

# University of Wollongong - Research Online

## Thesis Collection

Title: The importance of fine-scale environmental heterogeneity in determining levels of genotypic diversity and local adaptation

Author: Craig D H Sherman

Year: 2006

Repository DOI:

### Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

**Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.**

Research Online is the open access repository for the University of Wollongong. For further information contact the UOW Library: [research-pubs@uow.edu.au](mailto:research-pubs@uow.edu.au)

2006

## The importance of fine-scale environmental heterogeneity in determining levels of genotypic diversity and local adaptation

Craig D. H Sherman  
*University of Wollongong*

Follow this and additional works at: <https://ro.uow.edu.au/theses>

### University of Wollongong

#### Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

### Recommended Citation

Sherman, Craig D. H, The importance of fine-scale environmental heterogeneity in determining levels of genotypic diversity and local adaptation, PhD thesis, School of Biological Sciences, University of Wollongong, 2006. <http://ro.uow.edu.au/theses/505>

## **NOTE**

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

## **UNIVERSITY OF WOLLONGONG**

### **COPYRIGHT WARNING**

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

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

DOCTOR OF PHILOSOPHY

from the

UNIVERSITY OF WOLLONGONG

by

Craig D. H. Sherman B. Sc. (Hons)

SCHOOL OF BIOLOGICAL SCIENCES

2006

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

## Table of Contents

<b>List of Tables .....</b>	<b>vi</b>
<b>List of Figures .....</b>	<b>x</b>
<b>Abstract .....</b>	<b>xiv</b>
<b>Acknowledgements .....</b>	<b>xvii</b>
 <b>Chapter 1 General Introduction .....</b>	 <b>1</b>
1.1 Predicted Roles of Sexual and Asexual Propagules .....	1
1.2 The Study Species .....	5
1.3 Aims .....	7
1.4 Thesis Outline .....	8
 <b>Chapter 2 Asexual Reproduction does not Produce Clonal Populations within a Range of Reef Habitats for the Brooding Coral <i>Pocillopora damicornis</i> on the Great Barrier Reef, Australia .....</b>	 <b>10</b>
2.1 Introduction .....	10
2.2 Materials and Methods .....	13
2.2.1 Study Site and Sample Collection .....	13
2.2.2 Electrophoresis .....	14
2.2.3 Larval Collections .....	15
2.2.4 Testing for Cryptic Species .....	15
2.2.5 Statistical Analyses .....	16
2.2.5.1 Fine-Scale Population Structure and Levels of Genetic Subdivision .....	16
2.2.5.2 Assessing Relative Contributions of Sexual and Asexual Reproduction .....	17
2.3 Results .....	19
2.3.1 Genetic Variation Among Sites and Habitats .....	19
2.3.2 Population Subdivision .....	19

2.3.3 The Relative Importance of Sexual and Asexual Reproduction Among Habitats .....	23
2.3.4 Variation in Mode of Production of Brooded Larvae Among Habitats.....	24
2.3.5 Tests for the Presence of Cryptic Species.....	25
2.4 Discussion .....	27
2.4.1 Variation in Genotypic Diversity Across Habitats .....	27
2.4.2 Low Levels of Genotypic Diversity Within Disturbed Habitats .....	29
2.4.4 Genetic Variation and Subdivision .....	30
<b>Chapter 3 Intermediate Levels of Selfing in the Hermaphroditic Brooding Coral, <i>Seriatopora hystrix</i>, on the Great Barrier Reef.....</b>	<b>32</b>
Preamble to Chapter 3 .....	32
3.1 Introduction.....	33
3.2 Methods.....	36
3.2.1 Adult and Larval Collections.....	36
3.2.2 Electrophoresis.....	36
3.2.3 Statistical Analysis.....	37
3.3 Results.....	40
3.3.1 Mating System .....	40
3.3.2 Allelic Variation and Population Subdivision .....	40
3.4 Discussion .....	45
<b>Chapter 4 Mode of Reproduction Does Not Vary with Habitat Heterogeneity in the Sea Anemone <i>Actinia tenebrosa</i>.....</b>	<b>50</b>
4.1 Introduction.....	50
4.2 Methods.....	55
4.2.1 Habitat Heterogeneity .....	55
4.2.2 Collection of Specimens .....	55
4.2.3 DNA Extraction and Amplification .....	56
4.2.4 Allozyme Electrophoresis.....	58

4.2.5 Statistical Analysis .....	58
4.3 Results .....	59
4.3.1 Genetic Variation among Adult Anemones .....	59
4.3.2 Asexual Production of Brooded Juveniles .....	59
4.4 Discussion .....	64

**Chapter 5 Genotypic Diversity and the Distribution of Clones Varies among Habitats, within Populations of the Sea Anemone *Actinia tenebrosa* ..... 67**

5.1 Introduction .....	67
5.2 Methods .....	72
5.2.1 Collection of Samples .....	72
5.2.2 Microsatellite and Allozyme Genotyping .....	72
5.2.3 Analysis .....	73
5.3 Results .....	75
5.3.1 Identification of Genotypes .....	75
5.3.2 Genotypic Diversity .....	77
5.3.3 Genotypic Structure and the Distribution of Clones .....	83
5.3.4 Sexual Reproduction <i>versus</i> Somatic Mutation as Source of Genotypic Diversity .....	84
5.4 Discussion .....	87
5.4.1 Power to Identify Unique Genotypes .....	87
5.4.2 Genotypic Diversity and Habitat Heterogeneity .....	88
5.4.3 Distribution of Clones .....	89
5.4.4 Sources of Genotypic Diversity .....	92

**Chapter 6 Local Adaptation of the Clonal Sea Anemone *Actinia tenebrosa* to Fine-Scale Environmental Variation ..... 94**

6.1 Introduction .....	94
6.2 Methods .....	97
6.2.1 Collection and Re-Attachment of Anemones for Transplants .....	97



6.2.2 Transplant Design .....	98
6.2.3 Measurement of Fitness .....	101
6.2.4 Statistical Analysis .....	103
6.3 Results .....	105
6.3.1 Initial Survival of Transplanted Adults .....	105
6.3.2 Patterns of Reproduction and Sex Ratios .....	105
6.3.3 Within-Habitat Transplants .....	106
6.3.4 Between-Habitat Transplants .....	110
6.4 Discussion .....	115
6.4.1 Evidence for Local Adaptation to Fine-Scale Habitat Heterogeneity .....	115
6.4.2 Evolutionary Consequences .....	118
<b>Chapter 7 Scales of Genetic Subdivision and Genotypic Diversity in the Brooding Sea Anemone, <i>Actinia tenebrosa</i> .....</b>	<b>121</b>
7.1 Introduction .....	121
7.2 Methods .....	127
7.2.1 Collection of Specimens .....	127
7.2.2 Microsatellite and Allozyme Genotyping .....	128
7.2.3 Data Analysis .....	128
7.3 Results .....	132
7.3.1 Genetic Variation and Linkage Disequilibria .....	132
7.3.2 Genotypic Diversity .....	135
7.3.3 Population Subdivision .....	137
7.4 Discussion .....	146
7.4.1 Genotypic Variation .....	146
7.4.2 Genetic Subdivision and Gene Flow .....	149
<b>Chapter 8 General Discussion and Conclusions .....</b>	<b>153</b>
8.1 The Importance of Sexual and Asexual Reproduction in Different Habitats .....	153
8.1.1 Mode of Reproduction .....	153

8.1.2 Genotypic Diversity, the Distribution of Clones and Scale of Localised Adaptation .....	155
8.1.3 Future directions .....	158
<b>References</b> .....	162

## List of Tables

Table 2.1 Allele frequencies for colonies of <i>Pocillopora damicornis</i> collected from three to five sites within each of six reef habitats from the One Tree Island reef on the Great Barrier Reef.....	20
Table 2.2 Wrights fixation index ( $F_{IS}$ ) and significant departures from levels of heterozygosity expected under Hardy-Weinberg equilibrium for <i>Pocillopora damicornis</i> 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. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	21
Table 2.3 Hierarchical analysis of standardized genetic variation (calculated as Weir and Cockerham's $\theta$ ) 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 <i>Pocillopora damicornis</i> at the One Tree Island Reef, Great Barrier Reef.....	21
Table 2.4 Comparison of the observed and expected multi-locus genotypic diversity within collections of <i>Pocillopora damicornis</i> 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. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	24
Table 2.5 Multi-locus genotypes of <i>Pocillopora damicornis</i> colonies and their brooded larvae collected over two consecutive years from the One Tree Island Reef, Great Barrier Reef.....	25
Table 3.1 Allele frequencies for <i>Seriatopora hystrix</i> colonies collected from three sites (25m <sup>2</sup> ) within each of two habitats from the One Tree Island Reef on the Great Barrier Reef, Australia. ....	41
Table 3.2 Wright's (1978) fixation index ( $F_{IS}$ ) and significant departures from levels of heterozygosity expected under Hardy-Weinberg equilibrium for <i>Seriatopora hystrix</i>	

from three sites (25m <sup>2</sup> ) 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. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	43
Table 3.3 Hierarchical analysis of standardized genetic variation calculated using the formula of Weir and Cockerham (1984) for <i>Seriatopora hystrix</i> from three sites (25m <sup>2</sup> ) within each of two reef habitats from the One Tree Island Reef, Great Barrier Reef, Australia. ....	44
Table 3.4 Comparison of the observed and expected multi-locus genotypic diversity for <i>Seriatopora hystrix</i> from three sites (25m <sup>2</sup> ) within each of two reef habitats from the One Tree Island Reef, Great Barrier Reef, Australia. ....	44
Table 4.1 Primer sequence for the microsatellite loci used in this study. All loci were developed from a genomic library specifically designed for <i>Actinia tenebrosa</i> (Mitchellson and Ayre, unpublished).....	57
Table 4.2 Genotype data of adult <i>Actinia tenebrosa</i> and their brooded juveniles collected from eight locations along the east coast of mainland Australia and Tasmania. ....	61
Table 5.1 Comparison of the observed and expected multi-locus genotypic diversity within collections of <i>Actinia tenebrosa</i> 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. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	78
Table 5.2 Wrights fixation index ( $F_{IS}$ ) indicating significant departures from Hardy-Weinberg equilibria for <i>Actinia tenebrosa</i> collected from two habitats. Calculations made using all adult genotype frequencies. Significant departures determined after the application of a sequential Bonferroni correction. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	81
Table 5.3 Wrights fixation index ( $F_{IS}$ ) indicating significant departures from Hardy-Weinberg equilibria for <i>Actinia tenebrosa</i> collected from two habitats. Calculations made using only unique multi-locus genotypes. Significant departures determined after the application of a Bonferroni correction. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	81

Table 5.4 Comparison of the observed and expected multi-locus genotypic diversity within collections of <i>Actinia tenebrosa</i> 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. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	82
Table 5.5 Distribution of clones of <i>Actinia tenebrosa</i> collected from three 5 × 5 m sites within each of two habitats at two locations along the NSW coast, Australia. ....	83
Table 5.6 Wrights fixation index ( $F_{IS}$ ) indicating significant departures from Hardy-Weinberg equilibrium for <i>Actinia tenebrosa</i> collected from two locations. Calculations made using only unique multi-locus genotypes. Significant departures determined after the application of a sequential Bonferroni correction. * $P < 0.05$ , ** $P < 0.01$ , *** $P < 0.001$ .....	85
Table 7.1 Allele frequencies for collections of <i>Actinia tenebrosa</i> from 19 local populations along the east coast of Australia. (24) = Sample size. ....	133
Table 7.2 Genetic variation at four microsatellite and four allozyme loci in 19 populations of <i>Actinia tenebrosa</i> . $N = 24$ for each population. ....	134
Table 7.3 Levels of genotypic diversity observed within each of 19 populations of <i>Actinia tenebrosa</i> 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). ....	137
Table 7.4 Hierarchical analysis of standardised genetic variation (calculated as Weir and Cockerham 1984) for <i>Actinia tenebrosa</i> 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). All calculations made using the genotypes of all individual anemones within each local population. * indicates 95% confidence interval (CI) significantly different from zero.....	138

Table 7.5 Hierarchical analysis of standardised genetic variation (calculated as Weir and Cockerham 1984) for <i>Actinia tenebrosa</i> 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). All calculation made using only distinct multi-locus genotypes within each local population. * indicates 95% confidence interval (CI) significantly different from zero. ....	139
Table 7.6 Hierarchical analysis of standardised genetic variation excluding <i>Gpi1</i> (calculated as Weir and Cockerham 1984) for <i>Actinia tenebrosa</i> 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. ....	140
Table 7.7 The number of individuals of <i>Actinia tenebrosa</i> 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 <i>et al.</i> 1999). ....	145

## List of Figures

Figure 2.1 Map of One Tree Island Reef showing approximate locations of <i>Pocillopora damicornis</i> collection sites. Insert shows location of One Tree Island Reef along the Great Barrier Reef, Australia. ....	14
Figure 2.2 Dendrogram showing the genetic relationship between 17 collections of <i>Pocillopora damicornis</i> 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. ....	22
Figure 2.3 Principal coordinates analysis based on a genetic distance matrix (Nei 1978) for individual brooding and non-brooding colonies of <i>Pocillopora damicornis</i> collected from the One Tree Island Reef on the southern Great Barrier Reef, Australia. ....	26
Figure 4.1 Locations of collecting sites of brooding adult <i>Actinia tenebrosa</i> 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. ....	57
Figure 4.2 Mean ( $\pm$ SE) levels of gene diversity for four microsatellite loci and five allozyme loci for 37 <i>Actinia tenebrosa</i> samples collected across eight locations from the east coast of Australia. $N_a$ = mean number of alleles/locus; $N_e$ = effective number of alleles/locus; $H_E$ = mean heterozygosity. ....	59
Figure 4.3 PCR amplification products showing clonal genotypes of adult <i>Actinia tenebrosa</i> (A1 and A2) and their brooded juveniles (J1,...J 'n') which were heterozygous at two microsatellite loci ( <i>At1</i> and <i>At2</i> in alternate lanes). M = marker lanes, -ve = negative control. ....	60
Figure 5.1 Levels of genotypic diversity within three populations of the sea anemone <i>Actinia tenebrosa</i> 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 ( $N = 212$ ). ....	75

Figure 5.2 Levels of genotypic diversity identified for increasing number of loci for individuals of <i>Actinia tenebrosa</i> 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 ( $N = 212$ ).	76
Figure 5.3 The probability of identity ( $P_{ID}$ ) calculated for three populations of the sea anemone <i>Actinia tenebrosa</i> . $P_{ID}$ calculates the probability that two individuals drawn at random from a population will have the same genotype at multiple loci and is used to assess 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 <i>et al.</i> 2001). Sample sizes for each location were: Cape Banks ( $N = 123$ ), Bass Point ( $N = 223$ ), Bellambi ( $N = 212$ ).	77
Figure 5.4 The distribution of <i>Actinia tenebrosa</i> 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 *.	79
Figure 5.5 Frequency distribution of the pair-wise number of allele differences detected between unique multi-locus genotypes for <i>Actinia tenebrosa</i> within each of three populations along the NSW coast, Australia.	86
Figure 6.1 Rock pool and boulder habitat separated by < 200 meters at Cape Banks, NSW, Australia.	100
Figure 6.2 Seasonal patterns of sexual (gonad formation) and asexual (proportion brooding) reproduction in <i>Actinia tenebrosa</i> collected from rock pool and boulder habitats at two locations along the NSW coast, Australia.	107
Figure 6.3 Mean ( $\pm$ SE) proportion of <i>Actinia tenebrosa</i> 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.	108
Figure 6.4 Mean ( $\pm$ 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.....	109
Figure 6.5 Mean ( $\pm$ SE) change in size (as determined by initial and final column diameter) for native and foreign groups of the sea anemone <i>Actinia tenebrosa</i> reciprocally transplanted among three rock pools (i.e. <30m) within a single rocky headland. Each pool received half native and half foreign anemones. ....	110
Figure 6.6 Mean ( $\pm$ SE) proportion of adult <i>Actinia tenebrosa</i> surviving when reciprocally transplanted among habitats (<200m) within a rocky headland at two locations. ....	111
Figure 6.7 Mean ( $\pm$ SE) measures of asexual fecundity for <i>Actinia tenebrosa</i> 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.....	112
Figure 6.8 Mean ( $\pm$ SE) change in size (as determined by initial and final column diameter) for native and foreign groups of the sea anemone <i>Actinia tenebrosa</i> reciprocally transplanted between rock pool and boulder habitats (separated by < 200m) at two locations. ....	114
Figure 7.1 Map showing the location of collection sites for <i>Actinia tenebrosa</i> along the east coast of Australia. ....	128
Figure 7.2 The expected number of individuals with the identical genotype for increasing locus combinations within each of 19 local populations of <i>Actinia tenebrosa</i> .....	135
Figure 7.3. Dendrograms showing the genetic relationship among 19 populations of <i>Actinia tenebrosa</i> 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. ....	142
Figure 7.4 Dendrograms showing the genetic relationship among 19 populations of <i>Actinia tenebrosa</i> along the east coast of mainland Australia and Tasmania. Nei's genetic distance (1978) was calculated based on data for seven loci ( <i>Gpi1</i> 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.

..... 143

Figure 7.5 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..... 144

## 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 ( $r_p (\pm SE) = 0.32 \pm 0.43$ ), and almost half (46%) of the larvae resulting from self-fertilisation (mean outcrossing rates were  $t_m (\pm SE) = 0.54 \pm 0.22$ ). 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 ( $<1\text{m}^2$ ) 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 ( $25\text{m}^2$ ) 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  $F_{2, 4.77} = 4.77$ ,  $P = 0.014$ ).

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.

## Acknowledgements

First and foremost I would like to thank my supervisors, David Ayre and Karen Miller, who I cannot thank enough for their tireless support, never ending patience, and their thought provoking debates (both with me and between themselves). David, your encouragement and discussions have been inspirational, and your commitment to your students, even after life's unexpected challenges, is admirable. You have been a great mentor, and a good friend. Karen, your ability to encourage independent thought and question long standing views is a philosophy I will take with me in my future career (never just accept, always question). I will also fondly remember our numerous fieldtrips to the reef and the help and experience you have given me as a field biologist. Hopefully I will end up damaging fewer propellers in the future, but let's not count on it! This has been one of the most exciting and memorable experiences of my life and I wish you both, even more success in your future careers. It has been a pleasure to work with you and I look forward to the opportunity to collaborate with you in the future.

To my wonderful wife, Monica; without your support and encouragement I would not have been able to complete this thesis. Your tireless support and help with fieldwork, at some of the most beautiful locations on the east coast of Australia, will be memories I will always remember fondly. Your support and patience, especially during the long and often torturous writing phase is greatly appreciated and I promise to make up for all those beautiful sunny weekends when you were forced to stay at home so I could write. I cannot thank you enough.

To my parents, Rick and Loraine Sherman, and my sister Brenda Smith; your support from the far side of the world has meant more to me than you can imagine. I am grateful for your encouragement and continuous words of support, and for showing me the wonders of the natural world as a kid growing up in Africa. The love for the bush and nature you taught me while growing up, has led me to a wonderful and hopefully successful career in science, thank you.

I would like to thank all the numerous field assistants that help with fieldwork and made this project possible. In particular, Jodie Dunn, who helped collect, cut and freeze coral specimens at the One Tree Island Reef, always smiling and working hard no matter how much work I gave her. I hope I wasn't too much of a slave driver. Thanks to Pam and Korad Beinssen who were always helpful and accommodating at the One Tree Island Reef. I would like to thank Craig Mundy for his help and advice, especially when doing fieldwork at One Tree Island Reef. Craig has the most amazing ability to find the simplest and most practical solutions to seemingly complex problems. Your homemade viewing bucket certainly made the collection of corals from the reef flat much easier. I am grateful to Ezter Hidas and Trudy Costa for the collection of sea anemones from Victoria and to Karen Miller for the collection of sea anemones from Tasmania.

I am grateful to all my co-works in the molecular ecology laboratory for their assistance, advice, discussions and companionship – Paul Rymer, Dave Roberts, Annett Usher, Sam Lloyd, Chris Howard, Tom Celebrezze, Laurence Clarke, Kym Otterwell, Dave McKenna, Eszter Hidas, Beth Mott, Elizabeth Lindsay – your light hearted banter and patience with all my questions made my laboratory work enjoyable. I would also like to thanks other members of the Biology Department at the University of Wollongong for their stimulating discussions, advice and friendship.

I am indebted to Tonia Schwartz, Mo Healey and Monica Martin for the proofreading of this thesis and making helpful comments.

This work was supported, in part, by two student grants from the Great Barrier Reef Marines Park Authority and by an ARC Discovery Grant to D.J. Ayre and K.J. Miller. I am also grateful to the University of Wollongong for providing a University scholarship that helped support me during the PhD.

Fieldwork was carried out under scientific licences: G02/105 granted by Great Barrier Reef Marines Park Authority; P03/0085 granted by NSW Fisheries, and RP768 granted by Victoria Department of Primary Industries.