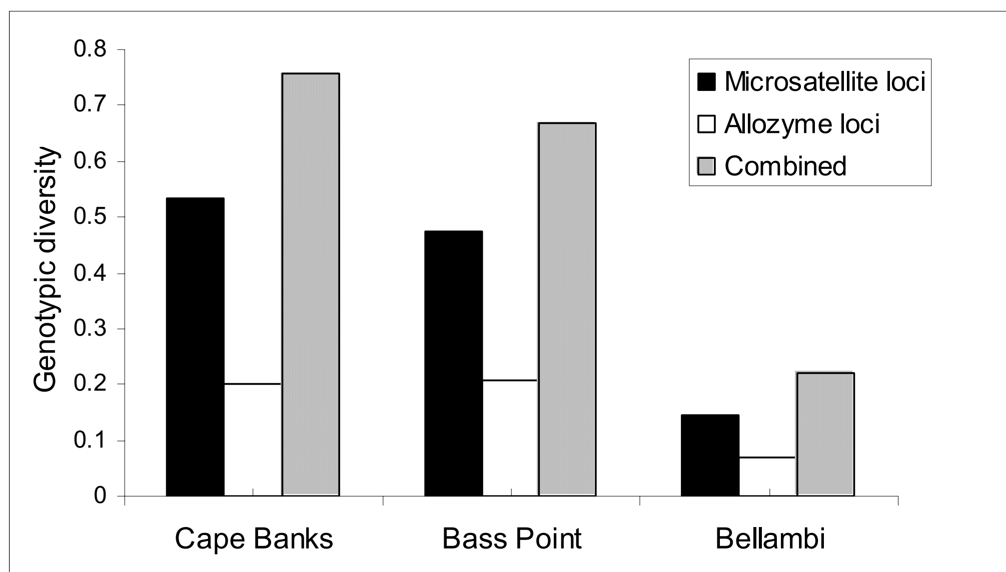

Figure 5.1 Levels of genotypic diversity within three populations of the sea anemone *Actinia tenebrosa* calculated for four microsatellite loci, four allozyme loci, and all loci combined. Genotypic diversity = $G - 1 / N - 1$, where G is the number of genotypes and N is the sample size. Cape Banks (N = 123), Bass Point (N = 223), Bellambi (N = 212).

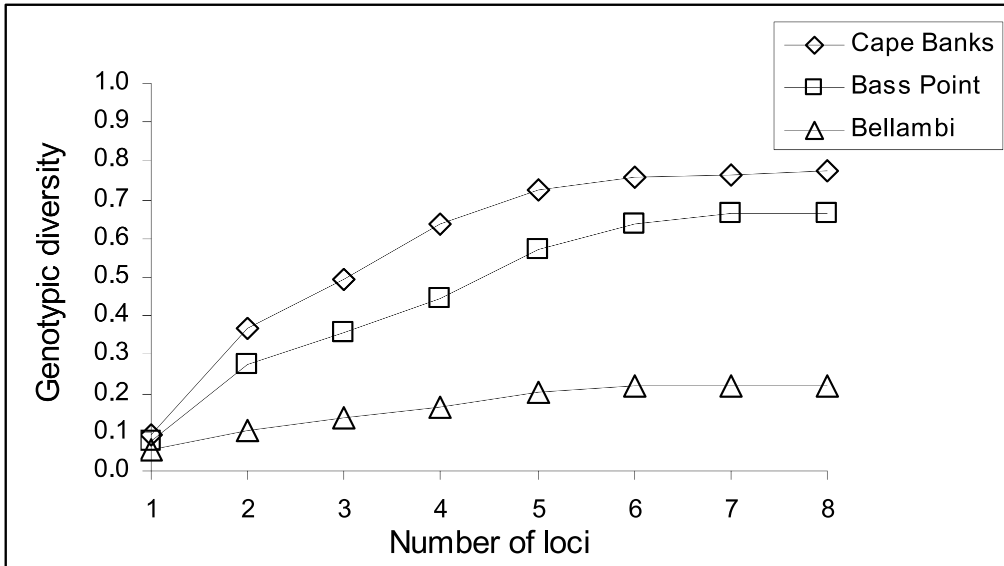

Figure 5.2 Levels of genotypic diversity identified for increasing number of loci for individuals of *Actinia tenebrosa* sampled from three populations. Loci added in increasing level of variability. Genotypic diversity = $G - 1 / N - 1$, where G is the number of genotypes and N is the sample size. Cape Banks (N = 123), Bass Point (N = 223), Bellambi (N = 212).

The probability that two individuals drawn at random from a population will share by chance the same eight multi-locus genotype was low for all three populations, with $P_{(ID)}$ ranging from 0.002 to 0.001 (Figure 5.3). The combination of microsatellite and allozyme loci used in this study thus allowed for the unambiguous assignment of individuals with the same genotype to a particular clone.

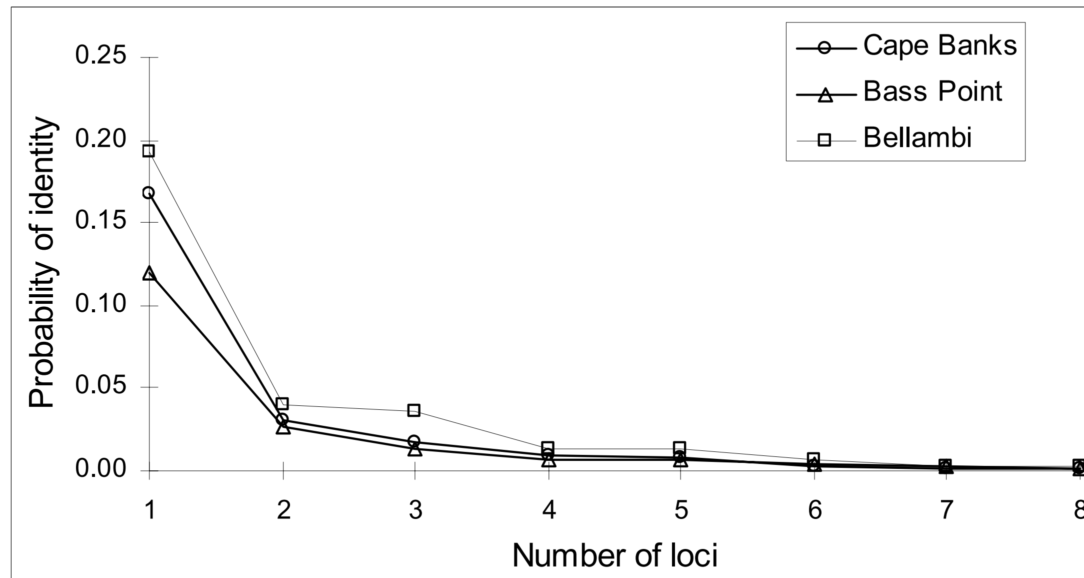

Figure 5.3 The probability of identity ($P_{(ID)}$) calculated for three populations of the sea anemone *Actinia tenebrosa*. $P_{(ID)}$ calculates the probability that two individuals drawn at random from a population will have the same genotype at multiple loci and is used to access the statistical confidence for individual identification. $P_{(ID)}$ was calculated for each locus using adult allele frequencies in the population and then multiplied across loci to give an overall P_{ID} (Waits *et al*. 2001). Sample sizes for each location were: Cape Banks (N = 123), Bass Point (N = 223), Bellambi (N = 212).

5.3.2 Genotypic Diversity

My analysis of fine-scale genotypic diversity within single rock pools and on individual boulders revealed distinct differences in the levels of genotypic diversity between these two habitats. Within individual rock pools at Bellambi I found that the number of unique genotypes (N_g) detected compared to the number of individuals sampled (N) was consistently low, with N_g/N ranging from 0.25 to 0.31 (Table 5.1). These rock pools showed only 7-10% of the genotypic diversity expected for random mating (G_o / G_e = 0.07 – 0.10) (Table 5.1). Approximately half (51%) of the genotypes detected in each pool were represented by two or more individuals, with just four of these clonal genotypes accounting for 52% of all individuals sampled at Bellambi (Figure 5.4). In contrast, I detected much higher levels of genotypic diversity on individual boulders at Bass Point (Table 5.1). Each boulder supported a relatively greater number of unique genotypes than detected in the rock pools at Bellambi. The number of unique genotypes (N_g) compared to the number of

individuals sampled (N) was much higher on boulders than in rock pools and ranged from 0.76 to 0.81 (*cf* 0.25 to 0.31 for the rock pools). Similarly, levels of genotypic diversity on boulders at Bass point were much higher than detected within the rock pools at Bellambi (G_o / G_e ranged from 0.51 – 0.64 *cf* 0.07 - 0.10 for the rock pools, Table 5.1), however, significant deviations from that expected for random mating were detected. No clone was numerically dominant on boulders with the most replicated clone represented by only 6 individuals (*cf* 36 for the rock pools) (Figure 5.4). This means that a high proportion of clones (78%) were represented by only one individual (Figure 5.4).

Table 5.1 Comparison of the observed and expected multi-locus genotypic diversity within collections of *Actinia tenebrosa* made from individual rock pools and boulders. Significant levels of G_o / G_e from panmixis are determined following a sequential Bonferroni correction for multiple tests. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Location	Habitat	N	N_g	N_g / N	G_o	$G_e \pm$ SD	G_o / G_e
Bellambi	Rock Pools						
	Pool 1	78	24	0.31	4.33	66 ± 6	0.07***
	Pool 2	53	15	0.28	3.27	47 ± 4	0.07***
	Pool 3	77	19	0.25	6.85	66 ± 6	0.10***
	Data pooled	208	47	0.23	12.41	141 ± 12	0.09***
Bass Point	Boulders						
	Boulder 1	25	19	0.76	15.24	24 ± 2	0.62**
	Boulder 2	47	36	0.77	28.69	45 ± 3	0.64**
	Boulder 3	31	25	0.81	15.25	30 ± 2	0.51**
	Data pooled	103	73	0.71	49.80	94 ± 6	0.78**

N = number of individuals sampled, N_g = number of unique eight-locus genotypes detected, G_o = observed multi-locus genotypic diversity, G_e = genotypic diversity expected for random mating (Stoddart and Taylor 1988).

Figure 5.4 The distribution of *Actinia tenebrosa* within rock pools at Bellambi and on boulders at Bass Point. Different numbers represent unique genotypes while the most common clones within each site are indicated by different colours. Genotypes represented by only one individual are given the symbol *.

Within individual rock pools at Bellambi the effects of asexual reproduction were reflected by the high proportion of statistically significant departures from Hardy-Weinberg equilibria, low levels of genotypic diversity and restricted distribution of clones among sites. Eight significant heterozygote excesses and three heterozygote deficits were detected within a total of 19 single-locus tests across eight loci, all of which remained significant after the application of a sequential Bonferroni correction (Table 5.2). Only one clone (clone number 74, Figure 5.4) was common to all three pools and was represented by two individuals in Pool 1, one individual in Pool 2, and 18 individuals in Pool 3. Pool 2 and 3 shared a further five clonal genotypes, while Pool 1 and 3 shared only a single additional genotype (Figure 5.4).

In contrast, samples collected from individual boulders at Bass point conformed closely to Hardy-Weinberg equilibria, with only two significant heterozygote excesses and two heterozygote deficits detected, within a total of 19 single-locus tests across eight loci, all of which remained significant after the application of a sequential Bonferroni correction (Table 5.2). There was only a single case of sharing of clonal genotypes between boulders 2 and 3 (clone number 58) and was represented by only one individual on each boulder.

When the effects of clonal reproduction were removed (i.e. replica genotypes removed from the data set), all samples collected from individual rock pools at Bellambi and boulders from Bass Point conformed closely to Hardy-Weinberg equilibrium suggesting that these populations are founded (at least initially) by sexually derived recruits (Table 5.3).

Table 5.2 Wrights fixation index (F_{IS}) indicating significant departures from Hardy-Weinberg equilibria for *Actinia tenebrosa* collected from two habitats. Calculations made using all adult genotype frequencies. Significant departures determined after the application of a sequential Bonferroni correction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

		Locus							
	N	*At1*	*At5*	*At21a*	*At38*	*Gpi*	*Mdh*	*Odh*	*6Pgd*
Bellambi Pools									
Pool 1	78	-0.123***	-0.010***	-0.048	-0.395*	-	0.016	0.760***	0.002
Pool 2	53	-0.174***	-0.186***	-	0.350	-	0.796***	0.306	0.070
Pool 3	77	-0.025***	-0.183***	-	0.957***	-	-0.418**	0.106	-0.086
Total	208	-0.093***	-0.150***	-0.017	0.393***	-	0.034	0.408***	-0.009
Bass Point boulders									
Boulder 1	25	0.126	-0.149	0.818**	0.073	-	-0.044	-0.174	-0.460
Boulder 2	47	-0.085**	-0.126	0.460	-0.280	-	-0.109	0.302	0.032
Boulder 3	31	-0.055*	-0.028	0.682**	-0.124	-	0.283	0.450	-0.073
Total	103	-0.027*	-0.072	0.638***	-0.147	-	0.110	0.192	-0.121

Table 5.3 Wrights fixation index (F_{IS}) indicating significant departures from Hardy-Weinberg equilibria for *Actinia tenebrosa* collected from two habitats. Calculations made using only unique multi-locus genotypes. Significant departures determined after the application of a Bonferroni correction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

		Locus							
	N	*At1*	*At5*	*At21a*	*At38*	*Gpi*	*Mdh*	*Odh*	*6Pgd*
Bellambi Pools									
Pool 1	24	-0.052	-0.020	-0.071	0.168	-	0.011	0.589	0.011
Pool 2	15	-0.068	-0.107	-	0.492	-	0.761	0.208	0.106
Pool 3	19	-0.066	-0.101	-	0.805	-	0.358	0.135	-0.246
Total	47	-0.076	-0.076	-0.034	0.292	-	0.343	0.380	-0.006
Bass Point boulders									
Boulder 1	19	0.155	-0.136	0.876*	0.079	-	-0.060	-0.165	-0.391
Boulder 2	36	-0.051	-0.103	0.431	-0.253	-	-0.112	0.291	-0.028
Boulder 3	25	-0.048	0.039	0.511	0.029	-	0.299	0.434	0.156
Total	73	-0.001	-0.031	0.522***	-0.670	-	0.152	0.197	-0.007

For samples collected at the larger spatial scales (i.e. 5 × 5 m sites and at the scale of the habitat) I detected relatively higher levels of genotypic diversity than that detected for the fine-scale sampling at both Cape Banks and Bass Point (Table 5.4). The number of unique genotypes (N_g) detected compared to the number of individuals sampled (N) ranged from 0.75 – 1.00 within each of the 5 × 5m sites (Table 5.4). At the scale of the habitat, samples collected from the boulder habitat at Bass Point showed a greater proportion of unique

genotypes compared to the rock pool habitat (82% *cf.* 62%), however, there was no difference in the proportion of unique genotypes between the rock pool and boulder habitat at Cape banks (78% *cf.* 78%) (Table 5.4). The levels of observed genotypic diversity to that expected for sexual reproduction varied greatly between sites and habitats (G_o /G_e. ranged from 0.50- 1.01, Table 5.4), however, pooled data for each habitat revealed that at Bass Point the boulder habitat displayed almost twice the level of genotypic diversity to that detected within the rock pool habitat (G_o /G_e = 0.78 *cf.* 0.44). In contrast, at Cape Banks levels of observed genotypic diversity were similar in the two habitats, with the rock pool habitat displayed only slightly higher levels of observed genotypic diversity in comparison to the boulder shore (G_o /G_e = 0.69 *cf.* 0.58).

Table 5.4 Comparison of the observed and expected multi-locus genotypic diversity within collections of *Actinia tenebrosa* made from 5 × 5m sites within rock pool and boulder habitats. Significant levels of G_o / G_e from panmixis are determined following a sequential Bonferroni correction for multiple tests. * P < 0.05, ** P < 0.01, *** P < 0.001

Location	Habitat	N	N_g	N_g /N	G_o	$G_e \pm SD$	G_o /G_e
Bass Point	Rock pool						
	Site 1	20	16	0.80	13.33	18.84 ± 2.06	0.71
	Site 2	20	14	0.70	9.52	18.97 ± 1.97	0.50*
	Site 3	20	16	0.80	14.29	19.59 ± 1.27	0.73*
	Data pooled	60	37	0.62	23.38	53.67 ± 4.66	0.44***
	Boulder						
	Site 1	20	15	0.75	13.33	18.52 ± 2.30	0.72
	Site 2	20	17	0.85	15.38	19.39 ± 1.54	0.79
	Site 3	20	16	0.80	13.33	19.47 ± 1.44	0.68**
	Data pooled	60	49	0.82	43.37	55.47 ± 4.21	0.78*
Cape Banks	Rock pool						
	Site 1	20	15	0.75	12.50	19.58 ± 1.28	0.64**
	Site 2	20	20	1.00	20.00	19.41 ± 1.51	1.03
	Site 3	20	19	0.95	18.18	19.85 ± 0.82	0.92
	Data pooled	60	47	0.78	40.00	57.90 ± 2.86	0.69**
	Boulder						
	Site 1	20	19	0.95	18.18	19.45 ± 1.46	0.93
	Site 2	20	17	0.85	12.90	19.12 ± 1.86	0.67*
	Site 3	20	18	0.90	15.38	18.06 ± 2.64	0.85
	Data pooled	60	47	0.78	30.51	52.91 ± 5.25	0.58**

N = number of individuals sampled, N_g = number of unique eight-locus genotypes detected, G_o = observed multi-locus genotypic diversity, G_e = genotypic diversity expected for random mating (Stoddart and Taylor 1988).

5.3.3 Genotypic Structure and the Distribution of Clones

The collection of samples from the 5 × 5 m sites at Cape Banks and Bass Point allowed for the identification of the distribution of clones within and between habitats on the same rocky shore. Within each location there was distinct structuring in the distribution of genotypes between rock pool and boulder habitats. I detected no sharing of genotypes between the rock pool and boulder habitat at Bass point and only one genotype (clone 41) common to both the rock pool and boulder habitat at Cape Banks (Table 5.5). For those genotypes represented by two or more individuals, I found that 76% were common to more than one site within a habitat indicating that, while different clones appear to be restricted to different habitats, most are able to disperse within each habitat patch. However, at Bass Point there was no sharing of clones between sites within the boulder habitat for samples collected from the 5 × 5m sites (Table 5.5).

Table 5.5 Distribution of clones of *Actinia tenebrosa* collected from three 5 × 5 m sites within each of two habitats at two locations along the NSW coast, Australia.

Bass Point							Cape Banks						
Clone	Boulder site			Pool site			Clone	Boulder site			Pool site		
ID	1	2	3	1	2	3	ID	1	2	3	1	2	3
7	2	-	-	-	-	-	41	-	-	1	-	1	1
12	2	-	-	-	-	-	39	1	3	3	-	-	-
56	2	-	-	-	-	-	4	1	1	-	-	-	-
73	2	-	-	-	-	-	52	1	1	-	-	-	-
1	-	2	-	-	-	-	37	1	-	1	-	-	-
19	-	2	-	-	-	-	43	1	-	1	-	-	-
54	-	2	-	-	-	-	44	2	-	1	-	-	-
48	-	-	3	-	-	-	70	-	2	-	-	-	-
67	-	-	2	-	-	-	6	-	-	-	1	1	-
77	-	-	2	-	-	-	53	-	-	-	1	1	-
66	-	-	-	2	2	1	55	-	-	-	2	1	-
71	-	-	-	1	2	2	79	-	-	-	1	1	-
29	-	-	-	3	1	-	15	-	-	-	2	-	-
39	-	-	-	1	1	-	16	-	-	-	3	-	-
28	-	-	-	2	-	-	18	-	-	-	2	-	-
46	-	-	-	-	2	2	17	-	-	-	1	-	1
63	-	-	-	-	1	1	10	-	-	-		1	1
78	-	-	-	-	3	2	27	-	-	-	-	-	2
72	-	-	-	-	2	-	*	12	13	13	7	14	15
81	-	-	-	-	-	2							
*	12	14	13	11	5	10							

* clones represented by only a single individual

5.3.4 Sexual Reproduction *versus* Somatic Mutation as Source of Genotypic Diversity

Because genotypes derived from somatic mutation from a common clonal lineage will share a large proportion of their alleles, I assessed the relative importance of somatic mutation to observed levels of genotypic diversity within each population by making pair-wise comparisons among all unique multi-locus genotypes. This revealed a low frequency of genotypes that shared the same allelic combinations at all but one or a few loci (Figure 5.5). There was no trend towards higher frequency of low values as might be expected if somatic mutation made a large contribution to genotypic diversity within these populations. Indeed, out of a total of 11 026 pair-wise comparisons of 149 clones detected at Bass Point (data pooled for fine-scale and large-scale collections), I found only 182 pairs (1.65%) of clones that differed by a single allele, of which 64 pairs differed by a single step-wise mutation at one of the four microsatellite loci. Similarly, out of a total of 1 081 pair-wise comparisons of the 47 unique genotypes detected within rock pools at Bellambi, I found only 29 pairs of clones that differed by a single allele, and only four pairs of clones that differed by a single step-wise mutation at one of the four microsatellite loci. At Cape Banks, out of a total of 4 278 pair-wise comparisons of the 93 unique genotypes detected, I found only 51 pairs of clones that differed by a single allele, and only nine pairs of clones that differed by a single step-wise mutation at one of the four microsatellite loci.

Analysis using only the unique genotypes detected within each population conformed closely to single-locus Hardy Weinberg expectations, indicating that genotypic frequencies closely matched that expected for sexual reproduction. I found only 10 cases of departures from expected values within a total of 125 tests across eight loci for all three populations (Table 5.3b and 5.6), with only four of these remaining significant after a sequential Bonferroni correction of significance levels (Table 5.3 and 5.6). In order to determine whether each locus assorted independently I tested each pair-wise combination of loci for linkage disequilibria (Weir 1979) at each location separately. Consistent associations are expected if a large number of genotypes are derived through mutation from a common clonal ancestor. From a total of 28 pair-wise tests, I detected only seven significant inter-locus associations at Bellambi and one at Bass Point ($P < 0.05$), however, only four of these

associations (all at Bellambi) remained significant after application of a Bonferroni correction of alpha values ($P < 0.0018$).

Table 5.6 Wrights fixation index (F_{IS}) indicating significant departures from Hardy-Weinberg equilibrium for *Actinia tenebrosa* collected from two locations. Calculations made using only unique multi-locus genotypes. Significant departures determined after the application of a sequential Bonferroni correction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

		Locus							
	N	*At1*	*At5*	*At21a*	*At38*	*Gpi*	*Mdh*	*Odh*	*6Pgd*
Bass Point Pools									
Site 1	20	0.020	0.267**	-	0.232	-	0.409	-0.088	-0.191
Site 2	20	0.124	0.048	-0.088	0.317	-	-0.191	-0.272	-0.316
Site 3	20	0.163	-0.152	-0.230	0.232	-	0.090	-0.042	0.023
Bass Point boulders									
Site 1	20	0.026	-0.113	0.875	-0.120	-	-0.191	-	0.090
Site 2	20	-0.067	-0.119	-0.001	0.090	-	-0.155	-0.028	0.779
Site 3	20	0.218	-0.018	0.090	0.164	-	0.164	-0.057	-0.272
Cape Banks Pools									
Site 1	20	0.130**	-0.024	0.090	-0.042	-0.120	0.164	-0.120	-0.414
Site 2	20	0.108	0.035	0.481	0.219	-0.120	0.376	0.023	0.164
Site 3	20	-0.075	0.443	0.667*	-0.060	-0.088	-0.169	0.090	-0.155
Cape Banks boulders									
Site 1	20	0.149	0.112	0.505	-0.272	-	0.317	0.468	-0.057
Site 2	20	-0.038	0.168	-0.028	-0.316	-	-0.234	0.057	-0.191
Site 3	20	-0.040	0.105	-	-0.088	-	-0.272	0.018	-

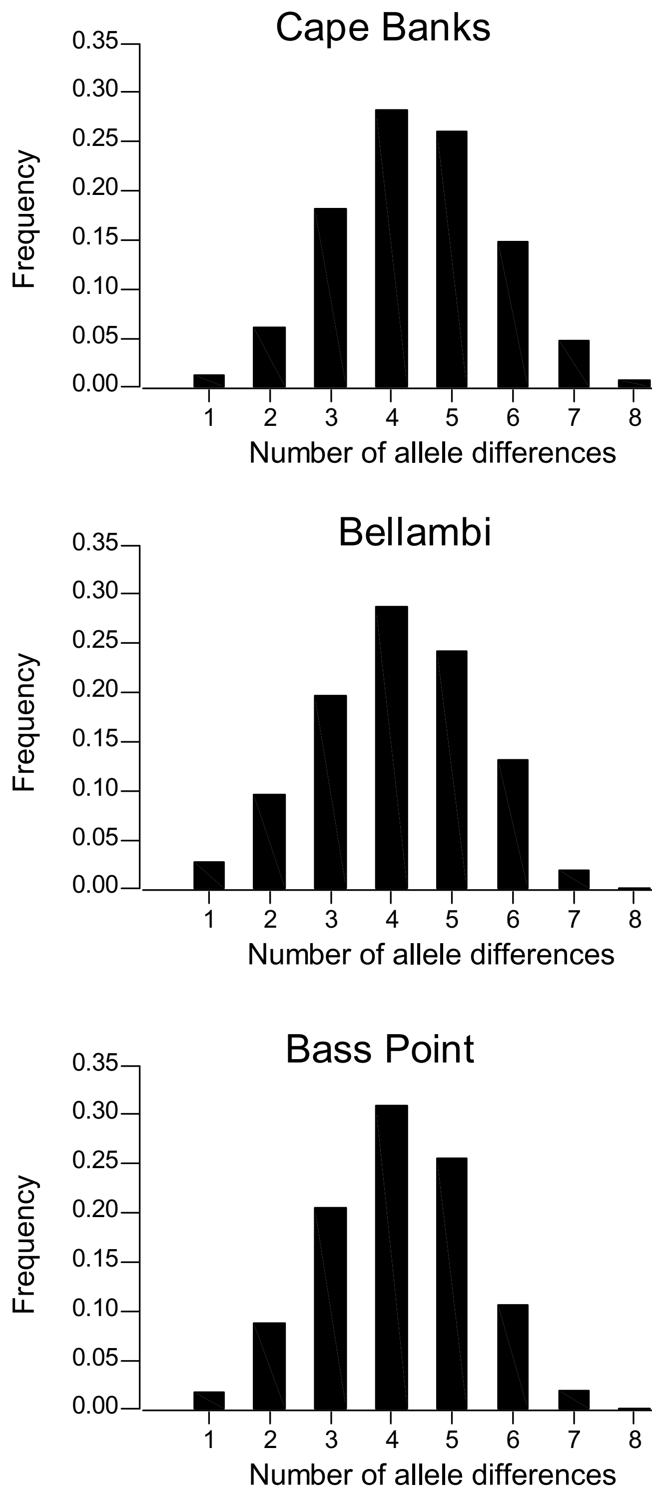

Figure 5.5 Frequency distribution of the pair-wise number of allele differences detected between unique multi-locus genotypes for *Actinia tenebrosa* within each of three populations along the NSW coast, Australia.

5.4 Discussion

My genetic surveys indicate that environmental heterogeneity may be important in determining the levels of genotypic diversity and the distribution of clones within populations of *A. tenebrosa*. Levels of genotypic diversity were significantly lower within the rock pool habitat compared with that detected within the more heterogeneous boulder habitat. Moreover, the finding that clones are generally restricted to particular habitats implies that the distribution of clones is determined by environment × genotype interactions.

5.4.1 Power to Identify Unique Genotypes

Much attention has been given to the ability of different types of markers to assess genotypic diversity in clonal organisms (Stoddart and Taylor 1988; Edmands 1995; Arnaud-Haond *et al.* 2005). Limited marker resolution can dramatically reduce the ability to detect genetic variation and may lead to incorrect inferences about population parameters, mating systems and levels of genotypic diversity within clonal populations. However, the low probability of identity values (Figure 5.3)., the asymptotic relationship observed between the number of loci used and the number of unique multi-locus genotypes detected in my study, indicate that my ability to distinguish between different clones was relatively high ($P_{(ID)}$ ranging from 0.002 to 0.001). Despite this, it is possible that at least a small number of distinct individuals displaying the same multi-locus genotype were incorrectly identified as the same clone. The sharing of some genotypes between the three locations may represent such instances. The asexual propagules of *A. tenebrosa* have limited dispersal abilities and typically settle within a few centimetres of the brood parent (Ayre 1983b) and at most some clones may potentially disperse distances of a few hundred meters of shore (Ayre 1984b). However, it is unlikely that clonal propagules are able to disperse between neighbouring headlands separated by several kilometres and as such the small number of multi-locus genotypes shared among different locations may in fact represent different clones. If however, genotypes shared among headland do represent the same clone, such clones may represent some type of general purpose genotype that have managed to disperse and survive over relatively large distance by some chance event.

5.4.2 Genotypic Diversity and Habitat Heterogeneity

Levels of genotypic diversity within populations of *A. tenebrosa* can vary dramatically between habitats with differing levels of environmental heterogeneity. Individual boulders appear to support a relatively greater diversity of genotypes in comparison to equivalent areas of rock pool habitats. Indeed, within samples collected from individual boulders, on average 71% of individuals displayed unique multi-locus genotypes compared with only 23% of individuals collected from rock pools. Rock pools were typically dominated by a small number of highly replicated genotypes forming distinct clonal aggregations. This is consistent with a previous study of *A. tenebrosa* that detected lower levels of genotypic diversity on rocky platforms compared with boulder shores in Western Australia (Ayre 1984b).

The higher level of genotypic diversity within boulder habitats may indicate either, i) greater sexual recruitment within these habitats, or ii) greater survival of sexual recruits. While sexual reproduction is likely to be important in generating genotypic diversity within these populations (at least initially), the higher level of genotypic diversity detected on boulder shores is unlikely to be the result of greater levels of sexual reproduction within this habitat. Genetic analysis of broods collected both from the boulder and rock pool habitats has shown that all brooded juveniles were genetically identical to their brood parent, and are the result of asexual reproduction (Chapter 4), suggesting no switch to sexual brooding within more heterogenous habitats.

The striking difference in the levels of genotypic diversity seen between boulder and rock pool habitats is more likely to reflect greater survivorship of sexually generated colonists in a spatially heterogeneous environment. Niche separation between ecologically distinct clones from the boulder shore would allow a large number of genotypes to coexist in such a heterogenous environment. In contrast, less heterogeneous rock pool habitats are less likely to support as large a diversity of genotypes. In such homogenous habitats it is predicted that inter-clonal competition will result in the fittest clones out-competing less fit clones such that only a small number of numerically dominant and locally adapted genotypes remain. Higher levels of clonal diversity associated with increased habitat heterogeneity

has been reported for a number of clonal plant (Linhart and Grant 1996; Lehmann 1997) and animal species (Hunter 1993; Geedey *et al.* 1996). The coexistence of a large number of different clones within populations has also been reported for a number of other clonal sea anemones including *Metridium senile* (Hoffmann 1976; Shick *et al.* 1979), *Anthopleura elegantissima* (Sebens 1982), *Sagartia elegans* (Shaw 1991) and *Nematostella vectensis* (Darling et al. 2004), although how levels of genotypic diversity varies with levels of environmental heterogeneity were not assessed in these studies.

Interestingly, levels of genotypic diversity collected over larger spatial scales (i.e. $5m^2$ sites) than the individual rock pool or boulder revealed little difference between the two habitats. However, this may be largely due to the sampling design of these collections which specifically targeted widely spaced individuals to maximise the detection of distinct genotypes and increase my ability to identify the potential of clones to disperse within and between habitats on the same rocky shore. Comparisons of genotypic diversity between boulder and rock pool habitats based on such data alone is therefore limited and highlights how quite different conclusions about levels of genotypic diversity can be reached using different sampling strategies. Nevertheless, this sampling strategy does reveal surprisingly high levels of genotypic diversity over the scale of the entire habitat for both boulder and rock pool habitats and highlights potential concerns over the interpretations of levels of genotypic diversity within clonal populations where samples may have been collected over inappropriate scales. Future sampling of populations of clonal organisms to assess genotypic diversity and population structure should carefully consider the likely scale of dispersal of asexual and sexual propagules and the question being addressed when collecting samples. Ideally samples should be collected over a variety of spatial scales, habitats and populations in order to tease apart the effects of dispersal, mating system and selection in determining levels of genotypic diversity in clonal populations.

5.4.3 Distribution of Clones

One of the most striking features revealed by my surveys on the distribution of genotypes between habitats on a single rocky shore is the apparent lack of sharing of genotypes between these two habitat types despite sites being separated by less than 200m at both

Cape Banks and Bass Point. I found only a single case of a genotype shared between the boulder and rock pool habitats at Bass Point and no sharing of genotypes between habitats at Cape Banks. While most clonal genotypes were restricted to a single site, I did detect some apparent sharing of genotypes among sites within a habitat (Table 5.5). Similar genotypic differentiation among habitat types has been reported for a number of clonal plant species in response to particular soil types, flooding regimes, pollution levels, and slope height (reviewed by Linhart and Grant 1996).

Two processes may potentially explain the lack of overlap of clones of *A. tenebrosa* between habitats separated by only a few hundred meters. Firstly, limited dispersal of asexual propagules may prevent clonal propagules from moving between neighbouring habitats. While it is clear from the fine-scale surveys of individual rock pools and boulders that the majority of asexual propagules recruit close to the brood parent, I did detect some clones spread among sites that were separated by up to 100m within a habitat. It therefore appears that clones can potentially disperse between these two habitats. However, both at Cape Banks and Bass Point, there were small areas (2 - 15m wide) of unsuitable shore that were completely devoid of anemones found between the two main habitat types. These small areas of unsuitable shore may be large enough to act as an effective barrier to dispersal between these two habitats.

Secondly, selection for different genotypes within each habitat type may occur, such that each habitat contains a set of distinct and locally adapted clones. Previous reciprocal transplant experiments have demonstrated that clones of *A. tenebrosa* can be locally adapted to conditions within headlands separated by up to just a few kilometres (Ayre 1985, 1995). The boulder and rock pool habitats targeted in this study differ dramatically in their biological, chemical and physical properties, thereby potentially providing different selective regimes for particular clones. Within the boulder habitat, the majority of anemones are found on the underside of boulders. This offers protection from desiccation and the potential harmful effects of high UVB radiation during periods of emersion on hot summer days. Conversely, on cold winter nights anemones exposed at low tide will have to cope with low temperatures. Anemones within rock pools may to some extent be buffered

by the large fluctuation in temperatures due to the high specific heat capacity of water, although temperatures may still increase dramatically on hot summer days and anemones will have to be tolerant of large fluctuations in salinity (high on hot days and near zero salinity during rainy periods). Additionally, anemones on the underside of boulders may have different metabolic processes for storing nitrogenous waste products that need to be stored during low tide. In contrast, anemones within tidal rock pools will not have to store nitrogenous wastes for such long periods and are also able to continue to respire until oxygen concentrations within rock pools fall too low. Physiological variation among different clones of other sea anemones have been reported (*Haliplanella lineata*, McManus *et al*. 1997).

The community composition of rock pool and boulder habitats is also quite different and hence biotic interactions may be quite different. Few, if any, macroalgae can be found on the underside of boulders but are often a dominant member within rock pool communities. As such, the communities associated with such macroalgae are also absent or rare on the underside of boulders (personal obs.).

While only a few studies of mainly sexually reproducing marine taxa have shown local adaptation to fine-scale habitat variation, much more work on local adaptation to microhabitat variation has been demonstrated in a number of clonal plant species (Linhart and Grant 1996; McManus *et al.* 1997; Lenssen *et al.* 2004). Local adaptation of clones to the parental habitat patch is also consistent with predictions based on life history theory for organisms with the potential to reproduce both sexually and asexually. Such mixed reproductive strategies often involve the use of genetically diverse sexual recruits for dispersal away from the parental habitat and asexual reproduction of locally adapted genotypes for the restocking of the parental habitat patch (Williams 1975; Bell 1982). However, such simplified views of the roles of sexual and asexual reproduction may not hold for some clonal organisms and a lack of genotypic differentiation and clonal structure has been reported for at least one clonal scleractinian coral species on the Great Barrier Reef (Chapter 2, Ayre and Miller 2004).

If the genotypic differentiation of anemones from boulder and rock pool habitats that I detected is indeed the result of selection, further studies that test for ecological difference between such clones are needed. Reciprocal transplant experiments offer one of the most promising experimental methods for testing for fitness differences between individuals from different environments with contrasting selection pressures (Ayre 1985, 1995; Bennington and McGraw 1995; Linhart and Grant 1996; Joshi *et al.* 2001; Hamerlynck *et al.* 2002).

5.4.4 Sources of Genotypic Diversity

My analysis on the source of genotypic diversity within single rock pools or on individual boulders indicates that the vast majority of genotypic diversity within populations of *A. tenebrosa* is the result of random sexual reproduction, although we cannot rule out the possibility that somatic mutation makes some small contribution to overall levels of genotypic diversity. Microsatellite markers may yield higher levels of mutation rates than functional gene markers, however, their value in identifying functionally different clones that have arisen by somatic mutation is likely to be limited as such variation is expected to be neutral. A number of studies have shown that microsatellites may be widespread in the genome (Condit and Hubbell 1991; Wu and Tanksley 1993), but there is little information regarding whether or not they are functionally important. Other molecular markers that survey a random sample of the genome and that include functionally important regions, (such as AFLP's) or target known functionally important genes (i.e. MHC) or gene combinations (i.e. QTL's), may offer a better means of identifying functionally different clones that have arisen from somatic mutation. While I was not able to identify a large number of clonal lineages that had potentially arisen from somatic mutation, this does not mean that somatic mutation is not an important source of genotypic diversity within populations of *A. tenebrosa*. It is possible that the variation detected within these populations results from a combination of somatic mutations and sexual reproduction.

The mode of production of clonal propagules will have an important effect in the expression and inheritance of mutations. For example, in parthenogenetic varieties, propagules develop from a single egg cell, so that any mutations in the genome of this cell

are passed on to all other cells during embryonic development. As such, this mutation can exert a major phenotypic effect and will also be inherited in the next generation. In contrast, propagules derived from a group of cells (such as budding, fragmentation or fission), mutation in a single cell will have limited spread in the cell population of the body, and may therefore be inherited unequally in the next asexual generation. This can result in organisms that are a mosaic of different cell types (i.e. intra-organismal genetic heterogeneity) with the degree of phenotypic effect dependent on the proportion of cells within the organism that have the new mutation (Hughes 1989; Pineda-Krch and Lehtila 2004).

Chapter 6 Local Adaptation of the Clonal Sea Anemone *Actinia tenebrosa* to Fine-Scale Environmental Variation

6.1 Introduction

In spatially heterogeneous environments, organisms that are sedentary or have restricted mobility could display adaptation to fine-scale environmental variation. However, sexual reproduction with widespread dispersal of propagules, coupled with temporal variation in environmental conditions, may limit the extent of adaptation to typical or average conditions at a site, even in the face of very strong selection (Hedgecock 1986; Slatkin 1987; Underwood and Fairweather 1989). This is because the combined effects of genetic recombination and assortment during sex are sufficient to breakdown site-specific adaptation each generation, although several reports of fine-scale adaptation (especially to conditions prevailing at the time of settlement) have been described (Johnson and Black 1982; Johnson and Black 1984). Surprisingly, many marine organisms have highly philopatric dispersal and have great potential for site-specific adaptation as multiple generations may compete within relatively static conditions. This includes a range of taxa with crawling larvae, direct development and localised settlement (Olson 1985; Grosberg 1987; Hellberg 1994; Miller 1998).

Although there is a large and growing body of empirical evidence of local adaptation to fine-scale environmental heterogeneity in plants (Linhart and Grant 1996) and some sexually reproducing marine taxa with restricted dispersal (Koehn *et al.* 1980; Yamada 1989; Johannesson *et al.* 1993; Rolan-Alvarez *et al.* 1997; Hughes and Taylor 1997; Riginos and Cunningham 2005), few tests for local adaptation have been carried out on clonal marine organisms. The potential for local adaptation to fine-scale environmental variation should be especially great for the many intertidal organisms with a complex life-cycle that involves the use of outcrossed sexual reproduction to generate diverse sets of colonists, followed by periods of asexual proliferation and prolonged inter-genotypic competition that should further reinforce local adaptation (Williams 1975). In such species it is expected that each habitat patch should be dominated by a small number of

numerically dominant and locally adapted genotypes (Williams 1975). Many sessile marine species lend themselves well to experimental manipulation enabling the fitness of different clones can be assessed under controlled experimental conditions (e.g. Ayre 1995; McManus 1997).

While environmental heterogeneity may lead to differential selection for locally adapted genotypes, some species appear to respond to environmental variation through phenotypic plasticity (Appleton and Palmer 1988; Palmer 1990; Gibbs 1993; DeWitt *et al.* 1998; Yeap *et al.* 2001). Reciprocal transplant experiments provide one of the most powerful methods for distinguishing between the effects of local adaptation and phenotypic plasticity (Yeap *et al.* 2001). If fitness traits of individuals from different environments with contrasting selection pressures are compared in reciprocal transplant experiments, individuals in their local habitat are likely to outperform less well-adapted individuals introduced from other sites (e.g. Ayre 1985; Yamada 1989; Ayre 1995; Bennington and McGraw 1995; Rolan-Alvarez *et al.* 1997; Joshi *et al.* 2001). However, if native and introduced individuals perform equally well in the same habitat, this suggests that individuals show phenotypic plasticity in response to different environmental conditions (Valverde and Pisanty 1999; Lorenzon *et al.* 2001; Hamerlynck *et al.* 2002).

One of the few examples where local adaptation has been shown to occur in a clonal marine organism is the intertidal sea anemone, *Actinia tenebrosa*. Reciprocal transplant experiments carried out between adjacent headlands (2-3 kilometre distance) and over larger regional scales (1000's kilometres) have demonstrated evidence of localised adaptation (Ayre 1985, 1995). In these studies Ayre (1985, 1995) found that native anemones were consistently fitter (as judged by survivorship and asexual fecundity) than imported anemones. Differences in fitness were greatest for anemones transplanted among distant rather than adjacent headlands and in trials that permitted direct interaction between native and imported individuals. However, more recent genetic studies using a combination of microsatellite and allozyme loci, have demonstrated fine-scale segregation of genotypes within a headland that may result from selection within different habitats and/or limited dispersal of asexual propagules (Chapter 5). In that study I found that in

general different clones occupy different habitats within the same rocky headland and typically form clonal aggregations within each habitat (although some individuals of a clone may be found spread across a habitat) (Chapter 5). This genotypic differentiation between habitats, separated by as little as 200m, may simply reflect the limited dispersal of asexual propagules, or may reflect the effects of differential selection of clones. This means that local adaptation may be occurring over much finer scales than has previously been established. A limitation of previous studies was that fine-scale reciprocal transplants that allow interaction between native and transplanted anemones could only be carried out for samples collected from different geographic regions (i.e. New South Wales and Victoria, Ayre 1995), which display different phenotypes at the glucose phosphate isomerase locus and thus allowed genetic identification of native and foreign anemones within mixed sites. However, insufficient fine-scale variability of allozyme markers and the inability to non-destructively determine the genotype of individuals prior to transplantation means that tests for local adaptation to fine-scale environmental heterogeneity (i.e. to sites within a habitat) have been limited. The recent development of microsatellite markers for *A. tenebrosa* (Mitchellson and Ayre, unpublished) that allow for greater fine-scale resolution of the distribution of genotypes and patterns of genotypic diversity (Chapter 5) means that fine-scale reciprocal transplants that allow direct competition between native and foreign anemones can now be carried out among sites within a habitat.

In order to test for evidence of local adaptation to fine-scale environmental variation I use a combination of reciprocal transplant experiments and molecular markers to follow the fate of individuals of *A. tenebrosa* transplanted over two spatial scales. Firstly, I carried out within-habitat reciprocal transplants among neighbouring rock pools separated by < 30 m on a single rocky shore in which native and foreign anemones were allowed to interact. Secondly between-habitat reciprocal transplants were conducted between boulder and rock pool within habitats at two locations to test for evidence of local adaptation to different habitat patches separated by < 200 m. Fitness of native and foreign groups of anemones, as judged by survival, growth and asexual fecundity, were used to test for evidence of site-specific adaptation.

6.2 Methods

6.2.1 Collection and Re-Attachment of Anemones for Transplants

I collected anemones by gently levering individuals off the substratum and scraping their pedal discs clean of any material to facilitate complete reattachment after transplantation. Any anemones that had suffered damage to their pedal discs during removal or cleaning were discarded and only adult anemones (i.e. >10mm pedal disc diameter) were used for transplants.

Anemones were transplanted at low tide and placed within sites previously occupied by anemones, with each site receiving anemones at approximately the same initial density found at that site (see below for details). All anemones were allowed a minimum of three hours to reattach before being exposed to the incoming tide, except in the case of anemones transplanted onto boulders. These anemones were reattached by encasing groups of 20-30 individuals in nylon mesh (pore diameter 0.5 mm) that was then fixed to the underside of a boulder using a marine epoxy resin (Zspar ®) and left for two full tidal cycles before the mesh was removed.

I allowed transplanted anemones to establish themselves for three weeks after transplantation to a new site before any initial fitness measurement were taken. Anemones that remained attached to the substratum after this three-week period were considered to have been transplanted successfully.

I cleared a one-metre wide buffer zone around each transplant site to ensure that anemones from surrounding areas did not migrate into the experimental sites. Sites were monitored monthly to maintain the one-metre wide buffer zone and to remove any recruits that had settled within the experimental sites. The removal of recruits was necessary to allow for the identification of transplanted anemones at the conclusion of the experiment.

6.2.2 Transplant Design

6.2.2.1 Within-Habitat Transplants

Within-habitat reciprocal transplant experiments were initiated in February 2002 among three neighbouring rock pools on a rocky intertidal platform at Bellambi, NSW, Australia. At low tide these rock pools are discrete with no connections to other rock pools and ranged in size from 30 to 85 litres. Distances between pools varied from three to thirty meters and each pool contained similar numbers of anemones (between 54 and 78 individuals).

I removed all anemones from each of the three rock pools, and all intact anemones suitable for transplantation were placed into separate zip-lock bags 1/3 filled with seawater and stored for up to 48 hours at 4ºC before transplantation. Prior to transplantation a small tissue sample (one to two tentacles) was collected from each anemone for microsatellite and allozyme genotyping analysis to enable me to determine the origin of surviving anemones at the end of the experiment.

Half the anemones from each pool were then randomly assigned to one of two treatment groups. Native groups of anemones consisted of between 27 and 35 adults that were transplanted back into their original pool. Foreign groups of anemones consisted of between 25 and 39 adults and were transplanted into a different pool from their pool of origin. Each rock pool therefore received half native and half foreign anemones at approximately equal densities. The experiment was allowed to run for 13 months before all anemones were removed for re-genotyping and evaluation of fitness traits (see below for details).

To determine the origin (i.e. native or foreign) of surviving anemones, all transplanted anemones were genotyped at four microsatellite and four allozyme loci at both the beginning and conclusion of the experiment. Of the 208 individuals used in the within-habitat transplant experiment, 46 unique genotypes were detected, most of which (76%) were unique to a particular rock pool. Only one genotype was detected that was common to all three pools and nine genotypes that were common to two of the three rock pools. As the allocation of individuals to native and introduced treatments was random, at the

conclusion of the experiment there were only 2 individuals from pool 1 and four individuals from rock pool 2 whose origin could not be determined with 100% confidence. These individuals were excluded from any further analysis.

Methodology for DNA extraction and amplification of microsatellite loci (*At1*, *At5*, *At21a* and *At38*) were carried out as described in Chapter 4. Tissue extracts and enzyme assays were the same as previously describe by Ayre (1982). Variation was consistently resolvable for four polymorphic enzymes. All enzymes were electrophoresed on a Tris-Citrate buffer (pH 8.0) and stain recipes followed Harris and Hopkinson (1976). Enzymes assayed were glucosephosphate isomerase (GPI*,* E.C. 5.3.1.9), 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44); malate dehydrogenase (MDH, E.C. 1.1.1.37); and octopine dehydrogenase (ODH, E.C. 1.5.1.11).

6.2.2.2 Between-Habitat Transplants

Between-habitat reciprocal transplants were initiated in February 2001 at two locations, Cape Banks and Bass Point, NSW, Australia. These two locations supported easily identifiable and discrete boulder and rock pool habitats (e.g. Figure 6.1) separated by no more than 200m. Transplant sites consisted of either a discrete rock pool (1.5 – 30 litres) or group of 1- 4 boulders (0.3 –0.8m diameter) containing between 50 and 85 anemones. The number of suitable sites within each habitat varied between locations with 6 and 10 suitable transplant sites within each habitat identified at Cape Banks and Bass Point respectively. Distances between sites varied from three to ten metres.

All anemones from a site were removed and transplanted as a single group. This differed from the fine-scale transplant design in that native and foreign anemones were not allowed to interact directly and meant that genetic identification of anemones was not required at the conclusion of the experiment as each experimental site contained anemones from only one of the three treatment groups. Each site served as both a source of anemones for transplantation and as a location for final transplants. At each location, sites within a habitat were randomly assigned to one of two treatment groups (three sites per treatment per habitat at Cape Banks and five sites per treatment per habitat at Bass Point). A native treatment group consisted of groups of anemones that had been removed from a site within

a habitat and then randomly assigned back to a site within the same habitat and were effectively a procedural control group. A foreign treatment group consisted of anemones that were removed from a site within a habitat and then randomly assigned to a site in the other habitat.

Figure 6.1 Rock pool and boulder habitat seperated by < 200 meters at Cape Banks, NSW, Australia.

In order to control for any effects from handling of anemones, additional undisturbed sites were also established within each habitat at both locations. At Cape Banks there were three undisturbed sites per habitat and at Bass Point there were five undisturbed sites per habitat. These sites consisted of anemones that had not been manipulated or disturbed in anyway, however, due to the limited number of suitable transplants sites at Bass Point these sites were typically smaller and contained on average fewer anemones than other experimental sites (18 - 39 *cf.* 50 – 85) and therefore were limited in their comparability with other experimental sites.

In addition to the one-metre cleared buffer zone around each site, a number of areas of approximate equal size to transplant sites within each habitat at each location were completely cleared of all anemones. These sites were used to assess the extent to which anemones from the surrounding areas moved across the one-metre buffer zone and into experimental sites. Throughout the entire experimental period only nine incursions of non-experimental anemones into the buffer zones surrounding each site were detected, seven for

the within-habitat and two for the between-habitat transplants respectively. There were only two incursions of anemones into the completely cleared sites. I therefore decided that the low numbers of incursions into cleared sites and buffer zones would have a negligible effect on experimental results given the large number of anemones transplanted into each experimental site (50-80 anemones per site).

The experiment ran for 23 months and terminated in February 2003. On completion of the experiment all surviving anemones were removed from each site and brought back to the laboratory where fitness traits were measured and compared.

6.2.3 Measurement of Fitness

The relative fitness and hence degree of local adaptation is often difficult to measure for many organisms. The fitness of an organism is typically quantified and expressed as the relative contribution of a given genotype to the subsequent generation relative to that of other genotypes. Given the rarity of sexual events and the apparent lack of female individuals within some populations on the east coast of Australia (see results) and the failure to detect any sexual recruitment into Western Australian populations during the preceding 22 years (Ayre unpublished data) measures of sexual reproductive output or success would be difficult, if not impossible to measure for *A. tenebrosa*. Additionally, measures such as the age of first reproduction (sexual or asexual) and lifetime reproductive success are difficult to assess for *A. tenebrosa* due to the great longevity of clones and our inability to determine the age of individual anemones (Ottaway 1979b; Ayre 1984a). I therefore used three measures of fitness that seemed the most relevant short-term measures for assessing the relative performance of native and foreign treatment groups and that have previously been shown to be good indicators of the relative success of transplanted anemones (Ayre 1985, 1995).

6.2.3.1 Survivorship

I assessed the proportion of anemones surviving at the conclusion of each transplant experiment by comparing the initial number of anemones transplanted with the final number of anemones surviving for each site.

6.2.3.2 Asexual Fecundity

Asexual reproduction in *A. tenebrosa* has been shown to follow an annual cycle with maximum fecundity occurring around early to mid-summer (Ottaway 1979a; Ayre 1984a). To ensure that asexual fecundity was assessed at or near its peak, and to ensure that there were no phase shifts in the reproductive cycle between habitats, asexual fecundity was monitored monthly throughout the experimental period in each habitat at Cape Banks and Bass Point.

I collected samples of 15 – 30 individuals from outside the experimental transplant sites each month over an 18 month period and determined the number of juveniles within each brooding adult by dissection. Additionally, sex ratios were noted during those summer months when mature gonads were present in some individuals, and which allowed the identification of male, female, and non-sexually reproductive polyps (Ottaway 1979a; Ayre 1984a; Ayre 1988). Monthly patterns of asexual fecundity were not monitored at Bellambi as this location was geographically situated between Cape Banks and Bass Point and it was assumed that these populations would be at approximately the same stage in their breeding cycles. This assumption is supported by previous studies that have reported that the timing and duration of the breeding cycle are synchronous throughout a large part of this species' range (Ottaway 1979a; Ayre 1984a, 1995). Based on this monthly data, both the within- and between-habitat transplant experiments were terminated in February 2003 when the proportion of brooding adults began to peak at Cape Banks and Bass Point simultaneously.

I assessed differences in asexual fecundity between treatment groups. These included the proportion of individuals brooding at the end of the experiment, the number of juveniles produced per site, and the number of juveniles produced per brooding adult. These were assessed by dissection of collected anemones at the end of the experimental period.

6.2.3.3 Change in Size

I determined the change in size of anemones by measuring the pedal disc diameter (to the nearest mm) of all successfully transplanted anemones to obtain a mean size for each group at the start, and then again at the conclusion of the experiment. Change in mean size may potentially be used as a measure of growth, however, caution in interpretation of such data needs to be taken as other factors such as the differential survival of small or large anemones may result in a significant change in mean size. Additionally, growth is not known to be a particularly good indicator of fitness in soft bodied organisms due to high levels of variability in size over short periods of time (i.e. up to 10% variation in size during a single tidal cycle, Ottaway, 1980), but was included here as a measure of fitness due to the relatively large numbers of individuals used within each experimental treatment. As anemones are soft bodied organisms they can have positive growth (i.e. increase in size) when conditions are favourable and negative growth (i.e. decrease in size) when conditions are unfavourable (Ottaway 1980; Ayre 1984a). Anemones were measured in the laboratory (except for undisturbed anemones which were measured in the field). All water within the coelenteron was expelled by gently applying pressure to the body wall and excess water as removed using absorbent paper towel. The pedal disc diameter was measured to the nearest mm and a mean of the longest and shortest diameters was used for individuals whose pedal disc was not uniformly round.

6.2.4 Statistical Analysis

Fitness response variables (survivorship, proportion of adults brooding, mean number of juveniles per site, mean number of juveniles per brooding adult, and growth) were compared for native and foreign treatment groups using a General Linear Model (GLM). Analyses were performed using the univariate analysis option in the statistical software program SPSS (V12). Significance tests were based on type III sum of squares and data required no transformation prior to analysis to satisfy the assumptions of normality and homogeneity of variances. Separate analyses were carried out for each of the within-habitat and between-habitat transplant experiments. Terms fitted to the model included location (random), habitat (fixed), treatment (fixed) and a habitat × treatment interaction term.

Tukey's pairwise comparisons of treatment means were done in cases where significant differences due to main effects were found in the between-habitat experiment but were not necessary for the within-habitat experiment due to only two treatment groups.

6.3 Results

6.3.1 Initial Survival of Transplanted Adults

Initial establishment success was high both for the within-habitat and between-habitat transplant experiments. For within-habitat transplants, 86 ± 11% (mean ± SE) of anemones survived the initial three-week establishment period. Therefore collection of a small tissue sample for genetic analysis before transplantation did not appear to adversely affect the viability of transplanted anemones with initial survival slightly higher for the within-habitat compared with the between-habitat transplants (77 ± 3% *cf.* 84 ± 4%). For between-habitat transplants initial establishment success was high at both locations and within both habitats (Cape Banks: Boulder = 86 ± 4%, pool = 85 ± 4%; Bass Point; Boulder = 83 ± 3%, pool = 82 ± 5%).

6.3.2 Patterns of Reproduction and Sex Ratios

Patterns of asexual reproduction followed a distinct seasonal trend with the highest proportion of brooding individuals detected during the mid-summer months (December-February) (Figure 6.2 a & b). This trend was consistent across locations, habitats and seasons. At the height of the reproductive period (January and February) on average 66 ± 4% (SE) and 55 ± 4% (pooled for locations) of adults were brooding within the rock pool and boulder habitats respectively. This dropped off during the winter periods (April-October) to an average minimum of 25 ± 4% (SE) and 27 ± 3% of adults brooding within the rock pool and boulder habitats respectively. This means that while *A. tenebrosa* has a reproductive peak during the summer months, some still continue to brood juveniles throughout the winter period. The monthly monitoring of asexual fecundity over the 17 month period enabled me to terminate transplant experiments when the proportion of brooding adults was at its height during 2003 (January / February), giving the best estimate of asexual fecundity.

Patterns of sexual maturity followed a distinct seasonal cycle, slightly out of phase with that observed for asexual reproduction (Figure 6.2 c & d). This trend was consistent across locations habitats and seasons, with a peak in the proportion of individuals with mature gonads greatest during the spring period (September to December). Surprisingly, out of the

total of 346 individuals identified with mature gonads sampled from 580 individuals during the two reproductive seasons (i.e. October to December 2002 and September to December 2003), were all identified as males and not a single female was detected within any of my samples. At the height of the sexual cycle (September to December) between 50 and 80% of individuals were identified as sexually mature males (Figure 6.2 c & d), with the remaining individuals identified as non-sexual or immature individuals for which their sex could not be determined.

6.3.3 Within-Habitat Transplants

6.3.3.1 Survival

Overall 73 ± 4% of all anemones used in the within-habitat transplants survived to the end of the experimental period. Microsatellite and allozyme electrophoretic identification of survivors was used to identify if they were native or foreign anemones. Native anemones performed only slightly better than foreign anemones within all three rock pools. Mean (± SE) survival of native anemones was 77 ± 5% compared with 68 ± 5% for foreign anemones (Figure. 6.3). However, mean survival was not significantly different between native and foreign treatment groups ($F_{1,\ 0.01}$ = 11.79, P = 0.075).

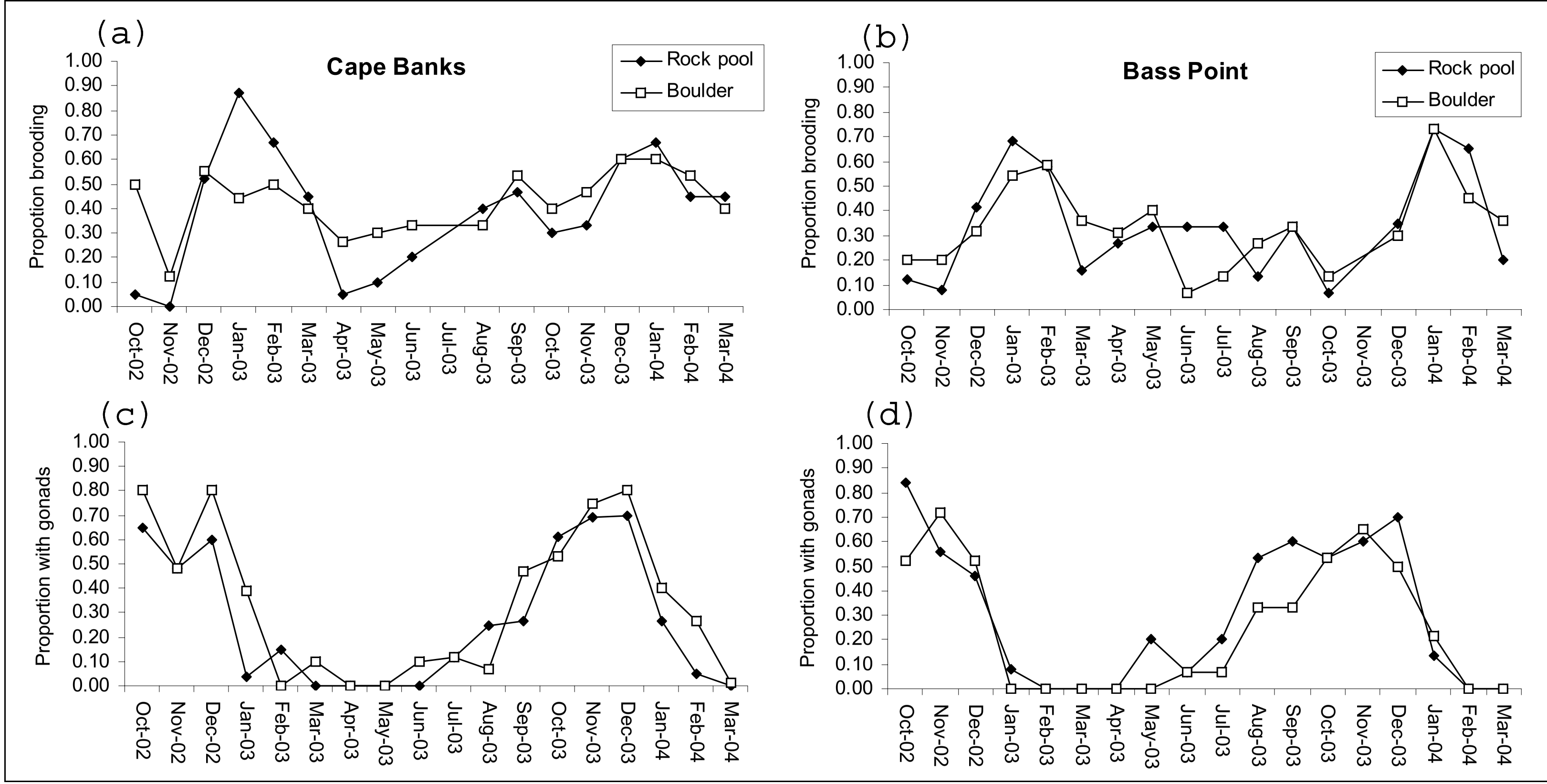

Figure 6.2 Seasonal patterns of sexual (gonad formation) and asexual (proportion brooding) reproduction in *Actinia tenebrosa* collected from rock pool and boulder habitats at two locations along the NSW coast, Australia.

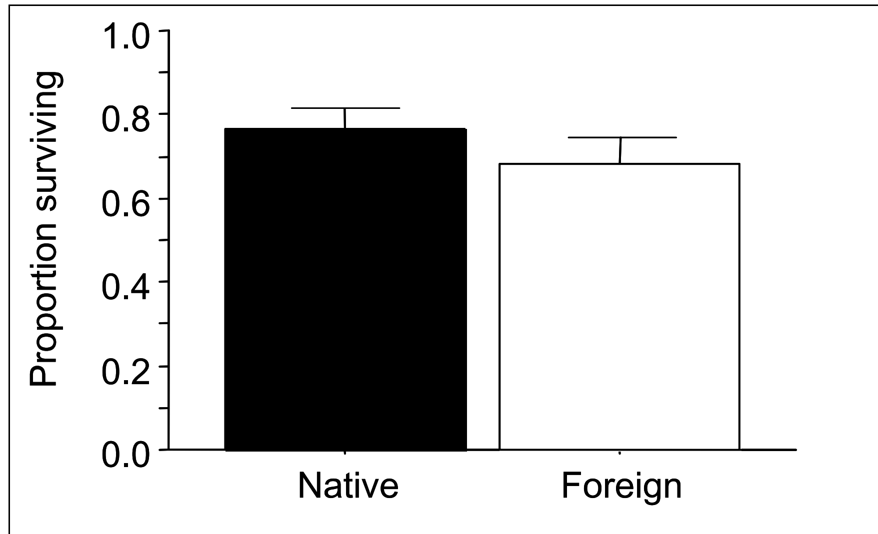

Figure 6.3 Mean (± SE) proportion of *Actinia tenebrosa* surviving after reciprocally transplanted among three rock pools (i.e. <30m) within a single rocky headland. Each pool received half native and half foreign anemones.

6.3.3.3 Asexual Fecundity

Overall I found no significant difference in the proportion of anemones brooding, the number of juveniles produced per brooding anemone, or the mean number of juveniles produced per site for native and foreign groups of anemones for the within-habitat transplants.

The mean (± SE) percentage of native anemones brooding was 45 ± 6% compared to 53 ± 10% for foreign anemones (Figure 6.4). Overall there was no significant difference in the proportion of adults brooding between the native and foreign treatment groups ($F_{1, 0.009}$ = 0.40, P = 0.592) (Figure 6.4).

The mean number of juveniles per brooded adult was almost twice as great in native anemones than in foreign anemones (6.5 ± 2.19 *cf.* 3.7 ± 0.52). However, due to the large variability among pools, no significant difference was detected between the two treatment groups ($F_{1, 12.2}$ = 1.238, P = 0.382) (Figure 6.4b). The mean number of juveniles produced by native anemones per site was again almost twice that produced by foreign anemones (81

± 37% *cf.* 42 ± 12%), although again this difference was not significant due to the large variability among pools ($F_{1, 2281}$ = 0.801, P = 0.465) (Figure 6.4c).

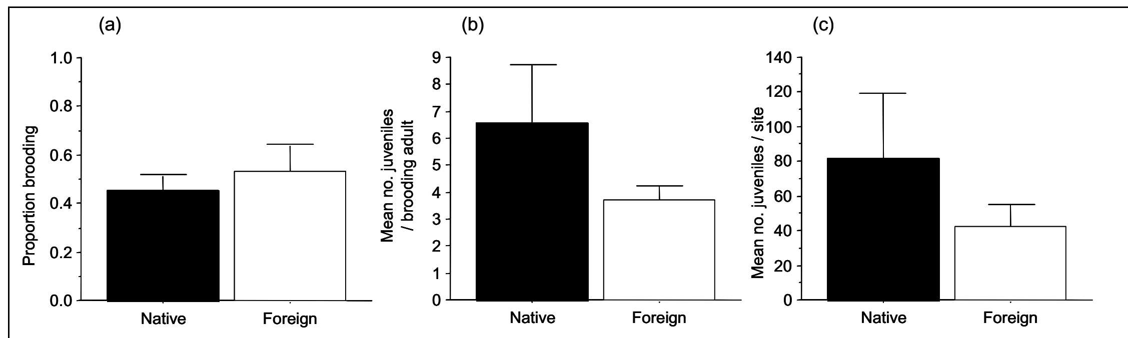

Figure 6.4 Mean (± SE) measures of asexual fecundity for anemones reciprocally transplanted among three rock pools (i.e. <30m) within a single rocky headland. Each pool received half native and half foreign anemones and measures of asexual fecundity determined at the peak of asexual reproductive season. (a) Mean proportion of adults brooding asexual juveniles, (b) mean number of asexual juveniles produced per brooding adult, (c) mean number of asexual juveniles produced per site.

6.3.3.2 Change in Size

There was little difference in the initial sizes of anemones within each of the three rock pools and consequently native and foreign groups of anemones at the start of the experimental period had very similar mean sizes (± SE) (native = 15.9 ± 2.2mm, foreign = 16.7 ± 2.0mm). At the end of the experimental period both native and foreign treatment groups showed a slight increase in size (native = 17.9 ± 1.5mm, foreign = 18.5 ± 1.9mm) although there was no significant difference between native and foreign treatment groups ($F_{1, 0.029}$ = 0.007, P = 0.942) (Figure 6.5).

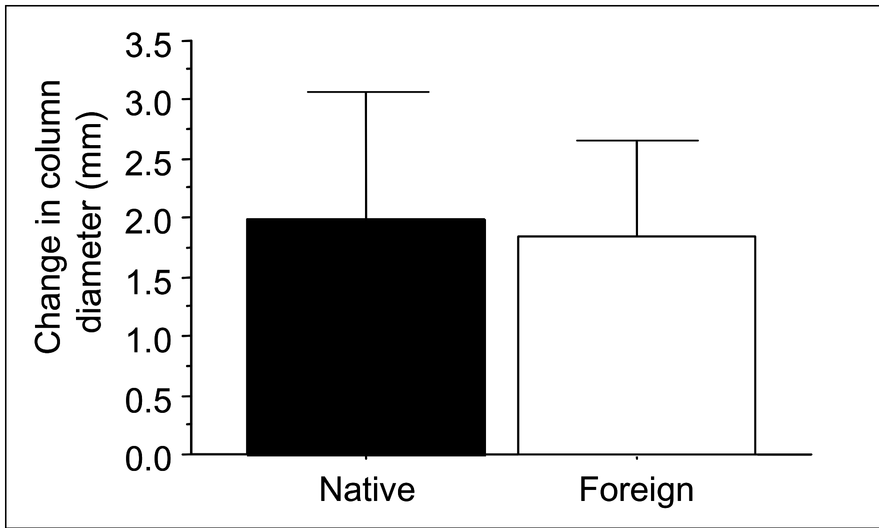

Figure 6.5 Mean (± SE) change in size (as determined by initial and final column diameter) for native and foreign groups of the sea anemone *Actinia tenebrosa* reciprocally transplanted among three rock pools (i.e. <30m) within a single rocky headland. Each pool received half native and half foreign anemones.

6.3.4 Between-Habitat Transplants

6.3.4.1 Survivorship

There was a significant difference in survival among treatment groups, with foreign anemones significantly less viable compared with native or undisturbed anemones ($F_{2,\,0.298}$ = 9.58, $P < 0.001$). This pattern was most striking at Cape Banks where survival of native anemones was 80 ± 5% compared with only 24 ± 7% for foreign anemones within rock pools, and 69 ± 16% and 42 ± 13% for native and foreign anemones on boulders (Figure 6.6). At Bass Point native anemones showed greater survival compared with foreign anemones within the rock pool habitat (native = 89 ± 5% *cf.* foreign = 66 ± 7%), however, foreign anemones showed slightly higher survivorship than native anemones within the boulder habitat (native = 78% ± 7% cf. foreign = 87% ± 6%). Survival of undisturbed anemones was consistently greater than foreign anemones except within the boulder habitat at Bass Point where survival was similar for all treatment groups. This resulted in a significant habitat × treatment interaction ($F_{2,\,0.116}$ = 3.72, $P = 0.033$) indicating a site effect and was driven by the lack of difference between treatment groups within the boulder habitat at Bass Point (Figure 6.6).

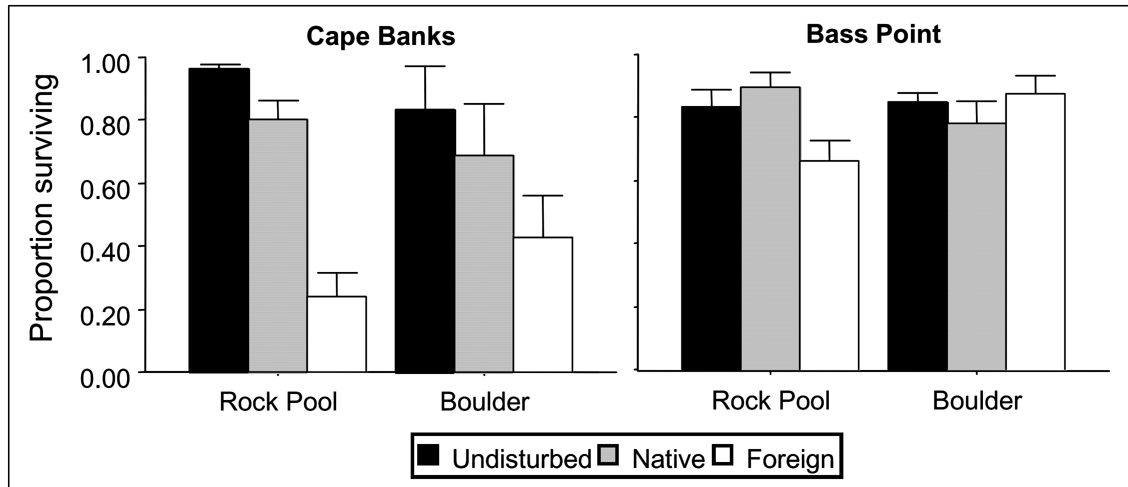

Figure 6.6 Mean (± SE) proportion of adult *Actinia tenebrosa* surviving when reciprocally transplanted among habitats (<200m) within a rocky headland at two locations.

6.3.4.3 Asexual Fecundity

The proportion of adults brooding at the conclusion of the experiment varied significantly among treatment groups ($F_{2, 0.139}$ = 3.12, P = 0.05). Significantly greater proportions of native *versus* foreign anemones were found to be brooding juveniles (Tukeys pair-wise test, P = 0.043). This result is most striking for native anemones within boulder habitats (for both Cape Banks and Bass Point) where on average 62 ± 5% of native anemones (pooled for locations) were brooding compared to only 35 ± 8% of foreign anemones (Figure 6.7a). Within the rock pool habitats native anemones again out-performed foreign anemones although the magnitude of the difference was smaller than that seen for the boulder habitat (local = 59 ± 6% *cf.* 49 ± 8%). There was no effect of habitat or any habitat × treatment interaction on the proportion of anemones brooding.

The mean number of juveniles produced per brooding adult was highly variable and no significant differences were detected between treatment groups ($F_{2, 12.6}$ = 0.56 P = 0.57) (Figure 6.7b). The mean number of juveniles per site (i.e. per pool or group of boulders) was highly variable, however, foreign sites produced significantly fewer ($F_{2, 14039}$ = 3.90, P = 0.028) juveniles compared to sites containing native anemones (Figure 6.7c).

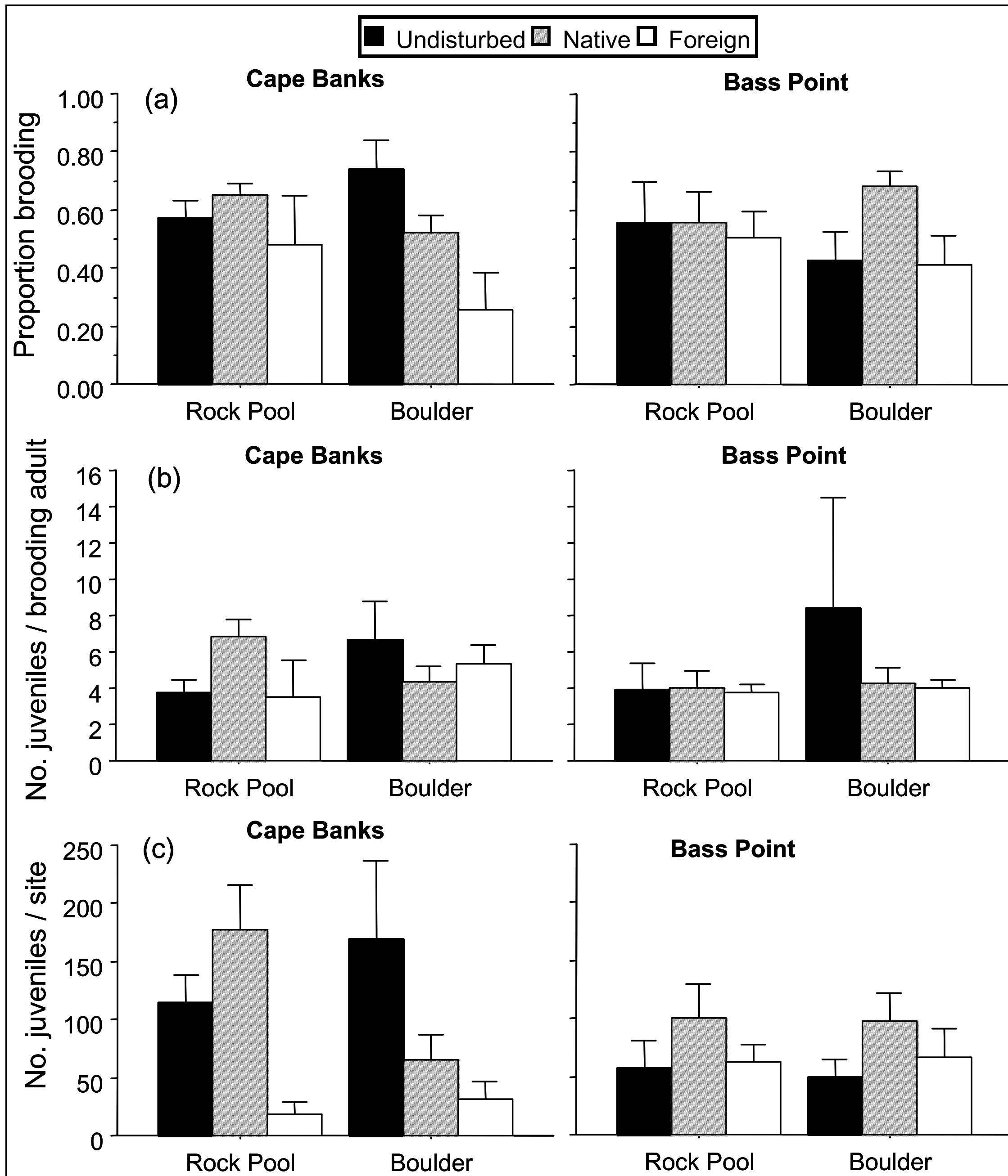

Figure 6.7 Mean (± SE) measures of asexual fecundity for *Actinia tenebrosa* reciprocally transplanted between rock pool and boulder habitats (separated by < 200m) at two locations. Measures of asexual fecundity determined at the peak of asexual reproductive season. (a) Mean proportion of adults brooding asexual juveniles, (b) mean number of asexual juveniles produced per brooding adult, (c) mean number of asexual juveniles produced per site.

6.3.4.2 Change in Size

Initial mean sizes of anemones were relatively homogenous across locations and habitats (Cape Banks: pools = 19.5 ± 5mm, Boulders = 22.4 ± 6mm; Bass Point: pools = 19.5 ± 5mm, Boulders = 21.3 ± 6mm). Growth was highly variable across all groups of anemones (Figure 6.8), however, I detected a significant treatment effect with local anemones consistently outperforming foreign anemones ($F_{2, 21.08} = 5.59$, $p = 0.007$). At Cape Banks native anemones transplanted back into their habitat of origin showed an increase in column diameter (rock pool = 0.64 ± 0.50 mm and boulder = 1.12 ± 0.25 mm) while foreign anemones showed a decrease in column diameter (rock pool = -2.50 ± 0.80 mm and boulder = -3.28 ± 1.29 mm). At Bass Point both local and foreign anemones showed negative growth (i.e. a decrease in column diameter). The decrease in column diameter was significantly larger for foreign anemones (rock pool = -3.22 ± 0.65 mm and boulder = -2.94 ± 0.53 mm) compared to native anemones (rock pool = -1.64 ± 0.92 mm and boulder = -2.37 ± 0.58 mm). There was no significant effect of habitat ($F_{1, 3.68} = 0.976$, $p = 0.329$) or habitat × treatment interaction ($F_{2, 3.47} = 0.919$ $P = 0.407$).

Growth for undisturbed anemones showed mixed responses between locations. At Cape Banks undisturbed anemones showed positive growth in the boulder habitat (2.60 ± 1.64 mm) but negative growth within the rock pool habitat (-0.48 ± 0.37 mm), however, this negative growth was still much smaller in comparison with foreign groups of anemones (Figure 6.8). At Bass Point undisturbed anemones displayed higher levels of negative growth compared to both local and foreign anemones across rock pool and boulder habitats (Figure 6.8).

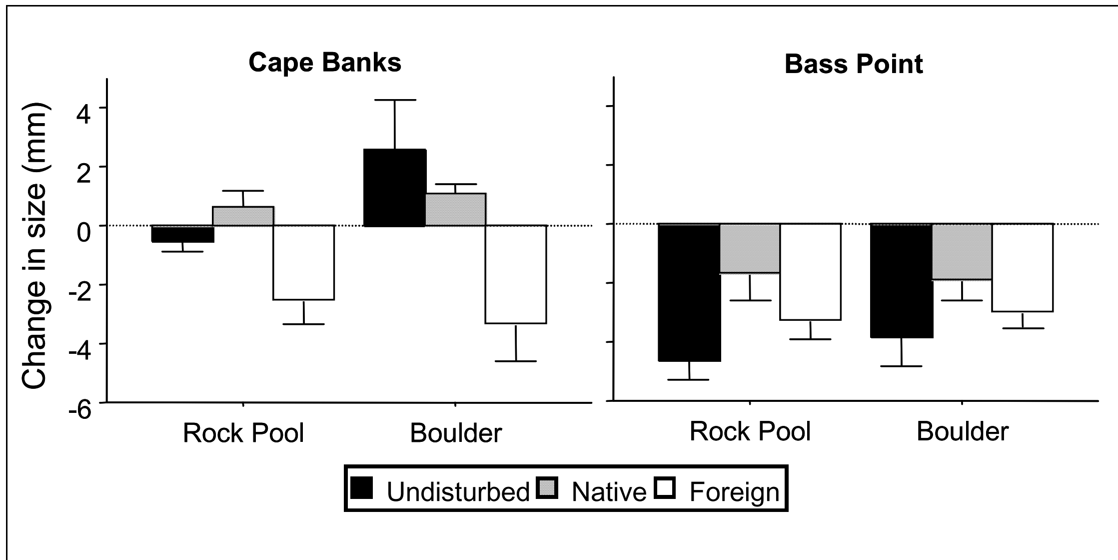

Figure 6.8 Mean (± SE) change in size (as determined by initial and final column diameter) for native and foreign groups of the sea anemone *Actinia tenebrosa* reciprocally transplanted between rock pool and boulder habitats (separated by < 200m) at two locations.

6.4 Discussion

6.4.1 Evidence for Local Adaptation to Fine-Scale Habitat Heterogeneity

Few studies of clonal marine organisms have used experimental approaches such as reciprocal transplant or common garden experiments to conclusively demonstrate local adaptation to fine-scale environmental heterogeneity. The results from this study provide some of the first such experimental evidence of local adaptation in response to fine-scale habitat heterogeneity in a clonal marine organism. My analysis of the fitness of native and foreign groups of anemones reciprocally transplanted between habitats on the same rocky shore (i.e. over distances of <200m) found significant differences in overall performance, with native anemones largely outperforming foreign anemones. However, I detected no significant home-site advantage for anemones transplanted between sites within a habitat (i.e. <30m), with foreign anemones performing equally well compared with native anemones.

These results confirm and extend those of Ayre (1985; 1995) who detected fitness differences (as judged by asexual reproductive output) between native and foreign groups of *A. tenebrosa* transplanted between adjacent headlands (i.e. 2 kilometres) and different geographic regions (1000's kilometres). Additionally, these results are also consistent with recent genetic surveys of genotypes of *A. tenebrosa* within and between boulder and rock pool habitats (Chapter 5). In these surveys I found that, with the exception of a single genotype, there was no overlap of genotypes between boulder and rock pool habitats on the same rocky shore. These findings, combined with the results from previous transplant studies, strongly suggest that sufficient environmental variation occurs between habitats within a headland for habitat-dependent selection to occur.

While it is generally thought that fine-scale adaptation of marine organisms is rare, the growing number of studies that have shown philopatric dispersal of sexual or asexual propagules of marine organisms (Jackson 1986; Miller 1998; Miller and Mundy 2003) and genetic differentiation over small spatial scales (Johnson and Black 1991; Hellberg 1994, 1996; Miller 1998; Miller and Ayre 2005), indicate that the opportunity for local adaptation may be much greater for marine organisms than has previously been recognised. Indeed,

evidence for local adaptation to fine-scale environmental heterogeneity has been inferred for the sexually reproducing marine snail, *Littorina saxatilis*, that has been shown to have different ecotypes from sites only a few meters apart, and is thought to be the result of limited dispersal of larvae and selection between lower and upper shore habitats (Johannesson *et al.* 1993; Rolan-Alvarez *et al.* 1997; Johannesson 2003; Perez-Figueroa *et al.* 2005); while genotype-environment interaction between foraging behaviour and habitat-specific shell morphology (which is known to be heritable) has been shown in the dogwhelk *Nucella lapillus* (Hughes and Taylor 1997). Clear fitness differences between clones of the byrozoan, *Celleporella hyalina*, from major habitat types have also been shown (Hughes 1992). Additionally, a number of studies have reported the attenuation of genotypic diversity and distortion of genotypic frequencies among settling recruits (Koehn *et al.* 1980; Johnson and Black 1984; Quicke *et al.* 1985). In these studies, post-settlement selection appears to severely alter allele frequencies of settled juveniles over fine-scales, indicating a strong and single bout of selection at an early stage. Such post-settlement selection is likely to be even more important for clonal marine organisms, as different episodes of asexual reproduction provide the opportunity for the same genotype to be exposed to numerous bouts of post-settlement selection during this sensitive life history stage and is likely to further reinforce the effects of local adaptation.

The scale of adaptation within the marine environment will be largely dependent on the intensity of selection within each habitat, the dispersal potential of propagules and the spatial separation between habitats. My within-habitat transplants were arguably the more sensitive of the two transplant experiments as these allowed direct inter-genotypic interaction and competition among clones from different sites within the same habitats. Inter-genotypic aggression and competition has been shown to be important in determining the distribution and extent of clones within populations of *A. tenebrosa* (Ottaway 1978; Ayre 1982, 1983b) and other clonal sea anemones (Ottaway 1978; Brace *et al.* 1979; Ayre and Grosberg 1995, 2005). Despite the potential for fine-scale adaptation mediated through inter-genotypic competition, I detected no statistical evidence of adaptation at this scale. However, the relative power of this experiment may have be low due to the lack of replicate sites and the relatively short duration of this trial (13 months *cf.* 24 months for

between-habitat transplants). While the use of molecular markers in this study were essential for the non-destructive identification of different clones within a site, the cost and labour intensive nature of genotyping a large number of individuals before and after the experiment means that this could only be done for a relatively small number of sites. Indeed, for the within-habitat transplant experiment the fitness of native anemones, as judged by survivorship, the number of juveniles produced per brooding adult, the number of juveniles per site and growth, were all slightly higher (but not statistically significant) compared with foreign anemones. Further studies that explore the potential for local adaptation of clones to within-habitat variation are clearly warranted and may become logistically more manageable as the cost of molecular techniques fall and increased throughput technologies allow for greater sample sizes.

While this study provides fairly strong evidence of local adaptation in response to habitat variation, I cannot completely rule out that the apparent greater fitness of native anemones may reflect the effects of prior acclimation or developmental canalisation (Waddington and Robertson 1966; Kerszberg 1989; Yeap *et al.* 2001; Piersma and Drent 2003; Huber *et al.* 2004). Transplantation of individuals into a new area may result in a physiological adjustment to local environmental conditions (acclimation). This is particularly relevant to reciprocal transplant experiments carried out between different latitudinal regions where there is likely to be marked differences in water temperature and/or water chemistry (McMahon 1996). The transplant experiments in this study were carried out over relatively small spatial scales (maximum distance between sites <200m) and while I cannot completely rule out any site-specific effects of prior acclimation, any differences in water temperature and chemistry were likely to be minimal. However, to reduce any effects of prior acclimation, I did allow transplanted anemones to establish themselves for three weeks after transplantation to a new site before any initial fitness measurements were taken. Additionally, experiments were allowed to run for between 13 and 24 months so that measures of fitness reflected at least one year's investment under local environmental conditions. Ayre (1995) tried to address the affects of prior acclimation in his transplant experiments by initially transplanting native and foreign groups of anemones to a common shore eight months before the start of the experiment. However, the result from this

experiment were similar to that of an earlier transplant experiment carried out over similar distances (Ayre 1985), indicating that prior acclimation is unlikely to greatly affect observed fitness difference between native and foreign treatment groups.

The effects of developmental canalisation (i.e. where a particular developmental trajectory is determined early on in an individuals development by local conditions) is however more problematic to assess (Waddington and Robertson 1966; Kerszberg 1989; Moller 1993; Wagner 1996) and is potentially a limiting factor of any transplant experiment involving adult individuals. In plants this has typically been addressed by using seeds for transplantation or in common garden experiments, rather than adult individuals (Kindell *et al.* 1996; Nagy and Rice 1997; Galloway and Fenster 2000). However, most marine organisms lack propagules whose development can be initiated as easily (i.e. the equivalent of inducing seed germination), and the only satisfactory way to address this issue would be to follow and compare the fitness of native and foreign recruits produced within each habitat after transplantation. As demonstrated in this study, the use of microsatellite markers allows for the non-destructive and unambiguous identification of transplanted genotypes, and therefore provides an ideal method for identifying native and foreign recruits and following them through to reproductive age. Only with such experiments can the issue of developmental canalisation be adequately addressed.

6.4.2 Evolutionary Consequences

The results from this study suggest that *A. tenebrosa* appears to have a life history similar to that envisaged by Williams' Strawberry-Coral Model. The model predicts that intense inter-clonal competition will lead to a high degree of local adaptation, with the scale and degree of local adaptation determined by the intensity of selection within each habitat, the scale of separation between habitats and the frequency and diversity of recruits (Williams 1975). Within populations of *A. tenebrosa* levels of genotypic diversity appear to be derived (at least initially) from widely dispersed and sexually produced recruits (Ayre 1983b, 1984b; Ayre *et al.* 1991a). Subsequent selection and inter-genotypic competition then result in the elimination of all but the fittest clones, which then propagate asexually and come to dominate particular habitat patches. The extent and distribution of clones is

determined by the spatial variability of the environment (i.e. Chapter 5), and the ability for clones to compete for space within each habitat patch (Ayre 1982, 1987). As such, *A. tenebrosa* appears to fit this life history model, however, the level of local adaptation observed in this and previous studies, and the diversity of genotypes detected within populations (Chapter 5), presents somewhat of a paradox. Local adaptation requires a sufficient input of genotypic diversity into populations for selection to act on. While clearly sufficient amounts of genotypic diversity exist within these habitats (Chapter 5), sexual recruitment appears to be rare within populations of *A. tenebrosa* (Ayre 1984b, a). Additionally, a 22-year monitoring program of east and west coast Australian populations has failed to detect any evidence of sexually recruitment (Ayre, unpublished data), and the apparent lack of females within the populations studied here, indicates that sex may have been completely lost from some populations. While it may be possible that females are cryptic within these populations (i.e. within the group of non-reproductive individuals), Ayre (1988) detected both female and male clones within Western Australian populations, although sex ratios were often highly skewed towards either males or females. Nevertheless, this means input of genotypic diversity into populations that lack females must either no longer be possible or must come from sources outside these populations. Considering the potential longevity of clones of *A. tenebrosa*, episodes of sexual recruitment and subsequent selection may be acting over relatively large time scales (i.e. hundreds of years), and continuing long term recruitment studies are needed to detect sexual events. However, the level of adaptation detected in this study suggests that environmental conditions must have remained stable within these populations during intervening periods of sexual recruitment.

Taken together, the results from this investigation provide evidence of localised adaptation in response to fine-scale environmental heterogeneity in a clonal marine organism. However, these results have potentially important implications for a large number of marine organisms, especially those with restricted dispersal. These results suggest that major life history traits are subject to selection at very fine spatial scales and supports a growing number of studies that indicate selection may be acting over very fine spatial

scales within the marine environment (Garrity 1984; Yamada 1989; Johannesson *et al.* 1993; Rolan-Alvarez *et al.* 1997; Riginos and Cunningham 2005).

While this study is an important starting point for providing evidence of the scale over which selection may act within populations of a clonal marine invertebrate, further studies are needed to identify those life history traits responsible for differences in fitness under different selective environments. *A. tenebrosa* provides an ideal model for exploring those life history traits that are under direct selection, as individuals of the same genotype could be exposed to a variety of different selective pressures under controlled laboratory conditions. A good starting point for evaluating such traits may include testing tolerances to desiccation and heat stress, which are likely to vary between boulder and rock pool habitats. Further physiological differences between clones may also yield information on selectively important traits (e.g. McManus *et al.* 1997).

Chapter 7 Scales of Genetic Subdivision and Genotypic Diversity in the Brooding Sea Anemone, *Actinia tenebrosa*

7.1 Introduction

Asexual reproduction is a common reproductive tactic and has been described in most plant and animal groups (Hughes 1989). However, most clonal organisms are rarely exclusively asexual, with many experiencing events of sexual recombination at least occasionally (Bell 1982; Hughes 1989). This mixed reproductive strategy is common to many benthic marine organisms, including sponges, cnidarians, ascidians, bryozoans, and echinoderms (Jackson 1986; Hughes 1989; Knowlton and Jackson 1993). The relative importance of sexual and asexual modes of reproduction, and the dispersal distance of propagules is expected to have important consequences for the distribution of genetic variation within and among populations. For the vast majority of marine species with the capacity to reproduce both sexually and asexually, asexual propagules typically have limited dispersal capabilities and are primarily used to maintain local populations (Hoffmann 1986; Jackson 1986; Ayre and Willis 1988; Ayre 1990; Lasker 1990; Coffroth and Lasker 1998b). In contrast sexually generated propagules tend to be more widely dispersed and are typically used for the colonisation of distant or unpredictable habitats (Williams 1975; Bell 1982), (however, see reviews by Jackson (1986) and Knowlton and Jackson (1993) where dispersal capabilities of asexual propagules may be similar or even exceed that of sexual propagules in some species).

The degree of genetic differentiation of populations of organisms with the capacity to reproduce both sexually and asexually will, therefore, depend largely on levels of dispersal (gene flow) of sexually produced propagules, the intensity of post-settlement selection of sexually produced colonists, and the effect of localised asexual proliferation of successful genotypes. Episodes of sexual reproduction appear to be rare in many partially clonal species (Fadlallah 1982; Ayre 1984b; Hoffmann 1986; McFadden 1991; Ayre and Miller 2004) and established individuals may be exposed to long periods of site-specific selection. Such populations may be dominated by a small number of highly replicated and successful

genotypes resulting in relatively small effective population sizes (e.g. Shick and Lamb 1977; Hoffmann 1986; Johnson and Threlfall 1987; Ayre and Willis 1988; McFadden 1997; Darling *et al.* 2004). Small effective population sizes, coupled with rare episodes of sexual reproduction should increase levels of genetic differentiation among clonal populations. Levels of genetic differentiation among populations will also be affected if the dispersal potential of propagules is interrupted or restricted by physical barriers resulting in disjunct genetic patterns across a species range (Slatkin 1987). This may take the form of large stretches of unsuitable habitat between populations or hydrological regimes that retain larvae within a localised area (e.g. Johnson and Black 1998; Goldson *et al.* 2001).

Direct estimates of the realised dispersal distance of either sexually or asexually generated propagules are problematic and often impossible to obtain. Firstly, many larvae are too small to enable mark and recapture techniques or direct observation to be used, and may be prone to biases. For example, rare or occasional long distance dispersal may be very hard to detect with such techniques, but may be very important biologically. Secondly, the production of sexual larvae in some species may only occur rarely, with the causes or cues that trigger such events unknown for most species. This means that it is often impossible or only rarely possible to be "in the right place at the right time" to directly observe sexual events and the subsequent dispersal of larvae (e.g. Hoffmann 1986). While some success has been made in identifying cues that trigger spawning in some exclusively sexual species in controlled laboratory situations (Strathmann 1987), for the vast majority of benthic marine invertebrates this remains problematic. Estimates of larvae dispersal are often made from observations of the maximum time larvae can remain competent to settle (Harrison and Wallace 1990; Wilson and Harrison 1998; Nozawa and Harrison 2005); however, estimates of the minimum time to settlement indicate that dispersal for some larvae may be much less than has been assumed previously (Miller and Mundy 2003). Additionally, developmental periods, behaviour and competency periods of laboratory reared larvae may not accurately reflect those seen under natural conditions. Finally, direct measures of gene flow may not reflect the true movement of genes, as effective migration requires larvae to successfully recruit and, more importantly, reproduce in a new location for gene flow to have occurred.

The difficulties in directly measuring gene flow have led to the common use of indirect measures, which use the underlying spatial distribution of genetic variation among populations (Wright 1931; Slatkin 1985). Wright's (1931) island model of population structure predicts that the variance in gene frequencies among populations should be related to the number of migrants that enter each population in each generation. However, this model makes biologically unrealistic assumptions, many of which real populations are likely to violate, thereby, causing much debate over the accuracy of gene flow estimates using gene frequency data (Hilbish 1996; Bohonak 1999; Whitlock and McCauley 1999). Additionally, estimates of gene flow from gene frequency data assume that gene flow among populations is in equilibrium and, therefore, does not distinguish between contemporary and historical gene flow and dispersal.

The more recent development of population assignment tests, which assign individuals probabilistically to candidate populations based on their multi-locus genotype, have the potential to identify possible migrants within samples and may therefore provide estimates of contemporary rates of gene flow and dispersal (Rannala and Mountain 1997; Waser and Strobeck 1998; Davies *et al.* 1999; Berry *et al.* 2004). However, the accuracy of such assignment tests is heavily dependent on the stringency of the test applied, the degree of genetic differentiation between populations (and hence levels of gene flow), and the number and levels of polymorphism of loci used (Davies *et al.* 1999; Berry *et al.* 2004).

The intertidal sea anemone, *Actinia tenebrosa*, has a wide geographical range and is common to rocky shores along the temperate coasts of mainland Australia, Tasmania and New Zealand (Ottaway 1979a; Ayre 1984b). The patterns and levels of genotypic diversity detected in the majority of populations of *A. tenebrosa* on the east and west coasts of Australia are consistent with the establishment of populations from widely dispersed sexually generated colonists with subsequent asexual proliferation of clones (Ayre 1983b; 1984a; Ayre *et al.* 1991a). However, as with other clonal cnidarians, episodes of sexual reproduction appear to be rare (Ayre 1984b), and may no longer be possible in some populations (e.g. Chapter 6). Experimental clearance of suitable recruitment space within

populations failed to detect any recruitment other than via asexual reproduction (Ayre 1984b). Indeed, ongoing recruitment studies of cleared areas have also failed to detect any sexual recruitment during 22 years of monitoring (Ayre, unpublished data).

Surveys of allozyme variation within populations of *A. tenebrosa* typically show low levels of genotypic diversity and populations often consist of a small number of numerically dominant genotypes (Ayre 1984a; Ayre *et al.* 1991a). There is little sharing of clones between neighbouring populations, implying that successful long distance dispersal of asexually produced juveniles rarely occurs. However, one limitation of previous estimates of genotypic diversity within populations of *A. tenebrosa* on the east and west coasts of Australia may have been the sampling of multiple individuals from clonal aggregations (Ayre 1983a, 1984b; Ayre *et al.* 1991a). While this was intentionally done to gain a meaningful assessment of the relative importance of asexual reproduction within local populations, it means that estimates of overall levels of genotypic diversity within populations may have been underestimated, as this sampling strategy is unlikely to capture the true extent of genotypic diversity within these populations. Additionally, these studies relied on the use of allozyme markers to identify different clones. A number of recent studies have demonstrated lower levels of genetic diversity and potential effects of balancing selection on some allozyme loci compared with microsatellite loci (Scribner *et al.* 1994; Tessier *et al.* 1995; Barker *et al.* 1997; Estoup *et al.* 1998; Colihueque *et al.* 2003). As such, the ability to distinguish between different clones of *A. tenebrosa* in previous studies may have been limited by the exclusive use of allozyme markers. The recent development of microsatellite markers for *A. tenebrosa* (Mitchellson and Ayre, unpublished) provides the opportunity to use microsatellite markers in conjunction with allozyme markers to dramatically increase the resolving power for the identification of different clones and the true extent of genotypic diversity within populations (Chapter 5).

Populations of *A. tenebrosa* on stable platforms along the east coast of Australia are highly genetically subdivided (F_{ST} = 0.294), and appear to reflect the combined effects of localised asexual reproduction and limited gene flow among populations (Ayre *et al.* 1991a). Interestingly, cluster analysis based on genetic distance among populations revealed a clear

subdivision between northern and southern populations along Australia's east coast indicating that gene flow may be limited by some physical barrier. However, much of this variation could be attributed to clinal variation at a single allozyme locus, glucose phosphate isomerase (*Gpi*). Similar genetic clines have been reported for *Gpi* along temperature gradients for the sea anemone *Mertridium senile* (Hoffmann 1986, 1987) and are believed to be related to the thermal properties of the allozymes. This genetic cline, therefore, appears to be maintained by selection acting on this or a closely linked locus and may account for the differences between the two regions.

Despite much of the observed differentiation among populations being attributed to clinal variation at the *Gpi* locus, the existence for a potential barrier to gene flow between the NSW and Victoria regions are partially supported by observations of ocean currents along Australia's east coast. Larval dispersal is likely to be strongly influenced by the magnitude and direction of prevailing currents and fine-scale topographical variation. The East Australian Current (EAC) flows southward along the east Australian coast but shows remarkable levels both of latitudinal and seasonal variation. The current flow is strongest between 25ºS and 40ºS during the summer periods; however, near Sydney the current begins to separate from the coast and move off shore into the Tasman Sea (Boland and Church 1981; Chiswell *et al.* 1997; Ridgway and Godfrey 1997). The point of separation is highly variable from year to year and the presence of anticyclonic cold core eddies result in northward-flowing currents and may allow for potential dispersal of larvae in both directions within a region (Chiswell *et al.* 1997; Ridgway and Godfrey 1997). While the offshore movement of the EAC may act as a potential barrier to larval dispersal between northern and southern regions, this is only partially supported by other studies of other marine invertebrates along this geographic range. The sexually brooding anemone *Anthothoe albocinta* (Billingham and Ayre 1996), and sexually brooding starfish *Patiriella exigua* (Hunt 1993) both show high levels of subdivision between NSW and Victorian populations consistent with restricted dispersal between these two geographic regions. In contrast, Hunt (1993) reported no genetic differentiation between populations from these two regions for the broadcast spawning starfish *P. calcar*. Low levels of genetic differentiation have also been reported among NSW populations of the surf bivalve *Donax*

deltoides (Murray-Jones and Ayre 1997), the ascidian *Pyura gibbosa* (Ayre *et al.* 1997a), and the intertidal anemone *Oulactis muscosa* (Hunt and Ayre 1989), all three of which produce planktonic larvae. However, these studies did not include samples from either side of the NSW/Victorian border and included only samples taken from within NSW. The level of differentiation between populations within the NSW and Victorian regions is, therefore, likely to be highly dependent on the length of planktonic phase of larvae and the degree of variability in the offshore movement of the EAC. Further studies that use highly variable and unambiguously neutral genetic markers are clearly needed to identify the extent to which the EAC acts as a potential barrier to gene flow between these two regions.

In this study I used a combination of microsatellite and allozyme markers to carry out surveys of 19 populations of *A. tenebrosa* separated by up to 2500 kilometres on the east coast of Australia and Tasmania. Specifically I ask: 1) do levels of genotypic diversity vary within and among populations along the east coast of mainland Australia and Tasmania; and 2) what is the extent of genetic differentiation among local populations, and between northern and southern regions.

7.2 Methods

7.2.1 Collection of Specimens

I collected 456 adult anemones from 19 local populations (24 / population) covering most of the known distribution of *A. tenebrosa* along the east coast of mainland Australia and Tasmania (Figure 7.1). The average distance between neighbouring populations was ~80 km, however, distances varied from 10km to 325km between some nearest neighbouring populations. The sampled populations spanned six geographic regions consisting of: (1) northern NSW (Byron Bay and Coffs Harbour); (2) central NSW (Fingal Bay, Anna Bay, Newcastle); (3) Sydney / Illawarra region (Cape Banks, Bellambi, Bass Point, Durras); (4) southern NSW (Merimbula, Ben Boyd, Green Glades, Mallacoota); (5) southern Victoria (Waratah Bay, Cape Paterson, Flinders); and (6) Tasmania (Bicheno, Orford, Tasman Peninsula) (Figure 7.1).

I collected samples from rock platforms, except for Fingal Bay and Durras where anemones were removed from the underside of large boulders. I sampled anemones from up to three 5m×5m areas spread across the length of each rocky shore; however, populations at Newcastle, Durras and Green Glades were confined to relatively small areas of shore (<50m) and so samples were a haphazard collection of available anemones from across the entire shore. Inter-population genetic variation may be biased by multiple copies of a single genotype due to clonal replication (e.g. Ayre 1984b; Hoffmann 1986; Stoddart 1988). The brooded juveniles of *A. tenebrosa* are asexually produced (Black and Johnson 1979, Chapter 4), and typically settle within a few centimetres from their brood parent (Ottaway 1979b; Ayre 1983b; 1984b, Chapter 5). Since a key aim of this study was to assess geographical patterns of alleleic and genotypic variation, I attempted to minimise the collection of clonemates by taking only widely spaced individuals within each aggregation of anemones. In those cases where anemones occurred in large continuous aggregations with no visible boundaries, I collected samples that were separated >50cm. Anemones were levered from the substratum using a spatula, or by hand, and transported back to the laboratory in separate zip-lock bags filled with seawater. Tissue samples were immediately frozen in liquid nitrogen prior to storage at –80ºC.

Figure 7.1 Map showing the location of collection sites for *Actinia tenebrosa* along the east coast of Australia.

7.2.2 Microsatellite and Allozyme Genotyping

I genotyped all anemones at four microsatellite loci (*At1*, *At5*, *At21a* and *At38*; see Chapter 4 for primer sequences) and five allozyme loci (*Gpi 1 & 2, 6Pgd, Mdh* and *Odh*). The methods used for DNA extraction, enzyme assay preparation, and genotyping of individuals at microsatellite and allozyme loci were the same as that described in Chapter 4.

7.2.3 Data Analysis

7.3.3.1 Allele Frequencies and Hardy-Weinberg

I carried out all computations using the software package GENEPOP V3.4 (Raymond and Rousset 1995), unless otherwise indicated. I calculated allele frequencies for each locus

and population. Observed and expected heterozygosity (Nei 1978) and number of alleles were calculated for each locus and as a multi-locus estimate for each of the 19 populations. Single and multi-locus F_{IS} were estimated as in Weir and Cockerham (1984). I assessed the fit of single locus genotype frequencies to Hardy-Weinberg expectations for each polymorphic locus (i.e. frequency of the most common allele <95%, (Hedrick 2000)) using Fisher's exact tests, with resultant *P*-values adjusted using the sequential Bonferroni method (Rice 1989). In order to determine whether each locus assorted independently I tested each pair-wise combination of loci for linkage disequilibrium (Weir 1979) within each population. Clonal reproduction generates non-random associations among loci and may mimic the affects of physical linkage over the entire genome. Even though my sampling was designed to minimise the collection of clonemates, it is still likely that I collected some clonemates, especially in those populations that are dominated by one or a few genets. Therefore, I calculated estimates of linkage disequilibria with and without repeated multi-locus genotypes. A sequential Bonferroni correction was applied to correct for the large number of similar tests (Rice 1989).

7.2.3.2 Genotypic Diversity

I assigned anemones to different genets based on their multi-locus genotypes. Those anemones with an identical multi-locus genotype were assumed to represent a single genet. I tested the statistical power to identify different genets by calculating the number of unique genotypes identified within each local population and the probability of identity, P_{ID}, for increasing locus combinations (Waits *et al.* 2001) using the program GenAlex (V6) (Peakall and Smouse 2005). P_{ID} was calculated as

$$P(_{ID}) = \Sigma p_i + \Sigma\Sigma\ (2p_i\, p_j)$$

where p_i and p_j are the frequencies of the *i*th and *j*th alleles and $i \neq j$ (Paetkau and Strobeck 1994). This identification (P_{ID}) estimator calculates the probability that two individuals drawn at random from a population will have the same genotype at multiple loci and is used to access the statistical confidence for individual identification. $P_{(\mathrm{ID})}$ was calculated for each locus using adult allele frequencies in the population and then multiplied across loci to give an overall P_{ID} (Waits *et al.* 2001).

I then used several methods to assess genotypic diversity within each population. Firstly, I compared the number of individuals sampled (N) to the number of unique multi-locus genotypes (N_g) detected. The ratio of N_g/N provides a maximum estimate of the contribution of asexual reproduction to local populations. Secondly, I compared the ratio of observed multi-locus genotypic diversity (G_o) to that expected under conditions of sexual reproduction with free recombination (G_e), as described by (Stoddart and Taylor 1988). Departures of G_o/G_e from unity should reflect the combined effects of departures from single-locus Hardy-Weinberg equilibria and of multi-locus linkage disequilibria. Such departures are a predicted consequence of asexual reproduction (but may also result from other factors such as population subdivision). In contrast, a population with high levels of sexually derived recruitment will display a G_o/G_e ratio of close to unity (Stoddart and Taylor 1988). I tested for significance departures from unity by determining if G_o lay outside the 95% confidence interval of G_e (Stoddart and Taylor 1988). To reduce the chance of type I errors I applied a sequential Bonferroni correction to correct for the large number of similar tests.

7.2.3.3 Geographical Variation and Population Subdivision

I used a number of analyses to characterise the genetic relationship among populations and regions. I conducted all analyses using: (1) the genotypes of all individual anemones within each local population; and (2) all distinct multi-locus genotypes within each local population. Firstly, I assessed the overall relatedness of populations using Nei's (1978) standard genetic distance and the genetic relationship among populations determined by UPGMA analysis using the program TFPGA (Miller 1997b). I evaluated the robustness of each node by bootstrapping allele frequencies 100 times (Miller 1997b). Secondly, I quantified levels of population and regional subdivision using an hierarchical analysis of standardised genetic variance (F) (Wright 1978). Genetic variation was partitioned within and among geographical regions with subscripts used to denote the source of variation: F_{ST}, total variation among all populations; F_{PR}, mean variation among populations within each region; and F_{RT}, mean variation among regions. I calculated these parameters using the formulations of Weir & Cockerham's (1984) with the program TFPGA (V1.3) (Miller 1997b). This program executes numerical re-sampling (jackknifing) to provide estimates of

variances across loci. Values of F were judged to be statistically significant when zero lay outside the 95% confidence interval of the mean. Finally, I test for isolation by distance through the correlation between matrices of pairwise $F_{ST}/(1 - F_{ST})$ values and the geographical distances (Km) between populations (Mantel test, 10 000 permutation; Rousset 1997). Geographic distances were calculated as the shortest distance connecting the populations by sea.

I used a Bayesian approach of population assignment to estimate the likelihood of an individual's multi-locus genotype occurring in a given population, which was implemented in the genetics program GENECLASS 1.0.02 (Cornuet et al. 1999). Miss-assignment of individuals to the wrong population may indicate possible migrants and has also been used to infer levels of population genetic differentiation. This analysis makes the assumption that populations are in Hard-Weinberg equilibrium and that loci are unlinked (Cornuet *et al*. 1999).

7.3 Results

7.3.1 Genetic Variation and Linkage Disequilibria

The total number of alleles per locus ranged from 2 to 18, with two microsatellite loci, *At1* and *At5*, showing the highest levels of polymorphism (18 and 6 alleles respectively) (Table 7.1). Levels of polymorphism for the remaining two microsatellite loci (*At21a* and *At38*) and all five allozyme loci were much lower, with only 2 to 3 alleles detected per locus (Table 7.1). The average number of alleles per locus within each population varied from 1.9 ± 0.3 to 2.8 ± 0.7 (SE), with an overall mean of 2.45 ± 0.42 (Table 7.2). The mean expected heterozygosity within each population varied between 0.21 ± 0.08 to 0.45 ± 0.07 (SE) with an overall mean of 0.33 ± 0.07 (Table 7.2).

Tests for linkage disequilibria between loci within each population revealed 117 significant associations out of 576 possible comparisons, with 46 remaining significant after the application of a sequential Bonferroni correction. Reanalysis only using unique multi-locus genotypes in the analysis revealed only 39 significant associations out of 568 possible comparisons, with only 16 remaining significant after the application of a sequential Bonferroni correction. The only consistent and significant associations ($P < 0.01$) detected across populations were between *Gpi1* and *Gpi2*, and occurred irrespective of whether replicate genotypes were included or excluded from the analysis. The genotypes inferred from *Gpi1* and *Gpi2* were identical in all except three individuals, indicating that these two loci are physically linked. *Gpi2* was therefore excluded from further analysis.

Tests for departures from Hardy-Weinberg equilibrium revealed 5 significant cases of heterozygote excesses and 17 heterozygous deficits out of 127 statistically valid (Hedrick 2000) single-locus tests across loci and populations. However, only 2 heterozygote excesses and 7 heterozygote deficits remained significant after a sequential Bonferroni correction of significance levels (Table 7.2). Mean multi-locus estimates of F_{IS} ranged from –0.301 to 0.298 however, only three populations (Byron Bay, Green Glades and Orford) deviated significantly from Hardy-Weinberg expectations, although none of these remained significant after reanalysis with replicate genotypes removed (data not shown).

Table 7.1 Allele frequencies for collections of *Actinia tenebrosa* from 19 local populations along the east coast of Australia. (24) = Sample size.

		Location																		
		BYB	CFH	FGB	ANB	NWC	CPB	BLM	BPT	DRS	MBA	BBD	GRG	MCT	WTB	CPT	FND	BCO	ORF	TSP
Locus	Allele	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)	(24)
At1	105	-	-	0.208	0.104	0.063	0.208	-	0.021	0.063	0.188	-	0.188	-	-	-	-	-	-	-
	111	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.065	-	-	-
	118	0.042	0.104	-	-	-	0.042	0.063	0.042	0.021	-	-	-	-	-	-	-	-	-	-
	125	0.521	0.708	0.708	0.708	0.667	0.458	0.271	0.396	0.625	0.125	0.250	0.313	0.313	0.479	0.271	0.174	0.109	-	0.217
	132	0.438	0.188	0.083	0.167	0.271	0.250	0.542	0.438	0.250	0.667	0.583	0.271	0.354	0.313	0.417	0.587	0.522	0.271	0.261
	140	-	-	-	0.021	-	-	-	0.063	-	0.021	-	-	-	-	0.083	-	-	-	-
	148	-	-	-	-	-	-	-	-	-	-	0.021	-	-	-	-	-	-	-	-
	150	-	-	-	-	-	-	-	-	-	-	0.042	-	-	-	-	-	-	-	-
	154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.022	-	-	-
	162	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.022	-	-	-
	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.021	0.022
	175	-	-	-	-	-	0.042	0.125	0.042	0.042	-	0.083	-	0.271	0.021	0.104	-	0.109	0.417	0.130
	182	-	-	-	-	-	-	-	-	-	-	0.021	0.229	0.063	0.125	0.042	0.087	0.087	-	-
	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.292	-
	197	-	-	-	-	-	-	-	-	-	-	-	-	-	0.021	0.042	0.043	0.109	-	0.261
	204	-	-	-	-	-	-	-	-	-	-	-	-	-	0.042	0.042	-	-	-	0.065
	209	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.043
	216	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.065	-	-
At5	139	0.667	0.396	0.708	0.583	0.625	0.313	0.313	0.729	0.521	0.542	0.708	0.333	0.500	0.438	0.250	0.087	0.543	0.682	0.457
	141	0.292	0.479	0.104	0.271	0.146	0.104	0.479	0.188	0.167	0.188	0.208	0.646	0.313	0.396	0.708	0.543	0.261	0.295	0.478
	143	-	0.083	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	150	-	-	-	-	0.146	0.021	0.021	0.063	-	-	-	-	-	-	-	-	-	-	-
	152	-	-	-	0.021	-	0.104	0.063	-	0.063	0.042	0.083	0.021	0.188	0.125	0.042	0.370	0.196	0.023	0.065
	154	0.042	0.042	0.188	0.125	0.083	0.458	0.125	0.021	0.250	0.229	-	-	-	0.042	-	-	-	-	-
At21	169	-	-	-	-	-	-	-	-	-	-	0.063	-	0.063	-	-	-	-	-	-
	171	1.000	1.000	0.896	0.958	0.857	0.804	0.979	0.896	0.935	0.500	0.688	0.979	0.479	0.979	0.875	0.750	1.000	0.979	1.000
	173	-	-	0.104	0.042	0.143	0.196	0.021	0.104	0.065	0.500	0.250	0.021	0.458	0.021	0.125	0.250	-	0.021	-
At38	104	0.313	0.333	0.227	0.208	0.208	0.271	0.375	0.313	0.152	0.063	0.146	0.021	0.458	0.521	0.354	0.396	0.543	0.479	0.604
	105	0.688	0.667	0.773	0.792	0.792	0.729	0.625	0.688	0.848	0.938	0.854	0.979	0.542	0.479	0.646	0.604	0.457	0.521	0.396
Gpi1	1	1.000	1.000	0.979	1.000	0.938	0.875	0.979	0.979	0.875	0.875	0.896	0.500	0.563	0.188	0.479	0.292	0.271	0.125	0.167
	2	-	-	0.021	-	0.063	0.125	0.021	0.021	0.125	0.125	0.104	0.500	0.438	0.813	0.521	0.708	0.729	0.875	0.833
Gpi2	1	1.000	1.000	0.979	1.000	1.000	0.875	0.979	0.979	0.875	0.896	0.896	0.500	0.563	0.479	0.292	0.188	0.125	0.271	0.167
	2	-	-	0.021	-	0.000	0.125	0.021	0.021	0.125	0.104	0.104	0.500	0.438	0.521	0.708	0.812	0.875	0.729	0.833
Mdh	1	-	0.229	0.333	0.146	0.229	0.229	0.146	0.208	0.167	0.292	0.250	0.271	0.250	0.125	0.146	0.208	-	0.271	0.042
	2	1.000	0.771	0.667	0.854	0.771	0.771	0.854	0.792	0.833	0.708	0.750	0.729	0.750	0.875	0.854	0.792	1.000	0.729	0.958
Odh	1	0.042	0.042	0.146	0.146	0.417	0.188	0.208	0.188	0.042	0.146	0.021	0.063	0.104	0.104	0.063	0.063	0.229	0.083	0.333
	2	0.958	0.896	0.771	0.771	0.583	0.813	0.792	0.813	0.958	0.854	0.979	0.938	0.854	0.896	0.938	0.938	0.771	0.917	0.667
	3	-	0.063	0.083	0.083	-	-	-	-	-	-	-	-	0.042	-	-	-	-	-	-
6pgd	1	0.896	0.917	0.688	0.771	0.771	0.708	0.896	0.792	0.833	0.771	0.771	0.896	0.938	0.979	1.000	1.000	0.938	0.896	1.000
	2	0.104	0.083	0.313	0.229	0.229	0.292	0.104	0.208	0.167	0.229	0.229	0.104	0.063	0.021	-	-	0.063	0.104	-

(BYB) Byron Bay, (CFH) Coffs Harbour, (FGB) Fingal Bay, (ANB) Anna Bay, (NWC) Newcastle, (CPB) Cape Banks, (BLM) Bellambi, (BPT) Bass Point, (DRS) Durras, (MBA) Merimbula, (BBD) Ben Boyd, (GGD) Green Glades, (MCT) Mallacoota, (WTB) Waratah Bay, (CPT) Cape Paterson, (FND) Flinders, (BCO) Bicheno, (ORF) Orford, (TSP) Tasman Peninsula.

Table 7.2 Genetic variation at four microsatellite and four allozyme loci in 19 populations of *Actinia tenebrosa*. $N = 24$ for each population.

	Location																		
Locus	BYB	CFH	FGB	ANB	NWC	CPB	BLM	BPT	DRS	MBA	BBD	GRG	MCT	WTB	CPT	FND	BCO	ORF	TSP
At1																			
Na	3	3	3	4	3	5	4	6	5	4	6	4	4	6	7	7	6	4	7
H_E	0.542	0.542	0.458	0.375	0.500	0.583	0.750	0.458	0.458	0.417	0.667	0.917	0.875	0.917	0.792	0.583	0.708	0.875	0.833
H_O	0.536	0.452	0.448	0.459	0.478	0.681	0.614	0.644	0.541	0.504	0.588	0.741	0.700	0.655	0.730	0.615	0.681	0.668	0.797
F_{IS}	0.010	-0.177	-0.002	0.204	-0.024	0.164*	-0.202	0.308*	0.173	0.194	-0.113	-0.216***	-0.231	-0.383*	-0.063	0.072	-0.020	-0.291	-0.024
At5																			
Na	3	4	3	4	4	5	5	4	4	4	3	3	3	4	3	3	3	3	3
H_E	0.500	0.458	0.375	0.417	0.583	0.708	0.833	0.333	0.792	0.708	0.583	0.500	0.542	0.708	0.542	0.250	0.583	0.625	0.458
H_O	0.469	0.605	0.452	0.570	0.560	0.670	0.653	0.429	0.635	0.617	0.448	0.471	0.617	0.635	0.434	0.559	0.601	0.442	0.556
F_{IS}	-0.045	0.262**	0.191	0.289	-0.021	-0.036	-0.257	0.243*	-0.228	-0.127	-0.283	-0.040	0.143*	-0.095	-0.228	0.567***	0.050	-0.397	0.197
At21																			
Na	1	1	2	2	2	2	2	2	2	2	3	2	3	2	2	2	1	2	1
H_E	0.000	0.000	0.125	0.083	0.250	0.125	0.042	0.208	0.125	0.500	0.250	0.042	0.708	0.042	0.167	0.417	0.000	0.042	0.000
H_O	0.000	0.000	0.187	0.080	0.219	0.305	0.041	0.187	0.117	0.500	0.461	0.041	0.556	0.041	0.219	0.375	0.000	0.041	0.000
F_{IS}	-	-	0.349	-0.022	-0.122	0.603*	-	-0.095	-0.045	0.021	0.474**	-	-0.253	-	0.258	-0.090	-	-	-
At38																			
Na	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_E	0.625	0.500	0.417	0.333	0.333	0.458	0.250	0.208	0.333	0.125	0.125	0.042	0.167	0.292	0.458	0.208	0.375	0.708	0.208
H_O	0.430	0.444	0.375	0.330	0.330	0.395	0.469	0.430	0.278	0.117	0.249	0.041	0.497	0.499	0.457	0.478	0.492	0.499	0.478
F_{IS}	-0.438	-0.104	-0.090	0.011	0.011	-0.140	0.483	0.531*	-0.179	-0.045	0.514*	-	0.676**	0.433*	0.019	0.579**	0.258	-0.401	0.579**
Gpi1																			
Na	1	1	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_E	0.000	0.000	0.042	0.000	0.125	0.250	0.042	0.042	0.250	0.250	0.208	0.917	0.458	0.375	0.792	0.333	0.542	0.250	0.250
H_O	0.000	0.000	0.041	0.000	0.117	0.219	0.041	0.041	0.219	0.219	0.187	0.500	0.492	0.305	0.499	0.413	0.395	0.219	0.278
F_{IS}	-	-	-	-	-0.045	-0.122	-	-	-0.122	-0.122	-0.095	-0.827***	0.090	-0.211	-0.572*	0.214	-0.353	-0.122	0.121
Mdh																			
Na	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
H_E	0.000	0.292	0.667	0.292	0.458	0.292	0.208	0.333	0.333	0.333	0.417	0.542	0.500	0.250	0.208	0.167	0.000	0.542	0.083
H_O	0.000	0.353	0.444	0.249	0.353	0.353	0.249	0.330	0.278	0.413	0.375	0.395	0.375	0.219	0.249	0.330	0.000	0.395	0.080
F_{IS}	-	0.195	-0.484*	-0.151	-0.278	0.195	0.184	0.011	-0.179	0.214	-0.090	-0.353	-0.314	-0.122	0.184	0.511*	-	-0.353	-0.022
Odh																			
Na	2	3	3	3	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2
H_E	0.083	0.208	0.458	0.292	0.417	0.375	0.083	0.375	0.083	0.125	0.042	0.125	0.292	0.125	0.125	0.125	0.208	0.167	0.500
H_O	0.080	0.192	0.378	0.378	0.486	0.305	0.330	0.305	0.080	0.249	0.041	0.117	0.258	0.187	0.117	0.117	0.353	0.153	0.444
F_{IS}	-0.022	-0.065	-0.193	0.248	0.164	-0.211	0.757**	-0.211	-0.022	0.514*	-	-0.045	-0.110	0.349	-0.045	-0.045	0.428	-0.070	-0.104
6pgd																			
Na	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	2	1
H_E	0.208	0.083	0.458	0.292	0.458	0.500	0.208	0.417	0.333	0.292	0.458	0.208	0.125	0.042	0.000	0.000	0.125	0.208	0.000
H_O	0.187	0.153	0.430	0.353	0.353	0.413	0.187	0.330	0.278	0.353	0.353	0.187	0.117	0.041	0.000	0.000	0.117	0.187	0.000
F_{IS}	-0.095	0.471	-0.045	0.195	-0.278	-0.190	-0.095	-0.243	-0.179	0.195	-0.278	-0.095	-0.045	-	-	-	-0.045	-0.095	-
Mean Na	1.9	2.3	2.4	2.5	2.4	2.8	2.6	2.8	2.6	2.5	2.8	2.4	2.6	2.6	2.8	2.6	2.4	2.4	2.5
Mean H_E	0.245	0.260	0.368	0.260	0.395	0.412	0.302	0.297	0.336	0.343	0.344	0.412	0.458	0.254	0.344	0.385	0.432	0.318	0.296
Mean H_O	0.213	0.275	0.341	0.302	0.365	0.419	0.323	0.337	0.301	0.373	0.338	0.312	0.452	0.361	0.323	0.338	0.327	0.332	0.329
F_{IS}	-0.135	0.074	-0.068	0.160	-0.058	0.036	0.085	0.140	-0.096	0.096	0.003	-0.301	0.006	-0.045	-0.119	0.298	0.058	-0.294	0.135
HWE	ns	*	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns

Na = number of alleles, H_E = expected heterozygosity (Nei 1978), H_O = observed heterozygosity, F_{IS} = inbreeding coefficient, HWE = departures from Hardy-Weinberg equilibrium. Significant values determined after the application of a sequential Bonferroni correction (*P < 0.05, **P < 0.01, ***P < 0.001, ns = nonsignificant). See Table 7.1 for location abbreviations

7.3.2 Genotypic Diversity

The combination of microsatellite and allozyme loci allowed a high degree of discrimination among genotypes within each population, with the majority of genotypes (77 - 98%) being identified using a combination of the six most variable markers. The probability that two individuals drawn at random within a population will share, by chance, the same eight-locus genotype was low for all 19 populations, with $P_{(ID)}$ ranging from 0.028 to <0.001 (Table 7.3). The expected number of individuals with the same eight-locus genotype within each population was therefore low (<1) (Figure 7.2), indicating that the combination of microsatellite and allozyme loci used in this study allowed for a high degree of discrimination between genotypes within each population.

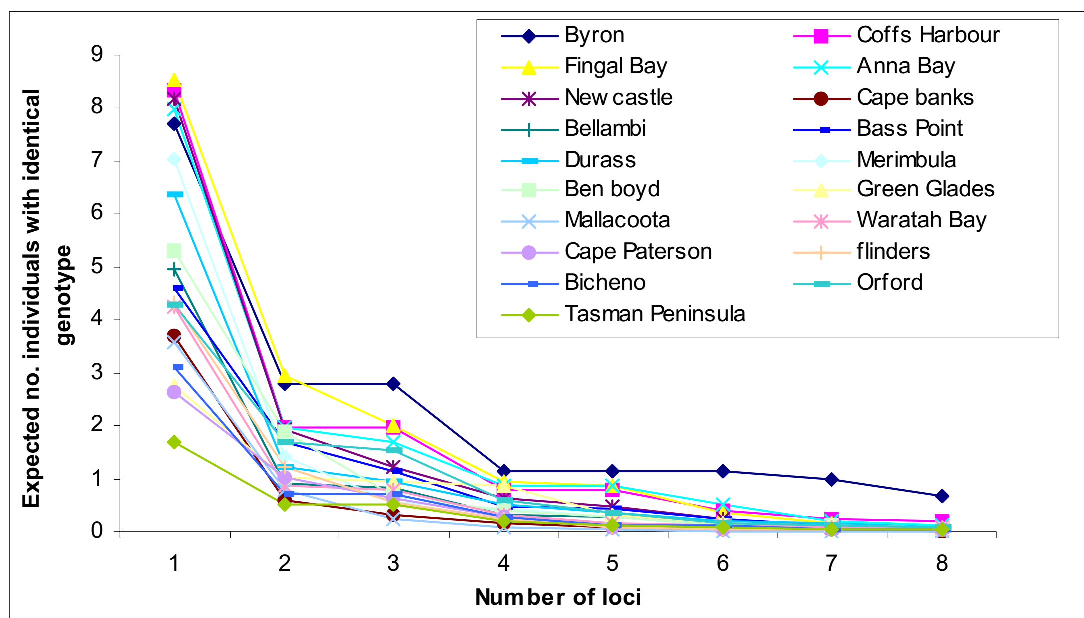

Figure 7.2 The expected number of individuals with the identical genotype for increasing locus combinations within each of 19 local populations of *Actinia tenebrosa*.

Genotypic diversity varied greatly among populations, with the ratio of unique multi-locus genotypes detected (N_g) compared to the number of individuals sampled (N), ranging from 0.42 to 0.96 (Table 7.2). There was no obvious geographic trend in the levels of genotypic diversity among populations indicating that populations at the margin of this species range

can still maintain high levels of genotypic diversity. In all 19 populations I detected some individuals with the same eight-multi-locus genotype (potential clones), although most replicated genotypes were represented by only one or two individuals. The three exceptions to this were populations sampled at Byron Bay, Green Glades and Orford, which showed high levels of clonality (N_g/N = 0.46, 0.42 and 0.58 respectively), with some clones being represented by up to seven individuals. Two of the three populations (Byron Bay and Green Glades) consisted of small isolated rocky platforms and the low levels of genotypic diversity detected in these populations may result from either founder events or extreme selection on recruits such that only a small number of highly locally adapted genotypes survive. The observed levels of genotypic diversity (G_o) compared to that expected for random mating (G_e) were significantly lower in 13 of the 19 populations sampled (G_o /G_e ranged from 0.25 – 1.09; Table 7.2), indicating that these samples contain far less genotypic diversity than would be expected if recruits were the product of outcrossed sexual reproduction. These data indicate that while sexually produced colonists are likely responsible for the founding of local populations, asexual reproduction appears to be the major source of recruitment into established populations, with some populations showing the extreme effects of founding events or selection resulting in low genotypic diversity.

A comparison of the number of genotypes shared among populations showed that of the 65 clonal genotypes detected from a total of 456 individuals sampled, 77% were confined to a single population and only 23% common to more than one population. Of those clones common to more than one population I found that 40% of these putative clones were shared by neighbouring populations. However, given the relatively large distances separating populations it is unlikely that these individuals represent clonal propagules that have dispersed between neighbouring populations and are more likely the products of sexual reproduction. I detected only a single genotype that was relatively widespread and common to four populations separated by up to 660km (Anna Bay, Bellambi, Durras and Ben Boyd).

Table 7.3 Levels of genotypic diversity observed within each of 19 populations of *Actinia tenebrosa* from the east coast of Australia. *N*, number of individuals sampled; N_g, number of unique multi-locus genotypes; P_{ID} probability of identity; G_o: observed multi-locus genotypic diversity; G_e: expected multi-locus genotypic diversity for random mating. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, non-significant. Significant values determined after the application of a sequential Bonferroni correction (Rice 1989).

Population	*N*	*Ng*	N_g/N	P_{ID}	G_o	G_e (SD)	G_o/G_e	*P*-value
Byron Bay	24	11	0.46	0.028	8.2	14.5 (3.4)	0.57	<0.010**
Coffs Harbour	24	23	0.96	0.008	22.2	20.4 (3.3)	1.09	ns
Fingal Bay	24	22	0.92	0.003	20.6	22.6 (2.3)	0.91	ns
Anna Bay	24	22	0.92	0.004	19.2	21.9 (2.8)	0.88	ns
Newcastle	24	17	0.71	0.002	12.3	23.0 (1.7)	0.53	<0.001***
Cape Banks	24	23	0.96	0.000	22.2	23.7 (0.8)	0.93	ns
Bellambi	24	16	0.67	0.002	9.9	22.6 (2.1)	0.44	<0.001***
Bass Point	24	18	0.75	0.002	13.7	22.5 (2.0)	0.61	<0.010**
Durras	24	21	0.88	0.004	18.0	22.0 (2.6)	0.82	ns
Merimula	24	20	0.83	0.001	15.6	23.1 (1.5)	0.67	<0.001***
Ben Boyd	24	21	0.88	0.002	19.2	22.8 (2.1)	0.84	ns
Green Glades	24	10	0.42	0.003	5.5	22.3 (2.3)	0.25	<0.001***
Mallacoota	24	19	0.79	0.000	10.7	23.7 (2.2)	0.45	<0.001***
Waratah Bay	24	15	0.63	0.003	11.5	22.5 (2.1)	0.51	<0.001***
Cape Paterson	24	20	0.83	0.002	15.2	22.9 (1.8)	0.66	<0.001***
Flinders	24	17	0.71	0.002	11.5	23.1 (1.7)	0.50	<0.001***
Bicheno	24	21	0.88	0.002	19.2	22.9 (1.8)	0.84	<0.020*
Orford	24	14	0.58	0.003	5.5	22.3 (2.4)	0.25	<0.001***
Tasman Peninsula	24	19	0.79	0.002	17.0	23.2 (1.9)	0.73	<0.001***

7.3.3 Population Subdivision

I detected considerable variation in allele frequencies among populations and geographical regions. Exact tests for homogeneity of allele frequencies among all samples indicated highly significant differences between all population pairs (Fishers exact test, $P < 0.0001$), both at individual loci, and over all loci combined. My hierarchical analysis of standardised genetic variance using all individual genotypes revealed significant differentiation among all populations (F_{ST} = 0.163, 95% CI = 0.088 to 0.276); however, each locus did not contribute equally to the interpopulation differentiation, with single locus F_{ST} values ranging from 0.040 (*Mdh*) to 0.497 (*Gpi1*) (Table 7.4). I detected high levels of genetic differentiation among the six geographic regions (F_{RT} = 0.174), although most of this variation (65%) was due to differences among populations within regions (F_{PR} = 0.113) (Table 7.4). As clonal reproduction within local populations can inflate estimates of subdivision, reanalyses using only distinct multi-locus genotypes within each local

population revealed only slightly lower but still significant levels of subdivision among populations and regions (Table 7.5).

The most suitable method for quantifying genetic differentiation among samples at microsatellite loci (i.e. infinite allele model using F_{ST}, (Kimura and Crow 1964) or a stepwise mutational model using R_{ST}, (Kimura and Ohta 1978)) is still a matter of debate (e.g. Estoup *et al.* 1995; Balloux and Lugon-Moulin 2002 and references therein). However, R_{ST} values calculated for the four microsatellite loci were similar to F_{ST} values calculated for those loci (mean R_{ST} = 0.173) (Table 7.4 & 7.5).

Table **Error! No text of specified style in document.**.1 Hierarchical analysis of standardised genetic variation using the genotypes of all individual anemones within each local population (calculated as Weir and Cockerham 1984) for *Actinia tenebrosa* showing F_{ST} (total variation among all populations), F_{RT} (variation among regions), and F_{PR} (variation among populations within regions). R_{ST} calculated for microsatellite loci assuming a stepwise mutation model (Kimura and Crow 1964). * indicates 95% confidence interval (CI) significantly different from zero.

Locus	F_{ST}	F_{RT}	F_{PR}	R_{ST}
At1	0.135	0.143	0.073	0.314
At5	0.123	0.125	0.035	0.126
At21	0.198	0.207	0.091	0.151
At38	0.099	0.108	0.072	0.099
Gpi1	0.497	0.527	0.472	-
Mdh	0.040	0.042	0.012	-
Odh	0.066	0.070	0.032	-
6pgd	0.069	0.076	0.061	-
Multi-locus	0.163*	0.174*	0.113*	0.173
(SE)	(0.05)	(0.060)	(0.061)	†
95% CI	0.088 to 0.276	0.093 to 0.312	0.041 to 0.245	†

† Bootstrapped and jackknifed values not calculated for R_{ST} due to only four loci, minimum of five required

Table 7.3 Hierarchical analysis of standardised genetic variation using only distinct multi-locus genotypes within each local population (calculated as Weir and Cockerham 1984) for *Actinia tenebrosa* showing F_{ST} (total variation among all populations), F_{RT} (variation among regions), and F_{PR} (variation among populations within regions). R_{ST} calculated for microsatellite loci assuming a stepwise mutation model (Kimura and Crow 1964). * indicates 95% confidence interval (CI) significantly different from zero.

Locus	F_{ST}	F_{RT}	F_{PR}	R_{ST}
At1	0.110	0.119	0.078	0.295
At5	0.107	0.113	0.055	0.103
At21	0.174	0.187	0.124	0.121
At38	0.094	0.107	0.112	0.094
Gpi1	0.465	0.494	0.429	-
Mdh	0.030	0.031	0.012	-
Odh	0.049	0.052	0.019	-
6pgd	0.061	0.068	0.062	-
Multi-locus	0.142*	0.155*	0.116*	0.153
(SE)	(0.049)	(0.054)	(0.051)	†
95% CI	0.071 to 0.243	0.082 to 0.274	0.051 to 0.227	†

† Bootstrapped and jackknifed values not calculated for R_{ST} due to only 4 loci, minimum of five required.

The most striking geographic pattern of genetic variation observed was that detected for *Gpi1*. I detected two alleles for *Gpi1*, a fast allele that occurred at a high frequency, or was completely fixed, within populations from NSW, while the slow allele occurred at a high frequency in Victorian and Tasmanian populations (Table 7.1). Two populations that occurred near the border of NSW and Victoria (Green Glades and Mallacoota) displayed almost equal frequencies of each allele. This distinct clinal variation in the frequency of the two alleles for *Gpi1* indicates that this locus (and the closely linked *Gpi2*) is likely to be under selection and made the largest contribution to genetic variation within and among regions and populations (Table 7.4 & 7.5). Reanalyses with *Gpi1* removed resulted in lower, but still significant levels of genetic subdivision within and among regions and populations regardless of whether all genotypes or only unique genotypes were used in the analysis (Table 7.6).

Table 7.6 Hierarchical analysis of standardised genetic variation excluding *Gpi1* (calculated as Weir and Cockerham 1984) for *Actinia tenebrosa* showing F_{ST} (total variation among all populations), F_{RT} (variation among regions), and F_{PR} (variation among populations within regions). * indicates 95% confidence interval (CI) significantly different from zero.

All individuals	95% CI
F_{ST} (SE) = 0.111* (0.015)	0.077 to 0.134
F_{RT} (SE) = 0.117* (0.015)	0.079 to 0.142
F_{PR} (SE) = 0.054* (0.010)	0.071 to 0.035
Only distinct multi-locus genotypes	
F_{ST} (SE) = 0.095* (0.013)	0.117 to 0.064
F_{RT} (SE) = 0.103* (0.014)	0.068 to 0.126
F_{PR} (SE) = 0.068* (0.013)	0.041 to 0.091

UPMGA analysis of Nei's (1979) genetic distance indicated a distinct division between two large geographic regions, NSW (which included northern, mid, central and southern NSW regions) and a southern region (including Victorian and Tasmanian populations) (Figure 7.3 A & B), and was again driven primarily by clinal variation at *Gpi1*. When *Gpi1* was removed from the analysis the division between these two regions was less pronounced although Tasmanian and Victorian populations still grouped together when only unique genotypes were included (Figure 7.4 A & B). Pairwise population comparisons of multi-locus F_{ST}, using only distinct genotypes, ranged from 0.008 to 0.338 (data not shown), with more geographically distant population showing higher levels of genetic differentiation. A Mantel test on the regression of $F_{ST} / (1 - F_{ST})$ values against geographical distance in kilometres, confirmed this relationship, showing a significant positive correlation ($P <$ 0.0001) between genetic and geographical distance (Figure 7.5).

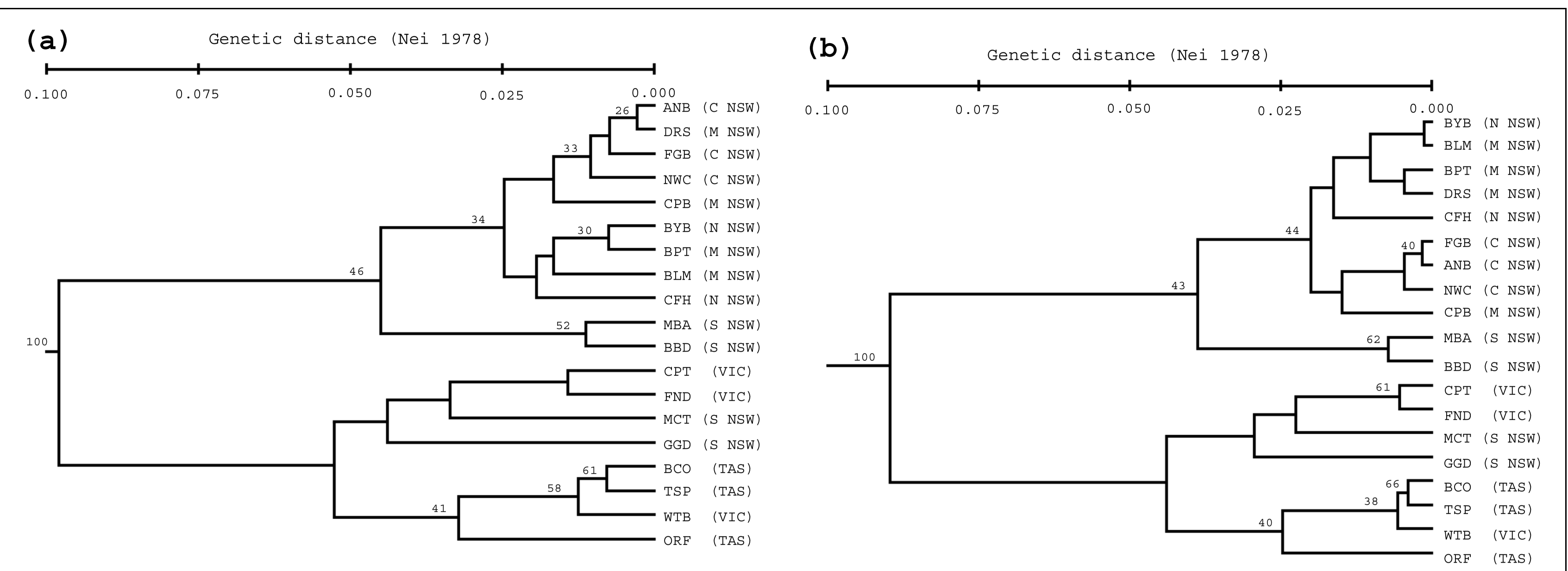

Figure 7.3. Dendrograms showing the genetic relationship among 19 populations of *Actinia tenebrosa* along the east coast of mainland Australia and Tasmania. Nei's genetic distance (1978) was calculated based on data for eight loci using gene frequencies derived from genotypes of all adults (a) and using only distinct multi-locus genotypes (b). Clustering determined by UPGMA with bootstrapped values over 20% (based on 100 randomisations) shown next to corresponding nodes.

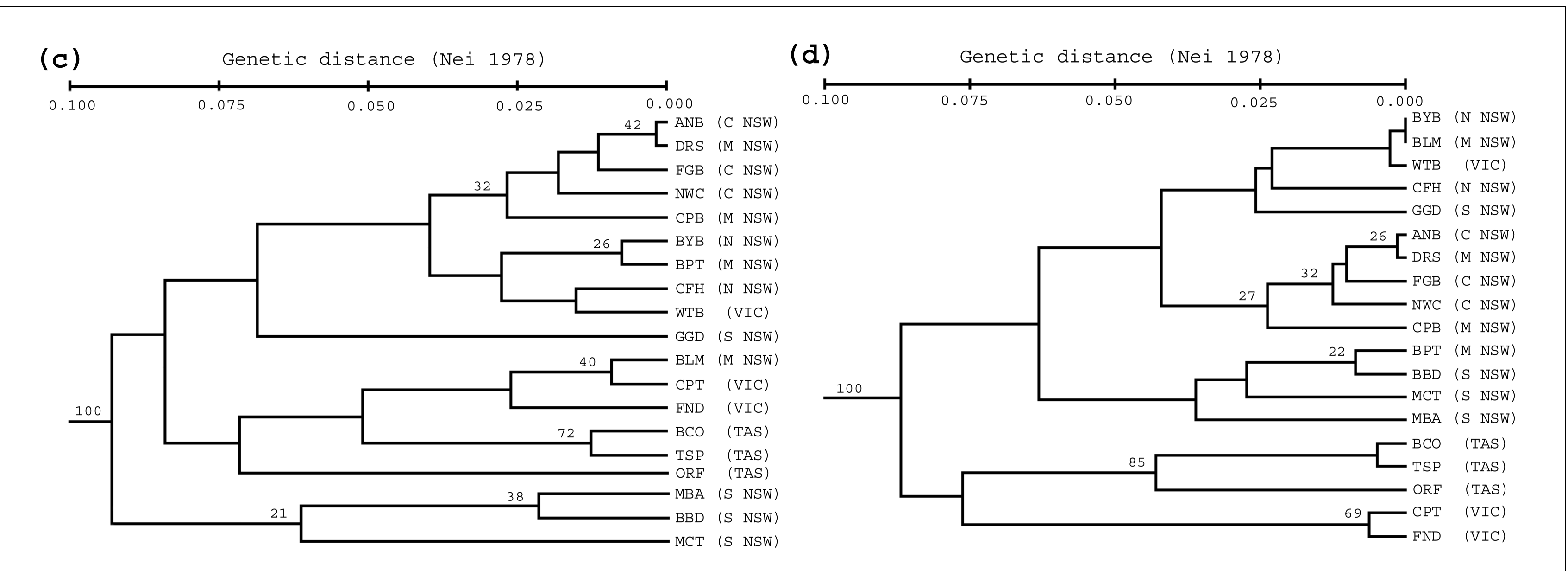

Figure 7.4 Dendrograms showing the genetic relationship among 19 populations of *Actinia tenebrosa* along the east coast of mainland Australia and Tasmania. Nei's genetic distance (1978) was calculated based on data for seven loci (*Gpi1* excluded) using gene frequencies derived from genotypes of all adults (c) and using only distinct multi-locus genotypes (d). Clustering determined by UPGMA with bootstrapped values over 20% (based on 100 randomisations) shown next to corresponding nodes.

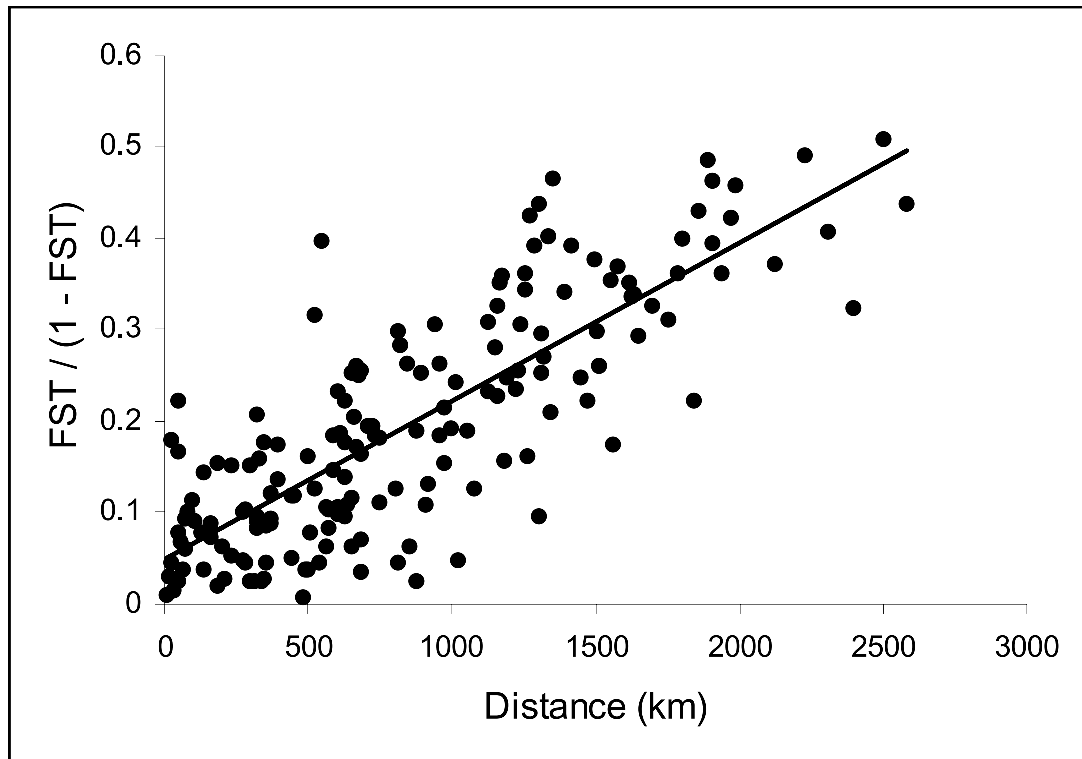

Figure 7.3 Relationship between genetic differentiation [calculated as (F_{ST}/ (1-F_{ST})] and geographic distance between populations of *Actinia tenebrosa* along the east coast of Australia. Pairwise F_{ST} values calculated using only unique multi-locus genotypes (*Gpi1* excluded). Correlation coefficient (R^2) = 0.66.

Using individual assignment tests, my data allowed correct assignment of 60% of anemones to their population of origin (Table 7.7). Of the 40% of individuals incorrectly assigned, 44% of these were assigned to the nearest or second nearest geographical population (Table 7.7). The percentage of individuals correctly assigned to larger geographical regions was much higher, with up to 90% of individuals correctly assigned to either the NSW or Victoria/Tasmania regions.

Table 7.5 The number of individuals of *Actinia tenebrosa* correctly assigned to their population of origin. Population assignment was implemented using the Bayesian approach of population assignment in the program GENECLASS 1.0.02 (Cornuet *et al.* 1999).

7.4 Discussion

My data on the patterns of genotypic diversity and genetic differentiation within and among populations of *A. tenebrosa* along the east coast of mainland Australia and Tasmania support theoretical predictions on the relative roles of sexually and asexually produced propagules (Williams 1975; Bell 1982). Local populations appear to be maintained by highly philopatric dispersal and recruitment of asexual propagules, while sexually derived recruits appear to be responsible for the initial establishment of each population. This confirms and extends similar patterns of genotypic diversity and genetic differentiation previously reported for *A. tenebrosa* (Ayre 1983a; 1984b; Ayre *et al.* 1991a) and other clonal sea anemones (*Anthothoe elegantissima* (Sebens 1982), *A. albocincta* (Billingham and Ayre 1996) and *Mertridium senile* (Hoffmann 1986;, 1987)).

7.4.1 Genotypic Variation

Levels of genotypic diversity varied greatly among populations, with several showing large numbers of distinct genotypes (Coffs Harbour, Fingal Bay, Anna Bay, Cape Banks, Ben Boyd and Bicheno), while others were dominated by only a small number of highly replicated genotypes (e.g. Byron Bay, Green Glades and Orford). While levels of genotypic diversity were generally lower than expected for exclusive sexual reproduction in the majority of the populations in this study, I still detected a large proportion of unique genotypes, with levels of genotypic diversity generally higher than that previously reported for this species (Ayre 1984b; Ayre *et al.* 1991a). The higher level of genotypic diversity reported here is likely to result from differences in the sampling designs between this and previous studies and the use of more variable microsatellite loci in this study. Ayre (1984b) and Ayre *et al.* (1991a) collected samples of *A. tenebrosa* from relatively small areas of rocky shore and sampled multiple individuals within aggregations of anemones. This sampling strategy was more likely to result in the collection of clonemates, which often form local aggregations (Ayre 1983b). Additionally, the higher levels of polymorphism of at least two of the four microsatellite loci allowed me to identify a greater number of clones compared with the exclusive use of allozyme markers (see Chapters 4 & 5 for a comparison of microsatellite and allozyme markers in *A. tenebrosa*).

The total amount of genotypic diversity within a local population should represent a balance between the addition of novel genotypes through sexually derived recruits (or, the accumulation of somatic mutations, though this is only thought to make a small contribution, Chapter 5), and the subsequent decline of genotypic diversity through the removal of less fit genotypes through natural selection. While sexual reproduction is likely to be the initial source of genotypic diversity within populations of *A. tenebrosa*, episodes of sexual reproduction appear to be extremely rare. Indeed, many populations along the east Australian coast appear to consist exclusively of male clones, prohibiting the possibility of sexual reproduction within these populations (Chapter 6), although the possibility of cryptic females among non-reproductive individuals (i.e. those lacking gonads) cannot be ruled out. Additionally, a three year recruitment study into Western Australian populations failed to detect any evidence of sexual recruitment into experimentally cleared areas (Ayre 1984a), and indeed sexual recruitment has not been detected in the subsequent 22 years (Ayre, unpublished data).

Considering the apparent rarity of sexual events combined with longevity of individuals, the continued coexistence of a large number of clones within many of the populations sampled here is surprising. Theoretical and empirical studies of other clonal organisms have suggested that infrequent sexual recruitment combined with individual longevity and inter-clonal competition will result in populations consisting of a small number of numerically dominant and locally adapted genotypes (Ayre 1985; Quicke *et al.* 1985; Hoffmann 1986; Ayre and Grosberg 1995; McFadden 1997). Fighting ability appears to be an important determinant of the outcomes of inter-clonal competition for space in a larger number of clonal cnidarians (Brace *et al.* 1979; Ayre 1987; Shaw 1991; Grosberg *et al.* 1996; Billingham and Ayre 1997), including *A. tenebrosa* (Ayre 1982, 1983b). Consequently, all else being equal, populations should come to be dominated by a small number of the most aggressive clones. Nevertheless, this and previous studies have shown that genotypic diversity may be very high in local populations. Sebens and Thorne (1985) proposed several mechanisms that may promote the coexistence of a number of clones within populations. Firstly, greater survivorship of sexually generated colonists may occur in a heterogeneous habitat that allows niche separation between clones (Solbrig 1971;

McManus *et al.* 1997; Vrijenhoek and Pfeiler 1997, Chapter 5). Reciprocal transplant experiments of *A. tenebrosa,* over a number of spatial scales, have shown that clones may be adapted to particular headlands (Ayre 1985, 1995) and even different habitats on the same rocky shore (Chapter 6). This indicates that high levels of genotypic diversity may indeed be maintained by niche separation over very fine spatial scales. This is further supported by reports that highly heterogeneous boulder shores support higher levels of genotypic diversity than less heterogeneous platform shores (Ayre 1984b) (Chapter 5). The higher levels of genotypic diversity seen on boulder shores may result from a greater variety of microhabitats. As boulder shores vary over very small spatial scales it is not surprising that higher levels of genotypic diversity were detected on these shores when compared to rocky platforms. While I did not detect higher levels of genotypic diversity of the two boulder shores surveyed in this study (Fingal Bay and Durras), this is again likely to be due to the collection of anemones over greater spatial scales than in previous studies and, therefore, unlikely to detect fine-scale variation in genotypic diversity seen within more heterogeneous habitats. This, however, suggests that even on seemingly homogenous stable shores, there is sufficient fine-scale environmental heterogeneity to support a diverse range of genotypes (Shaw 1991). Secondly, temporal variation, mediated by seasonal change, may also select for different genotypes at different times, allowing a greater range of established genotypes to coexist (Vrijenhoek 1979; Angus 1980; Hebert and Crease 1980). Thirdly, the coexistence of a number of clones may occur if genotypically distinct individuals are ecologically equivalent or similar, and unable to competitively exclude each other. Therefore, the coexistence of clones may also result from neutral stability (Angus 1980; Hebert and Crease 1980). This, however, seems an unlikely explanation for the high levels of genotypic diversity observed in this study as previous studies of *A. tenebrosa* have shown large variation in fighting ability among clones (Ottaway 1978; Ayre 1983b; 1987).

Despite the relatively high levels of genotypic diversity detected in most populations, some showed very low levels of genotypic diversity and were dominated by a small number of highly replicated genotypes (i.e. Byron Bay, Green Glades and Orford). Such populations may result from a small number of founding genotypes followed by asexual expansion (Hoffmann 1986; McFadden 1997; Darling *et al.* 2004). Alternatively these populations

may represent extreme environments where only a small number of recruits from a large and diverse set of settlers are able to survive. These highly locally adapted genotypes would then become numerically dominant through localised asexual reproduction. It is difficult to identify which of these processes are responsible for the low levels of genotypic diversity detected within these three populations without further sampling and experimentation, however, these factors may not be mutually exclusive and may be acting synergistically.

7.4.2 Genetic Subdivision and Gene Flow

I detected high levels of genetic differentiation among populations of *A. tenebrosa* along the east coast of mainland Australia and Tasmania. The average F_{ST} value based on individual genotypes was slightly lower than previous estimates of subdivision among populations of *A. tenebrosa* along the eastern Australian coast (F_{ST} = 0.163 *cf.* 0.294) (Ayre 1991), but were much larger than that reported for the exclusively sexually reproducing and co-occurring sea anemone *Oulactis muscosa* (Hunt and Ayre 1989), the sea star *Patiriella calcar* (Hunt 1993), and the broadcast-spawning solitary ascidian *Pyura gibbosa* (Ayre *et al.* 1997a). The levels of genetic subdivision reported here are also similar to that reported for the subtidal clonal sea anemone, *Anthothoe albocincta* (Billingham and Ayre 1996), and the brooding ascidians *Stolonica australis* and *Botrylloides magnicoecum* (Ayre *et al.* 1997a). Multiple copies of a genotype due to asexual reproduction can result in higher levels of genetic differentiation (Stoddart 1984a; Ayre *et al.* 1991a; Billingham and Ayre 1996). However, my estimates of variation within and among regions were only slightly lower when only clonal rather than individual genotype frequencies were used to estimate allelic variation in *A. tenebrosa*.

The significant correlation between genetic and geographical distance indicates that isolation by distance is an important mechanism of differentiation in *A. tenebrosa*. Such increasing genetic differentiation with geographical distance is likely to reflect the effects of dispersal of propagules among only neighbouring populations (Wright 1943; 1969; Slatkin 1987; 1993). This combined with infrequent sexual recruitment, the highly localised dispersal of asexual propagules, and intense inter-clonal competition for

recruitment space, is likely to account for the high levels of differentiation between populations. Although I detected high levels of genetic differentiation among populations within each of the six regions, my analysis revealed only two main clusterings of populations (Figures 7.3 and 7.4), with a possible genetic break near the NSW and Victorian border. This pattern of differentiation between NSW and Victorian populations has been reported for a number of species with limited dispersal capabilities (Ayre 1990; Hunt 1993; Billingham and Ayre 1996) and indicates the possible presence of a biogeographical barrier that prevents dispersal between these two geographic regions. The presence of a 200km stretch of sandy beach and the off-shore movement of the East Australian Current near this region (Chiswell *et al.* 1997; Ridgway and Godfrey 1997; Tilburg *et al.* 2001), have both been suggested as possible barriers to gene flow. However, in the present study each locus did not contribute equally to the interregional differentiation. *Gpi*1contributed significantly more than other loci and showed a strong latitudinal cline in the frequency of its two alleles. The fast allele occurs at a high frequency or is completely fixed in northern and central NSW populations, while the slow allele occurs at a much higher frequency in southern Victorian and Tasmanian populations. Populations at the border of these two geographical areas (i.e. Green Glades and Mallacoota) show almost equal frequencies of the fast and slow alleles. This strong clinal variation in the frequency of the two *Gpi1* alleles supports previous suggestions that selection is acting on this or a closely linked locus (Ayre *et al.* 1991a). Similar reports of a latitudinal cline in the frequency of some allozyme loci have been reported in a number of marine benthic invertebrates (Shick *et al.* 1979; Koehn *et al.* 1980; Hoffmann 1981; Hedgecock 1986) and invalidates such loci in estimates of population structure and gene flow. Reanalysis of levels of genetic differentiation with *Gpi1* removed still revealed significant levels of genetic subdivision among populations and regions (Table 7.5), although the regional break between NSW and Victorian was less pronounced when *Gpi1* was removed from this analysis.

The occurrence of widespread dispersal of asexual propagules is not supported by my data. Of the 65 clonal genotypes identified from the entire collection, 15 of these were common to more than one population, however, almost half of these clonal genotypes were common to only neighbouring populations. Only one genotype was found to be common to a maximum of four populations indicating that clones are not typically widely dispersed and do not represent generalist genotypes (Baker 1965; Lynch 1984; Vrijenhoek 1998). Despite the use of a combination both of microsatellite and allozyme markers in this study, I may still not have been able to identify all clones and therefore individuals identified as potential clones mates may actually be genotypically distinct. This was further confirmed with my assignment analysis, which revealed that miss-assigned individuals were most likely to be assigned to a neighbouring population. Individual-based assignment tests have increasingly been used by researchers to assess the level of natal dispersal between populations (Waser and Strobeck 1998; Davies *et al.* 1999; Berry *et al.* 2004). However, the use of assignment tests to identify possible migrants is highly dependent on the number of loci used, and the degree of differentiation between populations. My assignment test indicates that populations in NSW show higher levels of connectivity than those in Victoria and Tasmania and is consistent with the strong southerly movement of the East Australian Current along this section of coast. The presence of anti-cyclonic eddies also allows for the potential northerly movement of propagules between neighbouring populations. The division of the East Australian Current near the NSW/Victorian border results in substantially less current flow along the Victorian coast and may result in fewer migration events between established populations in Victoria. Samples from NSW populations were on average miss-assigned to 6.2 populations while Victoria and Tasmanian samples were on average only miss-assigned to 3.9 populations.

Taken together this data and previous studies of *A. tenebrosa* suggest that the effects of asexual reproduction determine fine-scale structure of local populations. Sexual reproduction appears to be the source of most of the genotypic diversity observed within populations but occurs very infrequently. High levels of genotypic diversity appear to be maintained in the majority of populations despite sexual episodes being rare and is likely to be maintained by either heterogeneity in the local environment allowing greater niche

separation between clones, selection for different clones during different periods or the coexistence of ecologically similar clones.

Chapter 8 General Discussion and Conclusions

8.1 The Importance of Sexual and Asexual Reproduction in Different Habitats

The aim of this thesis was to test several theoretical predictions on the relative importance of sexual and asexual reproduction and how this varies with environmental heterogeneity, using three anthozoan species as model organisms. Specifically, I assessed whether the mode of reproduction and levels of genotypic diversity varied among habitats and tested for evidence of local adaptation in response to fine-scale environmental variation. The prediction that organisms with mixed life history strategies will use sexual reproduction to produce genotypically diverse and widely dispersed propagules, enabling the colonisation of distant or heterogeneous habitats, while asexual reproduction will be used to restock or maintain locally adapted populations within the parental habitat patch (Williams 1975; Maynard Smith 1978; Bell 1982), appears to be overly simplistic. The body of data presented in this thesis suggests that for some species, such as the brooding sea anemone *Actinia tenebrosa*, the importance of sexual and asexual reproduction may indeed vary among habitats with different levels of heterogeneity in the manner predicted by evolutionary theory (Chapters 5 & 6). However, there are limitations to the generality of such theoretical predictions, which do not appear to hold for some species such as the brooding corals *Pocillopora damicornis* and *Seriatopora hystrix* (Chapters 2 & 3). In this final chapter, I discuss how these results relate to current understanding of life history theory, some of the limitations of these studies, and outline some exciting areas of future research within this field.

8.1.1 Mode of Reproduction

One possible explanation for the large amount of spatial and temporal variation in genotypic diversity reported for populations of some clonal organisms (Ellstrand and Roose 1987; Weider 1992; Widen *et al.* 1994), is the ability to shift from one mode of reproduction to another (i.e. from asexual to sexual) under changing environmental conditions. This strategy is common among many clonal organisms and has been shown to occur in response to seasonal changes in environmental conditions, or when conditions

become unfavourable. This is characteristic of many cyclical parthenogens (i.e.cladocerans & aphids) that use sexual reproduction to produce genotypically diverse dormant eggs during periods of harsh environmental conditions, and asexually when favourable conditions return (Weider 1992; Geedey *et al.* 1996; aphids: Rispe *et al.* 1998). Additionally, a number of species have been reported to show geographical variation in the mode of reproduction, with sexual and asexual lineages being associated with different environments or habitats (Ellstrand 1987, Peak *et al.* 1998). There are clear benefits in being able to shift from one mode of reproduction to another under different environmental conditions, however, I detected no such variation in the mode of reproduction within the three species studied here that could explain the large variation in genotypic diversity detected within different habitats in this and previous studies. My genetic analysis of the brooded larvae of *A. tenebrosa* and *P. damicornis* from different habitats suggested that all broods surveyed in these studies were produced asexually (Chapters 2 & 4). The brooded larvae of *P. damicornis*, collected from colonies from five reef habitats over a three-year period, all displayed identical electrophoretic banding patterns to that of their brood parent (Chapter 2). Similarly, I found that the brooded juveniles of *A. tenebrosa* collected from boulder and rock pool habitats displayed identical allozyme and microsatellite genotypes to that of their brood parent (Chapter 4). I also found that the mode of production of brooded larvae was consistent across a wide geographical range in *A. tenebrosa* along the east coast of Australia, including populations that varied widely in their levels of genotypic diversity (Chapters 4 & 7). This suggests that variation in the mode of reproduction cannot account for differences in the levels of genotypic diversity among habitats or populations in these two species, and other factors, such as the relative survival of the initial sexual colonists, potentially mediated through varying levels of inter-genotypic competition (Brace *et al.* 1979; Ayre 1983b) and the level and intensity of selection (Chapter 6, Ayre 1985, 1995), are likely to be responsible for variation in levels of genotypic diversity among habitats previously detected within these two species (Ayre 1984b; Stoddart 1984a; Ayre and Hughes 2004; Miller and Ayre 2004).

In contrast to *P. damicornis* and *A. tenebrosa*, I found that the brooded larvae of *Seriatopora hystrix* collected for a single site were sexually produced, a significant

proportion of which were likely to be the result of self-fertilisation. While the lack of broods collected from replica sites means I am unable to determine if the mode of reproduction in this species varies among habitats, my finding that broods are sexually produced is consistent with the only other study to assess the mode of production of a single brood within this species (Ayre and Resing 1986). Asexual reproduction via fragmentation appears to contribute little to local populations in *S. hystrix* (Ayre and Dufty 1994) and it appears that in contrast to the closely related coral *P. damicornis*, the mating system of *S. hystrix* is probably best characterised by a mixed mating model where a proportion of the progeny in a population are derived from self-fertilisation, while the remaining proportion represent outcrosses (e.g. Ritland and Jain 1981; Ritland 2002).

8.1.2 Genotypic Diversity, the Distribution of Clones and Scale of Localised Adaptation

Based on evolutionary theory, I predicted that the relative importance of sexual and asexual reproduction would vary among habitats with differing levels of environmental heterogeneity (Chapter 1). My genetic surveys of *A. tenebrosa* suggest that environmental heterogeneity is important in determining the levels of genotypic diversity and the distribution of clones for this species (Chapters 5 & 7). Levels of genotypic diversity appear to be greatest within the more heterogeneous boulder habitat than the more stable and homogenous rock pool habitat, and that almost exclusively different clones occupied each habitat (I found only a single case of a clone shared between boulder and rock pool habitats at Bass Point, Chapter 5). However, I did detect some apparent sharing of clones among sites within a habitat. Higher levels of genotypic diversity associated with increased habitat heterogeneity have been reported for a number of clonal plant (Linhart and Grant 1996; Lehmann 1997) and animal species (Hunter 1993; Geedey *et al.* 1996). For example, in four populations of the vegetatively propagating grass *Calamagrostis epigejos*, Lehmann (1997) detected only 5 and 10 unique genotypes within two stable and unpolluted populations compared with 59 and 92 unique genotypes detected at two polluted sites. The higher level of diversity within polluted sites was believed to be due to niche separation within a heterogeneous environment and a decrease in the importance of intraspecific competition among established genotypes. The coexistence of a large number of different

clones within populations has also been reported for a number of other clonal sea anemones including *Metridium senile* (Hoffmann 1976; Shick *et al.* 1979), *Anthopleura elegantissima* (Sebens 1982; Sebens and Thorne 1985; Ayre and Grosberg 1995), *Sagartia elegans* (Shaw 1991) and *Nematostella vectensis* (Darling et al. 2004), although only Shick *et al.* (1979) study assessed levels of genotypic diversity in response to habitat variation. In their study Shick *et al.* (1979) reported that the level of genotypic diversity within populations of *M. senile* varied in response to current velocity, with reduced levels of genotypic diversity detected in high flow areas. In high velocity habitats, asexual reproduction is believed to be favoured by a stable and consistent supply of food, while asexual proliferation within low velocity habitats was inhibited by a less reliable source of food, although individual anemones were larger.

The limited sharing of clones among habitats, and even among sites within a habitat in *A. tenebrosa*, could simply reflect the limited dispersal capabilities of asexual propagules, such that clones are restricted to a particular habitat because of their inability to disperse to a neighbouring habitat. Alternatively, the restricted distribution of clones may reflect selection for locally adapted genotypes within a particular habitat type. The results from my transplant experiments suggested that clones are indeed locally adapted at the scale of the habitat. Clones transplanted between habitats separated by less than 200 meters performed poorly in comparison to native anemones (Chapter 6). For *A. tenebrosa*, and potentially a large number of other clonal sea anemones such as *M. senile*, *A. elegantissima*, and *S. elegans*, theoretical predictions on the proximate roles of sexual and asexual reproduction appear to fit well with observations on the population structure, distribution of genotypic diversity, and the scale of localised adaptation.

In contrast, the results from my study on the population structure of *P. damicornis* challenge the established paradigm that asexual reproduction is used to maintain local populations, while sexual reproduction will be used to produce genotypically diverse and widely dispersed propagules. The results from this and previous studies of *P. damicornis* on the southern Great Barrier Reef (Benzie *et al.* 1995; Ayre and Miller 2004; Miller and Ayre 2004), suggest that the asexual propagules of this species do not recruit locally within

the majority of reef habitats, despite asexual reproduction being a major reproductive output within this species (Chapter 2). Variation in the relative contributions of sexual and asexual reproduction among the different reef habitats, or the presence of cryptic species failed to offer a plausible explanation for the high levels of genotypic diversity detected in this species (Chapter 2, Benzie *et al.* 1995; Ayre and Miller 2004; Miller and Ayre 2004). While I did find that clonal recruitment appears to be favoured within recently disturbed habitats, the function of asexual propagules in maintaining populations of *P. damicornis* in the majority of reef habitats, therefore, remains equivocal, and populations of *P. damicornis* on the southern Great Barrier Reef appear to be a poor fit to theoretical predictions of evolutionary theory.

Why two species such as *A. tenebrosa* and *P. damicornis*, which appear to have similar life histories, should have such different populations structures remains unclear. In *A. tenebrosa* asexual propagules appear to maintain local population, and levels of genotypic diversity vary predictably with levels of habitat heterogeneity. In *P. damicornis* there is no evidence of localised recruitment of asexual propagules and the function of asexual propagules in maintaining populations of *P. damicornis* in the majority of reef habitats therefore remains equivocal. One possible explanation for the lack of clonality within local populations is that asexual larvae disperse more widely than that predicted for asexual propagules. While widespread dispersal of asexual larvae may provide a potential explanation for the lack of clonal structure in local populations, understanding the evolution and maintenance both of sexual and asexual propagules for widespread dispersal remains problematic. Widespread dispersal of asexually generated propagules is typically reported for exclusively clonal species with populations dominated by a few generalist genotypes (Kirschner and Stepanek 1994; Chaplin and Ayre 1997; Rogstad, et al. 2002). However, I detected no evidence of widespread and common genotypes in my genotypic surveys of *P. damicornis* from six reef habitats (Chapter 2) to support the hypothesis that asexual propagules disperse widely and are adapted to a range of reef habitats. It is clear that sexual and asexual reproduction can be used in the manner predicted by theoretical models such as William's (1975) Strawberry Coral Model, and that this model may be particularly applicable to many clonal sea anemones such as *A. tenebrosa*. However, it is also clear that

this model may be overly simplistic for the brooding corals *P. damicornis* and *S. hystrix*, and may not be as widely applicable to many other coral species for which this model was initially envisaged. Many corals have branching structures that may make them susceptible to fragmentation (i.e. *S. hystrix*), however, it is becoming increasingly clear that successful recruitment of fragments can be low (Tunnicliffe 1981; Highsmith 1982) and that for at least some species, asexual recruitment via fragmentation is unimportant (Ayre and Dufty 1994; Ayre and Hughes 2000). For such species, predictions of the Strawberry Coral Model are unlikely to hold and it is becoming increasing clear that many coral species, which have the potential to reproduce both sexually and asexually, populations show little evidence of clonal recruitment (Ayre and Dufty 1994; Adjeroud and Tsuchiya 1999; Ridgway *et al.* 2001; Hellberg and Taylor 2002; Miller and Ayre 2004). However, there are also clearly a number of coral species where asexual reproduction does appear to be important for localised recruitment (Ayre and Willis 1988; McFadden 1997; Coffroth and Lasker 1998b; Chen *et al.* 2002) and further comparative research, on a wider range of partially clonal organisms, will be needed to identify the wider applicability of these models and predictions.

8.1.3 Future directions

8.1.3.1 The Role of Asexual Reproduction in P. damicornis

Clearly more intensive and extensive sampling of *P. damicornis* needs to be carried out for a range of different habitats, including habitats that have experienced varying levels and types of disturbance. This may provide a partial explanation for the persistence of sexual and asexual modes of reproduction for this species. Direct comparisons of the dispersal abilities and physiological differences of sexually and asexually generated larvae would ideally be needed to understand the roles that each of these types of propagules play in maintaining populations of *P. damicornis*, but is unlikely to be possible until better information on how and when sexual reproduction occurs in this species. Finally, studies that investigate genotype-environment interactions may also provide valuable information on the importance of genotypic diversity and the level of adaptation in populations of *P. damicornis*. The use of reciprocal transplant experiments offers one such powerful approach. The modular nature of coral colonies also makes them amenable to both field

and laboratory studies as individual colonies can be fragmented into multiple genetically identical units and assigned to different environmental treatments (e.g. Potts 1984; Hunter and Hughes 1995). This would then allow comparisons of the fitness of the same genotype under different environmental conditions.

8.1.3.2 The A. tenebrosa Paradox

While my study of genotypic diversity and the scale of local adaptation in *A. tenebrosa* support and confirm the apparent fit of this species to predictions for organisms with a mixed mode of reproduction, these results also present a paradox. Sexually derived colonists undoubtedly are the main source of genotypic diversity within populations (although somatic mutation may make a small contribution, Chapter 5) and will provide the raw material for natural selection. Yet despite the high levels of genotypic diversity seen in populations, and the high level of adaptation detected over fine spatial scales, the input of sexual recruits into these populations appears to be extremely rare. Recruitment into cleared areas during this study and previous studies of Western Australian populations indicate that sexual reproduction, or at least recruitment of sexual propagules, has not occurred during the last 22 years (Ayre, unpublished data).

Indeed, the apparent lack of functional females within populations of *A. tenebrosa* on the east coast of Australia could suggest that sexual reproduction may no longer be possible (Chapter 6). However, the presence of non-sexual individuals within populations may represent female clones that have not developed gonads and therefore remain undetectable during dissections and gonad observations. Gender in *A. tenebrosa* is genetically determined (Ayre 1988) and there is no evidence of sequential hermaphroditism within this species, therefore male and female clones should be genotypically distinct. Further genetic surveys are needed to determine if all individuals of a particular clone are either all male or all non-sexual. This may provide indirect evidence that females are present within these populations, but remain undetected. The identification of sex specific genetic markers would be extremely useful in testing this hypothesis, and may be identifiable from Western Australian populations which consist both of male and female clones. Amplified fragment length polymorphisms (AFLP's) provide a simple and relatively inexpensive technique that

has been useful in identifying sex specific bands in a number of other species (Witkowicz *et al.* 2003; Rahman and Ainsworth 2004; Stehlik and Blattner 2004; Hinckley *et al.* 2005). Alternatively, the apparent lack of females within east coast Australian populations may indicate selection against female clones (gender biased mortality) or gender differences in the rate of cloning such that female clones have been competitively excluded from most populations. Gender differences in the rate of cloning have been shown for the brittle star *Ophiactis savignyi* where male clones are significantly more like to reproduce asexually via fission than females, resulting in a significant male biased sex-ratio (McGovern 2002). The potential loss of functional females from east coast populations of *A. tenebrosa* has important implications for the long-term survival of these populations. Despite the high levels of genotypic diversity I detected within these populations, without further input of genotypic diversity via sexually derived recruits, the erosion of genotypic diversity through continued selection and inter-clonal competition means that populations may eventually become genetically poor, potentially reducing the ability of such populations to respond to major environmental changes. This is particularly concerning considering the potential effects of global climate change on marine species along the east coast of Australia (e.g. Ayre and Hughes 2004)

8.1.3.3 The Environmental and Biological Factors Driving Selection in A. tenebrosa

While this study has been important in identifying the scale of local adaptation, we still know little about the biological and environmental factors that are important in the selection of locally adapted clones. *A. tenebrosa* provides a model for determining those life history traits that are under selection. The two habitats used in the reciprocal transplant studies for *A. tenebrosa* differ in a number of physical and biological characteristics that may have lead to the localised adaptation observed (Chapter 6). The clonal nature of this species means that identical genotypes can be exposed to a range of different environmental and biological parameters under controlled laboratory conditions (i.e. Hunter and Hughes 1995). Laboratory experiments will also allow complex experimental designs that may allow the determination of synergistic effects on clonal fitness and other life history traits. Biological selection pressures may be more difficult to determine and may require field experiments that manipulate the community structure within the habitat of different clones.

Additionally, exclusion cages may also be used to prevent the re-colonisation of cleared areas or to identify the effects of excluding mobile organisms such as predators that may interact with these communities.

8.1.3.4 Importance of Self-Fertilisation in Corals

Much attention has been paid to the potentially detrimental effects of inbreeding (i.e. inbreeding depression) between closely related individuals within populations, including the extreme of self-fertilisation (Shields 1982; Charlesworth and Charlesworth 1999). However, despite the vast majority of scleractinian corals having the potential to self-fertilise (more than 68% of species are hermaphroditic, Harrison and Wallace, 1990), only a few studies have assed the potential for self-fertilisation within this group (Stoddart *et al.* 1988; Willis *et al.* 1997; Miller and Babcock 1997). Inbreeding depression has been demonstrated in Venetian populations of the ascidian, *Botryllus schlosseri*, where self-fertilised progeny displayed much higher levels of abnormality and mortality compared with cross-fertilised controls (Sabbadin 1979). However, the opposite situation of outbreeding depression has been reported for North American populations of *B. schlosseri* where success of fertilisation, embryogenesis and larval metamorphosis were inversely proportional to genetic dissimilarity between parental colonies (Grosberg 1987). My results show that levels of selfing may be important reproductive strategy in corals and that levels of selfing can vary widely among individuals. Levels of selfing are also likely to vary among habitats and populations due to differing levels of adult densities and water flow regimes. Further research that include a greater variety of habitats will be needed to establish the generality of these results and determine any potential detrimental effects of either inbreeding or outbreeding depression within populations of *S. hystrix*. Levels of outcrossing are likely to be affected by a range of environmental (i.e. local hydronamic patterns) and biological factors (i.e. adult densities). Experimental manipulation of adult densities and the placement of colonies in relation to current direction, provide a useful approach for determining some of the environmental and biological factors affecting outcrossing. This system also provides an opportunity to compare and assess the relative fitness of self-fertilised *versus* cross-fertilised offspring under controlled laboratory conditions.

References

Adjeroud, M. and Tsuchiya, M. (1999) Genetic variation and clonal structure in the scleractinian coral *Pocillopora damicornis* in the Ryukyu Archipelago, southern Japan. Marine Biology, 134: 753-760.

Amos, W., Sawcer, S.J., Feakes, R.W., and Rubinsztein, D.C. (1996) Microsatellites show mutational bias and heterozygote instability. Nature Genetics, 13: 390-391.

Angus, R.A. (1980) Geographic dispersal and clonal diversity in unisexual fish populations. American Naturalist, 115: 531-550.

Appleton, R.D. and Palmer, A.R. (1988) Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proceedings of the National Academy of Sciences of the United States of America, 85: 4387-4391.

Arnaud-Haond, S., Alberto, F., Teixeira, S., Procaccini, G., Serrao, E.A., and Duarte, C.M. (2005) Assessing genetic diversity in clonal organisms: Low diversity or low resolution? Combining power and cost efficiency in selecting markers. Journal of Heredity, 96, 434-440.

Atoda, K. (1951) The larva and postlarval development of some reef building corals. V. *Serriatopora hystrix* (Dana). Science reports of the Tohoku University, 4th series, XIX No. 1: 33-39.

Ayre, D.J. (1982) Inter-genotype aggression in the solitary sea anemone *Actinia tenebrosa*. Marine Biology, 68: 199-206.

Ayre, D.J. (1983a) The distribution of the sea anemone *Actinia tenebrosa* in southwestern Australia. Western Australian Naturalist., 15(6): 136-140.

Ayre, D.J. (1983b) The effects of asexual reproduction and inter genotypic aggression on the genotypic structure of populations of the sea anemone *Actinia tenebrosa*. Oecologia, 57: 158-165.

Ayre, D.J. (1984a) Effects of environment and population density on the sea anemone *Actinia tenebrosa*. Australian Journal of Marine and Freshwater Research, 35: 735-746.

Ayre, D.J. (1984b) The effects of sexual and asexual reproduction on geographic variation in the sea anemone *Actinia tenebrosa*. Oecologia, 62: 222-229.

Ayre, D.J. (1985) Localized adaptation of clones of the sea anemone *Actinia tenebrosa*. Evolution, 39: 1250-1260.

Ayre, D.J. (1987) The formation of clonal territories in experimental populations of the sea anemone *Actinia tenebrosa*. Biological Bulletin, 172: 178-186.

Ayre, D.J. (1988) Evidence for genetic determination of sex in *Actinia tenebrosa*. Journal of Experimental Marine Biology & Ecology, 116: 23-34.

Ayre, D.J. (1990) Population subdivision in Australian temperate marine invertebrates: Larval connections *versus* historical factors. Australian Journal of Ecology, 15: 403-411.

Ayre, D.J. (1995) Localized adaptation of sea anemone clones: Evidence from transplantation over two spatial scales. Journal of Animal Ecology, 64: 186-196.

Ayre, D.J., Davis, A.R., Billingham, M., Llorens, T., and Styan, C. (1997a) Genetic evidence for contrasting patterns of dispersal in solitary and colonial ascidians. Marine Biology, 130: 51-61.

Ayre, D.J. and Dufty, S. (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia Great Barrier Reef. Evolution, 48: 1183-1201.

Ayre, D.J. and Grosberg, R.K. (1995) Aggression, habituation, and clonal coexistence in the sea anemone *Anthopleura elegantissima*. American Naturalist, 146: 427-453.

Ayre, D.J. and Grosberg, R.K. (2005) Behind anemone lines: factors affecting division of labour in the social cnidarian *Anthopleura elegantissima*. Animal Behaviour, 70: 97-110.

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

Ayre, D.J. and Hughes, T.P. (2004) Climate change, genotypic diversity and gene flow in reef-building corals. Ecology Letters, 7*:* 273-278.

Ayre, D.J., Hughes, T.P., and Standish, R.S. (1997b) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier Reef, Australia. Marine Ecology Progress Series, 159*:* 175-187.

Ayre, D.J. and Miller, K.J. (2004) Where do clonal larvae go? Adult genotypic diversity conflicts with reproductive effort in the brooding coral *Pocillopora damicornis*. Marine Ecological Progress Series, 277*:* 95-105.

Ayre, D.J. and Miller, K.J. (in press) Random mating in the brooding coral *Acropora palifera.* Marine Ecological Progress Series.

Ayre, D.J., Read, J., and Wishart, J. (1991a) Genetic subdivision within the eastern Australian population of the sea anemone *Actinia tenebrosa*. Marine Biology, 109*:* 379-390.

Ayre, D.J. and Resing, J.M. (1986) Sexual and asexual production of planulae in reef corals. Marine Biology, 90*:* 187-190.

Ayre, D.J., Veron, J.E.N., and Dufty, S.L. (1991b) The corals *Acropora palifera* and *Acropora cuneata* are genetically and ecologically distinct. Coral Reefs, 10*:* 13-18.

Ayre, D.J. and Willis, B.L. (1988) Population structure in the coral *Pavona cactus* clonal genotypes show little phenotypic plasticity. Marine Biology, 99*:* 495-506.

Babcock, R.C., Bull, G.D., Harrison, P.L., Heyward, A.J., Oliver, J.K., Wallace, C.C., and Willis, B.L. (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef Australia. Marine Biology, 90*:* 379-394.

Babcock, R.C., Mundy, C.N., and Whitehead, D. (1994) Sperm diffusion models and in situ confirmation of long-distance fertilization in the free-spawning asteroid *Acanthaster planci*. Biological Bulletin, 186*:* 17-28.

Baird, A.H. and Babcock, R.C. (2000) Morphological differences among three species of newly settled Pocilloporid coral recruits. Coral Reefs, 19*:* 179-183.

Baird, A.H. and Marshall, P.A. (1998) Mass bleaching of corals on the Great Barrier Reef. Coral Reefs, 17*:* 376.

Baker, H.G. (1965). Charactersitics and modes of origins of weeds. In: Genetics of colonizing species (eds H.G. Baker and G.L. Stebbins), pp. 147-172. Academic Press, New York.

Balloux, F. and Lugon-Moulin, N. (2002) The estimation of population differentiation with microsatellite markers. Molecular Ecology, 11*:* 155-165.

Barker, J.S.F., Moore, S.S., Hetzel, D.J.S., Evans, D., Tan, S.G., and Byrne, K. (1997) Genetic diversity of Asian water buffalo (*Bubalus bubalis*): Microsatellite variation and a comparison with protein-coding loci. Animal Genetics, 28*:* 103-115.

Beck, M.W. (2000) Separating the elements of habitat structure: independent effects of habitat complexity and structural components on rocky intertidal gastropods. Journal of Experimental Marine Biology and Ecology, 249*:* 29–49.

Bell, G. (1982) The masterpiece of nature: the evolution and genetics of sexuality Croom Helm, London.

Bell, G. and Wolfe, L.M. (1985) Sexual and asexual reproduction in a natural population of *Hydra pseudoligactis*. Canadian Journal of Zoology, 63*:* 851-856.

Bennington, C.C. and McGraw, J.B. (1995) Natural selection and ecotypic differentiation in I*mpatiens pallida*. Ecological Monographs, 65*:* 303-323.

Benzie, J.A.H., Haskell, A., and Lehman, H. (1995) Variation in the genetic composition of coral (*Pocillopora damicornis* and *Acropora palifera*) populations from different reef habitats. Marine Biology, 121*:* 731-739.

Berry, O., Tocher, M.D., and Sarre, S.D. (2004) Can assignment tests measure dispersal? Molecular Ecology, 13*:* 551-561.

Billingham, M.R. and Ayre, D.J. (1996) Genetic subdivision in the subtidal, clonal sea anemone *Anthothoe albocincta*. Marine Biology, 125*:* 153-163.

Billingham, M.R. and Ayre, D.J. (1997) Asexual reproduction and genetic determination of colour patterns within populations of the subtidal sea anemone *Anthothoe albocincta*. Marine Ecology Progress Series, 156*:* 121-130.

Black, R. and Johnson, M.S. (1979) Asexual viviparity and population genetics of *Actinia tenebrosa*. Marine Biology, 53*:* 27-31.

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

Boland, F.M. and Church, J.A. (1981) The East Australian Current 1978. Deep-Sea Research Part a-Oceanographic Research Papers, 28*:* 937-957.

Booth, D.J. and Beretta, G.A. (2002) Changes in a fish assemblage after a coral bleaching event. Marine Ecology Progress Series, 245*:* 205-212.

Borsa, P., Jousselin, Y., and Delay, B. (1992) Relationships between allozymic heterozygosity, body size, and survival to natural anoxic stress in the Palourde, *Ruditapes decussatus* L (Bivalvia, Veneridae). Journal of Experimental Marine Biology and Ecology, 155*:* 169-181.

Brace, R.C., Pavey, J., and Quicke, D.L.J. (1979) Intraspecific aggression in the color morphs of the anemone *Actinia equina* - Convention governing dominance ranking. Animal Behaviour, 27*:* 553-&.

Brazeau, D.A., Gleason, D.F., and Morgan, M.E. (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: Evidence from molecular markers. Journal of Experimental Marine Biology and Ecology, 231*:* 225-238.

Bucklin, A., Hedgecock, D., and Hand, C. (1984) Genetic evidence of self-fertilization in the sea anemone *Epiactis prolifera*. Marine Biology, 84*:* 175-182.

Cain, A.J. (1974) Breeding system of a sessile animal. Nature, 247*:* 289-290.

Carlon, D.B. (1999) The evolution of mating systems in tropical reef corals. Trends in Ecology and Evolution, 14*:* 491-495.

Carter, M.A. and Miles, J. (1989) Gametogenic cycles and reproduction in the beadlet sea anemone *Actinia equina* (Cnidaria: Anthozoa). Biological Journal of the Linnean Society, 36*:* 129-155.

Carter, M.A. and Thorp, C.H. (1979) Reproduction of *Actinia equina* var. *mesembryanthemum*. Journal of the Marine Biological Association of the United Kingdom, 59*:* 989-&.

Carvalho, G.R. (1994). Genetics of aquatic clonal organisms. In Genetics and evolution of aquatic organisms (ed R. Beaumont A), pp. 291-322. Chapman and Hall, London.

Carvalho, G.R. and Wolf, H.G. (1989) Resting eggs of lake-*Daphnia* .1. Distribution, abundance and hatching of eggs collected from various depths in lake sediments. Freshwater Biology, 22*:* 459-470.

Chaplin, J.A. and Ayre, D.J. (1997) Genetic evidence of widespread dispersal in a parthenogenetic freshwater ostracod. Heredity, 78*:* 57-67.

Chapman, M.G. (2002) Early colonisation of shallow subtidal boulders in two habitats. Journal of Experimental Marine Biology and Ecology, 275*:* 95-116.

Charlesworth, B. and Charlesworth, D. (1999) The genetic basis of inbreeding depression. Genetical Research, 74*:* 329-340.

Chen, C.A., Wei, N.V., and Dai, C.F. (2002) Genotyping the clonal population structure of a gorgonian coral, *Junceella fragilis* (Anthozoa : Octocorallia : Ellisellidae) from Lanyu, Taiwan, using simple sequence repeats in ribosomal intergenic spacer. Zoological Studies, 41*:* 295-302.

Chia, F.S. and Rostron, M.A. (1970) Some aspects of reproductive biology of *Actinia equina.* (Cnidaria : Anthozoa). Journal of the Marine Biological Association of the United Kingdom, 50*:* 253-259

Chiswell, S.M., Toole, J., and Church, J. (1997) Transports across the Tasman Sea from WOCE repeat sections: the East Australian Current 1990-94. New Zealand Journal of Marine and Freshwater Research, 31*:* 469-475.

Coffroth, M.A. and Lasker, H.R. (1998a) Larval paternity and male reproductive success of a broadcast-spawning gorgonian, *Plexaura kuna*. Marine Biology, 131*:* 329-337.

Coffroth, M.A. and Lasker, H.R. (1998b) Population structure of a clonal gorgonian coral: The interplay between clonal reproduction and disturbance. Evolution, 52*:* 379-393.

Cohen, C.S. (1996) The effects of contrasting modes of fertilization on levels of inbreeding in the marine invertebrate genus *Corella*. Evolution, 50*:* 1896-1907.

Colihueque, N., Vergara, N., and Parraguez, M. (2003) Genetic characterization of naturalized populations of brown trout *Salmo trutta* L. in southern Chile using allozyme and microsatellite markers. Aquaculture Research, 34: 525-533.

Coll, J.C., Leone, P.A., Bowden, B.F., Carroll, A.R., Konig, G.M., Heaton, A., De Nys, R., Maida, M., Alino, P.M., Willis, R.H., Babcock, R.C., Florian, Z., Clayton, M.N., Miller, R.L., and Alderslade, P.N. (1995) Chemical aspects of mass spawning in corals. II. :epi-thunbergol, the sperm attractant in the eggs of the soft coral *Lobophytum crassum* (Cnidaria: Octocorallia). Marine Biology, 123: 137-143.

Condit, R. and Hubbell, S.P. (1991) Abundance and DNA sequence of two-base repeats in plant genomes. Genome, 34: 66-71.

Connell, J.H. (1978) Diversity in tropical rain forests and coral reefs - High diversity of trees and corals is maintained only in a non-equilibrium state. Science, 199: 1302-1310.

Connell, J.H. (1997) Disturbance and recovery of coral assemblages. Coral Reefs, 16(SUPPL.): S101-S113.

Connell, J.H., Hughes, T.P., and Wallace, C.C. (1997) A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecological Monographs, 67: 461-488.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A., and Solignac, M. (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics, 153, 1989-2000.

Darling, J.A., Reitzel, A.M., and Finnerty, J.R. (2004) Regional population structure of a widely introduced estuarine invertebrate: *Nematostella vectensis* Stephenson in New England. Molecular Ecology, 13: 2969-2981.

Davis, A.N. and Wilce, R.T. (1987) Algal diversity in relation to physical disturbance: a mosaic of successional stages in a subtidal cobble habitat. Marine Ecology Progress Series, 37: 229–237.

Davies, N., Villablanca, F.X., and Roderick, G.K. (1999) Determining the source of individuals: multilocus genotyping in nonequilibrium population genetics. Trends in Ecology & Evolution, 14: 17-21.

DeWitt, T.J., Sih, A., and Wilson, D.S. (1998) Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution, 13: 77-81.

Done, T.J. (1982) Patterns in the distribution of coral communities across the central Great Barrier Reef. Coral Reefs, 1: 95-107.

Douek, J., Barki, Y., Gateno, D., and Rinkevich, B. (2002) Possible cryptic speciation within the sea anemone *Actinia equina* complex detected by AFLP markers. Zoological Journal of the Linnean Society, 136: 315-320.

Doyle, J.J. and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11-15.

Duran, S., Pascual, M., Estoup, A., and Turon, X. (2004) Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Molecular Ecology, 13: 511-522.

Eckert, C.G., Lui, K., Bronson, K., Corradini, P., and Bruneau, A. (2003) Population genetic consequences of extreme variation in sexual and clonal reproduction in an aquatic plant. Molecular Ecology, 12: 331-344.

Edmands, S. (1995) Mating systems in the sea anemone genus *Epiactis*. Marine Biology, 123: 723-733.

Edmands, S. and Potts, D.C. (1997) Population genetic structure in brooding sea anemones (*Epiactis spp.*) with contrasting reproductive modes. Marine Biology, 127: 485-498.

Ellegren, H. (2004) Microsatellites: Simple sequences with complex evolution. Nature Reviews Genetics, 5: 435-445.

Ellstrand, N.C. and Roose, M.I. (1987) Patterns of genotypic diversity in clonal plant species. American Journal of Botany, 74: 123-131.

Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J.M., Adriamanga, M., and Guyomard, R. (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). Molecular Ecology, 7: 339-353.

Estoup, A., Tailliez, C., Cornuet, J.M., and Solignac, M. (1995) Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). Molecular Biology and Evolution, 12: 1074-1084.

Fadlallah, Y.H. (1982) Reproductive ecology of the coral *Astrangia lajollaensis*: sexual and asexual patterns in a kelp forest habitat. Oecologia, 55: 379-388.

Foltz, D.W. (1986) Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica* and other bivalves. Evolution, 40: 869-870.

Fuji, A. and Nomura, H. (1990) Community structure of the rocky shore macrobenthos in southern Hokkaido, Japan. Marine Biology, 107, 471–477.

Fuller, S.J., Chavigny, P., Lapchin, L., and Vanlerberghe-Masutti, F. (1999) Variation in clonal diversity in glasshouse infestations of the aphid, *Aphis gossypii* (Glover) in southern France. Molecular Ecology, 8: 1867-1877.

Galloway, L.F. and Fenster, C.B. (2000) Population differentiation in an annual legume: Local adaptation. Evolution, 54: 1173-1181.

Gashout, S.E. and Ormond, R.F.G. (1979) Evidence for parthenogenetic reproduction in the sea anemone *Actinia equina* L. Journal of the Marine Biological Association of the United Kingdom, 59: 975-987.

Geedey, C.K., Tessier, A.J., and Machledt, K. (1996) Habitat heterogeneity, environmental change, and the clonal structure of Daphnia populations. Functional Ecology, 10: 613-621.

Gibbs, P.E. (1993) Phenotypic changes in the progeny of *Nucella lapillus* (Gastropoda) transplanted from an exposed shore to sheltered inlets. Journal of Molluscan Studies, 59: 187-194.

Goffredo, S., Mezzomonaco, L., and Zaccanti, F. (2004) Genetic differentiation among populations of the Mediterranean hermaphroditic brooding coral *Balanophyllia europaea* (Scleractinia : Dendrophylliidae). Marine Biology, 145: 1075-1083.

Goldson, A.J., Hughes, R.N., and Gliddon, C.J. (2001) Population genetic consequences of larval dispersal mode and hydrography: A case study with bryozoans. Marine Biology, 138, 1037-1042.

Granovitch, A. and Johannesson, K. (2000) Digenetic trematodes in four species of Littorina from the west coast of Sweden. Ophelia, 53: 55-65.

Grosberg, R.K. (1987) Limited dispersal and proximity dependent mating success in the colonial ascidian *Botryllus schlosseri*. Evolution, 41: 372-384.

Grosberg, R.K., Levitan, D.R., and Cameron, B.B. (1996) Evolutionary genetics of allorecognition in the colonial hydroid *Hydractinia symbiolongicarpus*. Evolution, 50: 2221-2240.

Hamerlynck, E.P., Csintalan, Z., Nagy, Z., Tuba, Z., Goodin, D., and Henebry, G.M. (2002) Ecophysiological consequences of contrasting microenvironments on the desiccation tolerant moss *Tortula ruralis*. Oecologia, 131: 498-505.

Hamilton, W.D. (1980) Sex vs non-sex vs parasite. Oikos, 35: 282-290.

Harii, S., Kayanne, H., Takigawa, H., Hayashibara, T., and Yamamoto, M. (2002) Larval survivorship, competency periods and settlement of two brooding corals, *Heliopora coerulea* and *Pocillopora damicornis*. Marine Biology, 141: 39-46.

Harris, H. and Hopkinson, D.A. (1976) Handbook of enzyme electrophoresis in human genetics North-Holland Publishing Co Inc, Amsterdam.

Harrison, P.L., Babcock, R.C., Bull, G.D., Oliver, J.K., Wallace, C.C., and Willis, B.L. (1984) Mass Spawning in Tropical Reef Corals. Science, 223: 1186-1189.

Harrison, P.L. and Wallace, C.C. (1990) Coral Reefs. Ecosystems of the world Elsevier, Amsterdam.

Hebert, P.D.N. (1974) Ecological differences between genotypes in a natural population of *Daphnia magna*. Heredity, 33: 327-337.

Hebert, P.N. (1978) Adaptive significance of cyclomorphosis in *Daphnia*: More possibilities. Freshwater Biology, 8: 313-320.

Hebert, P.D.N. and Crease, T.J. (1980) Clonal coexistence in *Daphnia pulex* (Leydig): Another planktonic paradox. Science, 207: 1363-1365.

Hebert, P.D.N. and Ward, R.D. (1976) Enzyme variability in natural populations of *Daphnia magna* .IV. Ecological differentiation and frequency changes of genotypes at Audley End. Heredity, 36: 331-341.

Hebert, P.D.N., Ward, R.D., and Weider, L.J. (1988) Clonal diversity patterns and breeding-system variation in *Daphnia pulex*, an asexual-sexual complex. Evolution, 42: 147-159.

Hedgecock, D. (1986) Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bulletin of Marine Science, 39(2): 550-564.

Hedrick, P.W. (2000) Genetics of populations, 2nd edn. Jones and Bartlett, Boston.

Hedrick, P.W. and Kalinowski, S.T. (2000) Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics, 31: 139-162.

Hellberg, M.E. (1994) Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. Evolution, 48(6): 1829-1854.

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

Hellberg, M.E. and Taylor, M.S. (2002) Genetic analysis of sexual reproduction in the dendrophylliid coral *Balanophyllia elegans*. Marine Biology, 141: 629-637.

Henry, P-Y., Bousset, L., Sourrouille, P., and Jarne, P. (2005) Partial selfing, ecological disturbance and reproductive assurance in an invasive freshwater snail. Heredity, 95: 428-436.

Heyward, A.J. and Babcock, R.C. (1986) Self and cross-fertilization in scleractinian corals. Marine Biology, 90: 191-196.

Highsmith, R.C. (1982) Reproduction by fragmentation in corals. Marine Ecology Progress Series, 7: 207-226.

Hilbish, T.J. (1996) Population genetics of marine species: The interaction of natural selection and historically differentiated populations. Journal of Experimental Marine Biology and Ecology, 200: 67-83.

Hily, C. and Jean, F. (1997) Macrobenthic biodiversity in intertidal habitats of the Iroise Biosphere Reserve (Brittany, France). Journal of the Marine Biological Association of the United Kingdom, 77: 25–38.

Hinckley, J.D., Park, R.L., Xiong, S., Andersen, W.R., and Kooyman, D.L. (2005) Identification and development of sex specific DNA markers in the ostrich using polymerase chain reaction. International Journal of Poultry Science, 4: 663-669.

Hoare, K. and Hughes, R.N. (2001) Inbreeding and hermaphroditism in the sessile, brooding bryozoan *Celleporella hyalina*. Marine Biology, 139, 147-162.

Hoffmann, R.J. (1976) Genetics and asexual reproduction in the sea anemone *Mertidium senile*. Biological Bulletin, 151: 478-488.

Hoffmann, R.J. (1981) The evolutionary genetics of *Metridium senile*. I. Kinetic differences in the phosphoglucose isomerase allozymes. Biochemical Genetics, 19: 129-144.

Hoffmann, R.J. (1986) Variation in contributions of asexual reproduction to the genetic structure of populations of the sea anemone *Metridium senile*. Evolution, 40: 357-365.

Hoffmann, R.J. (1987) Short-term stability of genetic structure in populations of the sea anemone *Metridium senile*. Marine Biology, 93: 499-507.

Huber, H., Whigham, D., and O'Neill, J. (2004) Timing of disturbance changes the balance between growth and survival of parent and offspring ramets in the clonal forest understory herb *Uvularia perfoliata*. Evolutionary Ecology, 18: 521-539.

Hughes, R.N. and Cancino, J.M. (1985). An ecological overview of cloning in metazoa. In Population biology and evolution of clonal organisms (eds J.B.C. Jackson, L.W. Buss and C. Cook), pp. 153-186. Yale University Press, New Haven and London.

Hughes, R.N. (1989) A functional biology of clonal animals Chapman and Hall, New York.

Hughes, D.J. (1992) Genotype-environment interactions and relative clonal fitness in a marine bryozoan. Journal of Animal Ecology, 61: 291-306.

Hughes, R.N. and Taylor, M.J. (1997) Genotype-environment interaction expressed in the foraging behaviour of dogwhelks, *Nucella lapillus* (L.), under simulated environmental hazard. Proceedings of the Royal Society of London - Series B: Biological Sciences, 264, 417-422.

Hughes, T.P., Ayre, D., and Connell, J.H. (1992) The evolutionary ecology of corals. Trends in Ecology & Evolution, 7: 292-295.

Hughes, T.P. and Connell, J.H. (1999) Multiple stressors on coral reefs: A long-term perspective. Limnology & Oceanography, 44: 932-940.

Hunt, A. (1993) The effects of contrasting patterns of larval dispersal strategies on populations of two species of intertidal starfish, *Patiriella calcar* and *Patiriella exigua*. PhD Thesis, University of Wollongong.

Hunt, A. and Ayre, D.J. (1989) Population structure in the sexually reproducing sea anemone *Oulactis muscosa*. Marine Biology, 102: 537-544.

Hunter, C.L. (1993) Genotypic variation and clonal structure in coral populations with different disturbance histories. Evolution, 47: 1213-1228.

Hunter, E. and Hughes, R.N. (1993) Self-fertilisation in *Celleporella hyalina*. Marine Biology, 115, 495-500.

Hunter, E. and Hughes, R.N. (1995) Environmental and genetic components of variation in sexual allocation by an epialgal bryozoan. Marine Ecology-Progress Series., 120, 193-201.

Isomura, N. and Nishihira, M. (2001) Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. Coral Reefs, 20: 309-315.

Jackson, J.B.C. (1985). Distribution and ecology of clonal and aclonal benthic invertebrates. In: Population biology and evolution of clonal organisms (ed J.B.C. Jackson), pp. 297-355. Yale University Press, New Haven.

Jackson, J.B.C. (1986) Modes of dispersal of clonal benthic invertebrates: consequences for species distributions and genetic structure of local populations. Bulletin of Marine Science, 39(2): 588-606.

Jarne, P., Njiokou, F., Bellec, C., and Delay, B. (1996) Population genetic structure and mating systems in hermaphrodite freshwater snails: The examples of *Bulinus globosus* and *B. truncatus*. Malacological Review Supplement, 6: 115-120.

Jarne, P., Perdieu, M.A., Pernot, A.F., Delay, B., and David, P. (2000) The influence of self-fertilization and grouping on fitness attributes in the freshwater snail *Physa acuta*: population and individual inbreeding depression. Journal of Evolutionary Biology, 13: 645-655.

Johannesson, K. (2003) Evolution in Littorina: ecology matters. Journal of Sea Research, 49: 107-117.

Johannesson, K., Johannesson, B., and Rolanalvarez, E. (1993) Morphological differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail *Littorina saxatilis*. Evolution, 47: 1770-1787.

Johnson, M.S. and Black, R. (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria sp*. Marine Biology, 70: 157-164.

Johnson, M.S. and Black, R. (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution, 38: 1371-1383.

Johnson, M.S. and Black, R. (1998) Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littoraria cingulata*. Marine Biology, 132(2): 295-303.

Johnson, M.S. and Black, R. (1991) Genetic subdivision of the intertidal snail *Bembicium vittatum (*Gastropoda: Littorinidae) varies with habitat in the Houtman Abrolhos Islands Western Australia. Heredity, 67(2): 205-214.

Johnson, M.S. and Threlfall, T.J. (1987) Fissiparity and population genetics of *Coscinasterias calamaria*. Marine Biology, 93: 517-525.

Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R., Hector, A., Huss-Danell, K., Jumpponen, A., Minns, A., Mulder, C.P.H., Pereira, J.S., Prinz, A., Scherer-Lorenzen, M., Siamantziouras, A.S.D., Terry, A.C., Troumbis, A.Y., and Lawton, J.H. (2001) Local adaptation enhances performance of common plant species. Ecology Letters, 4: 536-544.

Kerszberg, M. (1989) Developmental canalization can enhance species survival. Journal of Theoretical Biology, 139: 287-309.

Kimura, M. and Crow, J.F. (1964) The number of alleles that can be maintained in a finite population. Genetics, 49: 725-738.

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

Kindell, C.E., Winn, A.A., and Miller, T.E. (1996) The effects of surrounding vegetation and transplant age on the detection of local adaptation in the perennial grass *Aristida stricta*. Journal of Ecology, 84: 745-754.

Kirschner, J. and Stepanek, J. (1994) Clonality as a part of the evolution process in *Taraxacum*. Folia Geobotanica & Phytotaxonomica, 29: 265-275.

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

Knowlton, N. and Jackson, J.B.C. (1993). Inbreeding and outbreeding in marine invertebrates. In The natural history of inbreeding and outbreeding (ed N. Thornhill), pp. 200-249. University of Chicago Press, Chicago.

Knowlton, N., Mate, J.L., Guzman, H.M., Rowan, R., and Jara, J. (1997) Direct evidence for reproductive isolation among the three species of the *Montastraea annularis* complex in Central America (Panama and Honduras). Marine Biology, 127: 705-711.

Knowlton, N., Weil, E., Weigt, L.A., and Guzman, H.M. (1992) Sibling species in *Montastraea annularis*, coral bleaching, and the coral climate record. Science, 255: 330-333.

Koehn, R.K., Newell, R.I.E., and Immermann, F. (1980) Maintenance of an aminopeptidase allele frequency cline by natural-selection. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences, 77: 5385-5389.

Kolasa J. and Rollo C.D. 1991. Introduction: the heterogeneity of heterogeneity: a glossary. In: Kolasa J. and Pickett S.T.A. (eds) Ecological Heterogeneity. Springer-Verlag, Berlin, Germany, pp. 1–23.

Ladle, R.J. (1992) Parasites and sex - catching the Red Queen. Trends in Ecology & Evolution, 7: 554-557.

Lande, R., Schemske, D.W., and Schultze, S.T. (1994) High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. Evolution, 48: 965-978.

Lasker, H.R. (1990) Clonal propagation and population dynamics of a Gorgonian coral. Ecology, 71: 1578-1589.

Lehmann, C. (1997) Clonal diversity of populations of *Calamagrostis epigejos* in relation to environmental stress and habitat heterogeneity. Ecography, 20: 483-490.

Lenssen, J.P.M., Van Kleunen, M., Fischer, M., and De Kroon, H. (2004) Local adaptation of the clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. Journal of Ecology, 92: 696-706.

Levin, S.A. (1992) The problem of pattern and scale in ecology. Ecology, 73: 1943–1967.

Levitan, D.R. and Petersen, C. (1995) Sperm Limitation in the sea. Trends in Ecology & Evolution, 10: 228-231.

Linhart, Y.B. and Grant, M.C. (1996) Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics, 27: 237-277.

Lorenzon, P., Clobert, J., and Massot, M. (2001) The contribution of phenotypic plasticity to adaptation in *Lacerta vivipara*. Evolution, 55: 392-404.

Lubbock, R. and Allbut, C. (1981) The sea anemone *Actinia equina* tolerates allogeneic juveniles but alters their phenotype. Nature, 293: 474-475.

Lynch, M. (1984) Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. Quarterly Review of Biology, 56: 257-290.

Magalon, H., Samadi, S., Richard, M., Adjeroud, M., and Veuille, M. (2004) Development of coral and zooxanthella-specific microsatellites in three species of Pocillopora (Cnidaria, Scleractinia) from French Polynesia. Molecular Ecology Notes, 4: 206-208.

Maier, E., Tollrian, R., and Nuernberger, B. (2001) Development of species-specific markers in an organism with endosymbionts: Microsatellites in the scleractinian coral *Seriatopora hystrix*. Molecular Ecology Notes, 1: 157-159.

Marshall, D.J., Semmens, D., and Cook, C. (2004) Consequences of spawning at low tide: limited gamete dispersal for a rockpool anemone. Marine Ecology-Progress Series, 266: 135-142.

Maynard Smith, J. (1978) The evolution of sex Cambridge University Press, Cambridge.

McCoy E.D. and Bell S.S. 1991. Habitat structure: the evolution and diversification of a complex topic. In: Bell S.S., McCoy E.D. and Mushinsky H.R. (eds) Habitat Structure: The Physical Arrangement of Objects in Space. Chapman & Hall, London, pp. 3–27.

McFadden, C.S. (1991) A comparative demographic analysis of clonal reproduction in a temperate soft coral. Ecology, 72: 1849-1866.

McFadden, C.S. (1997) Contributions of sexual and asexual reproduction to population structure in the clonal soft coral, *Alcyonium rudyi*. Evolution, 51: 112-126.

McGuinness, K.A. and Underwood, A.J. (1986) Habitat structure and the nature of communities on intertidal boulders. Journal of Experimental Marine Biology and Ecology, 104: 97–123.

McMahon, R.F. (1996) The physiological ecology of the zebra mussel, *Dreissena polymorpha*, in North America and Europe. American Zoologist, 36: 339-363.

McManus, M.G., Place, A.R., and Zamer, W.E. (1997) Physiological variation among clonal genotypes in the sea anemone *Haliplanella lineata*: Growth and biochemical content. Biological Bulletin, 192: 426-443.

Michod, R.E. and Levin, B.R. (1988) The evolution of sex. Sinauer Associates, Sunderland, Massachusetts.

Miller, K. and Babcock, R. (1997) Conflicting morphological and reproductive species boundaries in the coral genus Platygyra. Biological Bulletin, 192: 98-110.

Miller, K. and Mundy, C. (2003) Rapid settlement in broadcast spawning corals: implications for larval dispersal. Coral Reefs, 22: 99-106.

Miller, K.J. (1997) Genetic structure of black coral populations in New Zealand's fiords. Marine Ecology Progress Series, 161: 123-132.

Miller, K.J. (1998) Short-distance dispersal of black coral larvae: inference from spatial analysis of colony genotypes. Marine Ecology Progress Series, 163: 225-233.

Miller, K.J. and Ayre, D.J. (2004) The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity, 92: 557-568.

Miller, K.J. and Benzie, J.A.H. (1997) No clear genetic distinction between morphological species within the coral genus Platygyra. Bulletin of Marine Science, 61: 907-917.

Miller, K.J. and Howard, C.G. (2004) Isolation of microsatellites from two species of scleractinian coral. Molecular Ecology Notes, 4: 11-13.

Miller, K.J. and Mundy, C.N. (2005) In situ fertilisation success in the scleractinian coral *Goniastrea favulus*. Coral Reefs, 24: 313-317.

Miller, M.P. (1997b) Tools for population genetic analysis (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Moller, A.P. (1993) Developmental Stability, Sexual Selection and Speciation. Journal of Evolutionary Biology, 6: 493-509.

Monteiro, F.A., Russo, C.A.M., and Solé-Cava, A.M. (1998) Genetic eveidence for the asexual origin of small individuals found in the coelenteron of the sea anemone *Actinia bermudensis* Mcmurrich. Bulletin of Marine Science, 63: 257-264.

Monteiro, F.A., SoleCava, A.M., and Thorpe, J.P. (1997) Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands. Marine Biology, 129: 425-433.

Motta, J.J.C., Underwood, A.J., Chapman, M.G., and Rossi, F. (2003) Benthic assemblages in sediments associated with intertidal boulder-fields. Journal of Experimental Marine Biology and Ecology, 285: 383-401.

Murray-Jones, S.E. and Ayre, D.J. (1997) High levels of gene flow in the surf bivalve *Donax deltoides* (Bivalvia: Donacidae) on the east coast of Australia. Marine Biology, 128: 83-89.

Nagy, E.S. and Rice, K.J. (1997) Local adaptation in two subspecies of an annual plant: Implications for migration and gene flow. Evolution, 51: 1079-1089.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

Neigel, J.E. and Avise, J.C. (1983) Clonal diversity and population structure in a reef-building coral, *Acropora cervicornis*: self-recognition analysis and demographic interpretation. Evolution, 37: 437-453.

Nozawa, Y. and Harrison, P.L. (2005) Temporal settlement patterns of larvae of the broadcast spawning reef coral *Favites chinensis* and the broadcast spawning and brooding reef coral *Goniastrea aspera* from Okinawa, Japan. Coral Reefs, 24: 274-282.

O'Connell, L.M. and Eckert, C.G. (2001) Differentiation in reproductive strategy between sexual and asexual populations of *Antennaria partinii* (Asteracae). Evolutionary Ecological Research, 3: 311-330.

Olson, R.R. (1985) The consequences of short-distance larval dispersal in a sessile marine invertebrate. Ecology, 66: 30-39.

Orr, J., Thorpe, J.P., and Carter, M.A. (1982) Biochemical genetic confirmation of the asexual reproduction of brooded offspring in the sea anemone *Actinia equina*. Marine Ecology-Progress Series, 7: 227-229.

Ottaway, J.R. (1978) Population ecology of the intertidal anemone *Actinia tenebrosa* .I. Pedal locomotion and intraspecific aggression. Australian Journal of Marine and Freshwater Research, 29: 787-802.

Ottaway, J.R. (1979a) Population ecology of the intertidal anemone *Actinia tenebrosa* .II. Geographical distribution, synonymy, reproductive-cycle and fecundity. Australian Journal of Zoology, 27: 273-290.

Ottaway, J.R. (1979b) Population ecology of the intertidal anemone *Actinia tenebrosa* .III. Dynamics and environmental factors. Australian Journal of Marine and Freshwater Research, 30: 41-62.

Ottaway, J.R. (1980) Population ecology of the intertidal anemone *Actinia tenebrosa* .IV. Growth rates and longevities. Australian Journal of Marine and Freshwater Research, 31: 385-395.

Ottaway, J.R. and Kirby, G.C. (1975) Genetic relationships between brooding and brooded *Actinia tenebrosa*. Nature, 255: 221-223.

Paetkau, D. and Strobeck, C. (1994) Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology, 3: 489-495.

Palmer, A.R. (1990) Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L). Hydrobiologia, 193: 155-182.

Peakall, R. and Smouse, P.E. (2005) GenAlEx V6: Genetic analysis in Excel. Populations genetic software for teaching and research. Australian National University, Canberra, Australia. http://www.anu.edu.au/BoZo/GenALEx/.

Peck, J.R., Yearsley, J.M., and Waxman, D. (1998) Explaining the geographic distributions of sexual and asexual populations. Nature, 391, 889–892.

Pemberton, A.J., Sommerfeldt, A.D., Wood, C.A., Flint, H.C., Noble, L.R., Clarke, K.R., and Bishop, J.D.D. (2004) Plant-like mating in an animal: sexual compatibility and allocation trade-offs in a simultaneous hermaphrodite with remote transfer of sperm. Journal of Evolutionary Biology, 17: 506-518.

Perez-Figueroa, A., Cruz, F., Carvajal-Rodriguez, A., Rolan-Alvarez, E., and Caballero, A. (2005) The evolutionary forces maintaining a wild polymorphism of *Littorina saxatilis*: model selection by computer simulations. Journal of Evolutionary Biology, 18: 191-202.

Permata, W.D., Kinzie R A, III, and Hidaka, M. (2000) Histological studies on the origin of planulae of the coral *Pocillopora damicornis*. Marine Ecology Progress Series, 200: 191-200.

Perrin, M.C., Thorpe, J.P., and Sole-Cava, A.M. (1999) Population structuring, gene dispersal and reproduction in the *Actinia equina* species group. Oceanography and Marine Biology, 37: 129-152.

Piersma, T. and Drent, J. (2003) Phenotypic flexibility and the evolution of organismal design. Trends in Ecology & Evolution, 18: 228-233.

Pineda-Krch, M. and Lehtila, K. (2004) Costs and benefits of genetic heterogeneity within organisms. Journal of Evolutionary Biology, 17: 1167-1177.

Potts, D.C. (1984) Natural selection in experimental populations of reef-building corals scleractinia. Evolution, 38: 1059-1078.

Quicke, D.L.J., Donoghue, A.M., and Brace, R.C. (1983) Biochemical-genetic and ecological evidence that red brown individuals of the anemone *Actinia equina* comprise two morphs in Britain. Marine Biology, 77: 29-37.

Quicke, D.L.J., Donoghue, A.M., Keeling, T.F., and Brace, R.C. (1985) Littoral distributions and evidence for differential post-settlement selection of the morphs of *Actinia equina*. Journal of the Marine Biological Association of the United Kingdom, 65: 1-20.

Rahman, M.A. and Ainsworth, C.C. (2004) AFLP analysis of genome difference between male and females in dioecious plant *Rumex acetosa*. Journal of Biological Sciences, 4: 160-169.

Rannala, B. and Mountain, J.L. (1997) Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the United States of America, 94: 9197-9201.

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

Resing, J.M. and Ayre, D.J. (1985) The usefulness of the tissue grafting bioassay as an indicator of clonal identity in Scleractinian corals (Great Barrier Reef, Australia). In Proceedings of the Fifth International Coral Reef Congress, Tahiti, 6: 75-81.

Reusch, T.B.H. (2001) Fitness-consequences of geitonogamous selfing in a clonal marine angiosperm (*Zostera marina*). Journal of Evolutionary Biology, 14: 129-138.

Rice, W.R. (1989) Analyzing tables of statistical tests. Evolution, 43: 223-225.

Richmond, R.H. (1987) Energetics, competence, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. Marine Biology, 93: 527-533.

Richmond, R.H. and Jokiel, P.L. (1984) Lunar periodicity in larva release in the reef coral *Pocillopora damicornis* at Enewetak, Marshall slands and Hawaii, USA. Bulletin of Marine Science, 34: 280-287.

Ridgway, K.R. and Godfrey, J.S. (1997) Seasonal cycle of the East Australian Current. Journal of Geophysical Research-Oceans, 102: 22921-22936.

Ridgway, T., Hoegh-Guldberg, O., and Ayre, D.J. (2001) Panmixia in *Pocillopora verrucosa* from South Africa. Marine Biology, 139: 175-181.

Riginos, C. and Cunningham, C.W. (2005) Local adaptation and species segregation in two mussel (*Mytilus edulis* x *Mytilus trossulus*) hybrid zones. Molecular Ecology, 14: 381-400.

Rios, C. and Mutschke, E. (1999) Community structure of intertidal boulder-cobble fields in the Straits of Magellan, Chile. Sciencia Marina, 63: 193–201.

Rispe, C., Pierre, J.S., Simon, J.C., and Gouyon, P.H. (1998) Models of sexual and asexual coexistence in aphids based on constraints. Journal of Evolutionary Biology, 11: 685-701.

Ritland, K. (2002) Extensions of models for the estimation of mating systems using n independent loci. Heredity, 88: 221-228.

Ritland, K. and Jain, S. (1981) A model for the estimation of outcrossing rate and gene frequencies using n independent loci. Heredity, 47: 35-52.

Rolan-Alvarez, E., Johannesson, K., and Erlandsson, J. (1997) The maintenance of a cline in the marine snail *Littorina saxatilis*: The role of home site advantage and hybrid fitness. Evolution, 51: 1838-1847.

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

Ruggiero, M.V., Reusch, T.B.H., and Procaccini, G. (2005) Local genetic structure in a clonal dioecious angiosperm. Molecular Ecology, 14: 957-967.

Sabbadin, A. (1979). Colonial structure and genetic patterns in Ascidians. In Biology and systematics of colonial organsims (eds G. Larwood and B.R. Rosen), pp. 433-444. Academic Press, London and New York.

Schama, R., Sole-Cava, A.M., and Thorpe, J.P. (2005) Genetic divergence between east and west Atlantic populations of *Actinia spp*. sea anemones (Cnidaria : Actinidae). Marine Biology, 146: 435-443.

Scribner, K.T., Arntzen, J.W., and Burke, T. (1994) Comparative analysis of intra- and interpopulation genetic diversity in *Bufo bufo*, using allozyme, single-locus microsatellite, minisatellite, and multilocus minisatellite data. Molecular Biology and Evolution, 11: 737-748.

Sebens, K.P. (1982) Asexual reproduction in *Anthopleura elegantissima* (Anthozoa, Actiniaria): seasonality and spatial extent of clones. Ecology, 63: 434-444.

Sebens, K.P. and Thorne, B.L. (1985). Coexistence of clones, clonal diversity, and the effects of disturbance. In: Population biology and evolution of clonal organisms (ed J.B.C. Jackson), pp. 357-398. Yale University Press, New Haven.

Selander, R.K., Smith, M.H., Yank, S.Y., Johnston, W.E., and Gentry, J.B. (1971) Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in genetics, 6: 49-90.

Shaw, P.W. (1991) Effects of asexual reproduction on population structure of *Sagartia elegans* (Anthozoa, Actiniaria). Hydrobiologia, 216: 519-525.

Shick, J.M. (1991) A functional biology of sea anemones, 1st edn. Chapman & Hall, London.

Shick, J.M., Hoffmann, R.J., and Lamb, A.N. (1979) Asexual reproduction, population structure, and genotype environment interactions in sea anemones. American Zoologist, 19: 699-713.

Shick, J.M. and Lamb, A.N. (1977) Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. Biological Bulletin, 153: 604-617.

Shields, W.M. (1982) Philopatry, inbreeding, and the evolution of sex State University of New York Press, Albany.

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

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

Slatkin, M. (1993) Isolation by distance in equilibrium and nonequilibrium populations. Evolution, 47: 264-279.

Solbrig, O.T. (1971) Population biology of Dandelions. American Scientist, 59: 686-695

Sousa, W.P. (1979) Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. Ecology, 60: 1224-1239.

Stehlik, I. and Blattner, F.R. (2004) Sex-specific SCAR markers in the dioecious plant *Rumex nivalis* (Polygonaceae) and implications for the evolution of sex chromosomes. Theoretical and Applied Genetics, 108: 238-242.

Stephenson, T.A. (1929) On methods of reproduction as specific characters. Journal of the Marine Biological Association of the United Kingdom, 16: 131-172.

Stephenson, T.A. (1935) The British sea anemones, Vol II The Ray Society, London.

Stoddart, J.A. (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. Marine Biology, 76: 279-284.

Stoddart, J.A. (1984a) Genetic differentiation among populations of the coral *Pocillopora damicornis* off southwestern Australia. Coral Reefs, 3: 149-156.

Stoddart, J.A. (1984b) Genetic structure within populations of coral *Pocillopora damicornis*. Marine Biology, 81: 19-30.

Stoddart, J.A. (1988) Coral populations fringing islands larval connections. Australian Journal of Marine and Freshwater Research, 39: 109-116.

Stoddart, J.A., Babcock, R.C., and Heyward, A.J. (1988) Self-fertilization and maternal enzymes in the planulae of the coral *Goniastrea favulus*. Marine Biology, 99: 489-494.

Stoddart, J.A. and Black, R. (1985) Cycles of gametogenesis and planulation in the coral *Pocillopora damicornis*. Marine Ecology Progress Series, 23: 153-164.

Stoddart, J.A. and Taylor, J.F. (1988) Genotypic diversity - Estimation and prediction in samples. Genetics, 118: 705-711.

Strathmann, M.F. (1987) Reproduction and development of marine invertebrates of the Northern Pacific Coast. University of Washington Press, Seattle, WA.

Szmant, A.M., Weil, E., Miller, M.W., and Colon, D.E. (1997) Hybridization within the species complex of the scleractinan coral *Montastraea annularis*. Marine Biology, 129: 561-572.

Takada, Y. (1999) Influence of shade and number of boulder layers on mobile organisms on a warm temperate boulder shore. Marine Ecology Progress Series, 189: 171–179.

Takabayashi, M. and Hoegh-Guldberg, O. (1995) Ecological and physiological differences between two colour morphs of the coral *Pocillopora damicornis*. Marine Biology, 123: 705-714.

Tanner, J.E. (1996) Seasonality and lunar periodicity in the reproduction of Pocilloporid corals. Coral Reefs, 15: 59-66.

Tessier, N., Bernatchez, L., Presa, P., and Angers, B. (1995) Gene diversity analysis of mitochondrial DNA, microsatellites and allozymes in landlocked Atlantic salmon. Journal of Fish Biology, 47: 156-163.

Tilburg, C.E., Hurlburt, H.E., O'Brien, J.J., and Shriver, J.F. (2001) The dynamics of the East Australian Current system: The Tasman Front, the East Auckland Current, and the East Cape Current. Journal of Physical Oceanography, 31: 2917-2943.

Tunnicliffe, V. (1981) Breakage and propagation of the stony coral *Acropora cervicornis*. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences, 78: 2427-2431.

Tokkeshi, M. (1999) Species coexistence: Ecological and evolutionary perspectives. Cambridge University Press, Cambridge, UK.

Underwood, A.J. and Fairweather, P.G. (1989) Supply-side ecology and benthic marine assemblages. Trends in Ecology and Evolution, 4: 16-20.

Valverde, T. and Pisanty, I. (1999) Growth and vegetative spread of *Schizachyrium scoparium var. littoralis* (Poaceae) in sand dune microhabitats along a successional gradient. Canadian Journal of Botany, 77: 219-229.

Veron, J.E.N. (1986) Corals of Australia and the Indo-Pacific Angus and Robertson, Sydney.

Vianna, P., Schama, R., and Russo, C.A.M. (2003) Genetic divergence and isolation by distance in the West Atlantic sea anemone *Actinia bermudensis* (McMurrich, 1889). Journal of Experimental Marine Biology and Ecology, 297: 19-30.

Viard, F., Bremond, P., Labbo, R., Justy, F., Delay, B., and Jarne, P. (1996) Microsatellites and the genetics of highly selfing populations in the freshwater snail *Bulinus truncatus*. Genetics, 142: 1237-1247.

Viard, F., Justy, F., and Jarne, P. (1997) The influence of self-fertilization and population dynamics on the genetic structure of subdivided populations: A case study using microsatellite markers in the freshwater snail *Bulinus truncatus*. Evolution, 51: 1518-1528.

Vrijenhoek, R.C. (1979) Factors affecting clonal diversity and coexistence. American Zoologist, 19: 787-797.

Vrijenhoek, R.C. (1998) Animal clones and diversity. Bioscience, 48: 617-628.

Vrijenhoek, R.C. and Pfeiler, E. (1997) Differential survival of sexual and asexual Poeciliopsis during environmental stress. Evolution, 51: 1593-1600.

Waddington, C.H. and Robertson, E. (1966) Selection for developmental canalisation. Genetical Research, 7, 303-309

Wagner, A. (1996) Does evolutionary plasticity evolve? Evolution, 50: 1008-1023.

Wahlund, S. (1928) Composition of populations from the perspective of the theory of heredity. Hereditas, 11: 65-105.

Waits, L.P., Luikart, G., and Taberlet, P. (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology, 10: 249-256.

Ward, S. (1992) Evidence for broadcast spawning as well as brooding in the scleractinian coral *Pocillopora damicornis*. Marine Biology, 112: 641-646.

Waser, P.M. and Strobeck, C. (1998) Genetic signatures of interpopulation dispersal. Trends in Ecology & Evolution, 13: 43-44.

Weider, L.J. (1992) Disturbance, competition and the maintenance of clonal diversity in *Daphnia pulex*. Journal of Evolutionary Biology, 5: 505-522.

Weir, B.S. (1979) Inferences about linkage disequilibrium. Biometrics, 35: 235-254.

Weir, B.S. and Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. Evolution, 38(6): 1358-1370.

Weismann, A. (1889). The significance of sexual reproduction in the theory of natural selection. In: Essays on the heredity and kindred biological subjects (eds E.B. Poulton, S. Schönland and A.E. Shipley). Oxford University Press, Oxford.

Whitlock, M.C. and McCauley, D.E. (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity, 82: 117-125.

Widen, B., Cronberg, N., and Widen, M. (1994) Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. Folia Geobotanica & Phytotaxonomica, 29: 245-263.

Williams, G.C. (1975) Sex and evolution Princeton University Press, Princeton.

Willis, B.L. and Ayre, D.J. (1985) Asexual reproduction and genetic determination of growth form in the coral *Pavona cactus*: biochemical genetic and immunogenic evidence. Oecologia, 65: 516-525.

Willis, B.L., Babcock, R.C., Harrison, P.L., and Wallace, C.C. (1997) Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. Coral Reefs, 16: S53-S65.

Wilson, J.R. and Harrison, P.L. (1998) Settlement-competency periods of larvae of three species of scleractinian corals. Marine Biology, 131: 339-345.

Witkowicz, J., Urbanczyk-Wochniak, E., and Przybecki, Z. (2003) AFLP marker polymorphism in cucumber (*Cucumis sativus* L.) near isogenic lines differing in sex expression. Cellular & Molecular Biology Letters, 8: 857-857.

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

Wright, S. (1943) Isolation by distance. Genetics, 28: 139-156.

Wright, S. (1969) The evolution and genetics of populations. Vol. 2. The theory of gene frequencies University of Chicago Press, Chicago.

Wright, S. (1978) Evolution and the genetics of populations. IV. Variability within and among natural populations University of Chicago Press, Chicago.

Wu, K.S. and Tanksley, S.D. (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Molecular Genetics and Genomics, 241: 225–235.

Wulff, J.L. (1985) Dispersal and survival of fragments of coral reef sponges. In Proceedings of the Fifth International Coral Reef Congress, Tahiti, 5: 119-124.

Wulff, J.L. (1991) Asexual fragmentation, genotype success, and population dynamics of erect branching sponges. Journal of Experimental Marine Biology and Ecology, 149: 227-247.

Yamada, S.B. (1989) Are direct developers more locally adapted than planktonic developers. Marine Biology, 103: 403-411.

Yanagi, K., Segawa, S., and Tsuchiya, K. (1999) Early development of young brooded in the enteron of the beadlet sea anemone *Actinia equina* (Anthozoa : Actiniaria) from Japan. Invertebrate Reproduction & Development, 35: 1-8.

Yeap, K.L., Black, R., and Johnson, M.S. (2001) The complexity of phenotypic plasticity in the intertidal snail *Nodilittorina australis*. Biological Journal of the Linnean Society, 72(1): 63-76.

Ziegenhagen, B., Bialozyt, R., Kuhlenkamp, V., Schulze, I., Ulrich, A., and Wulf, M. (2003) Spatial patterns of maternal lineages and clones of *Galium odoratum* in a large ancient woodland: inferences about seedling recruitment. Journal of Ecology, 91: 578-586.