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JAMES COOK UNIVERSITY AUSTRALIA

Genetic assessment of population structure and the origin of recruits in brooding corals: understanding population connectivity on the Great Barrier Reef on various timescales

Thesis submitted by
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for the degree of Doctor of Philosophy
in the School of Marine and Tropical Biology
James Cook University

Statement on the contribution of others

Nature of assistance	Contribution	Contributor
Intellectual support	Proposal writing, assistance in data analysis and interpretation, editorial assistance	Dr. Madeleine van Oppen[1, 2, 3] Prof. Bette Willis[2, 3] Dr. Petra Lundgren[1, 4]
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Abstract

Ecological connectivity (i.e., population connectivity over ecological rather than evolutionary timescales) in the marine environment, including coral reefs, is poorly understood as few studies have specifically estimated contemporary dispersal. This limits our ability to evaluate the design and potential benefits of novel conservation and resource management strategies. Most population genetic studies on reef corals have estimated population connectivity over evolutionary time scales (i.e., over thousands of generations) using conventional statistical methods (F-statistics), as statistical methods to estimate connectivity on ecological time scales from genetic data have only recently been developed. Further to advances in statistical methods, a novel and promising approach to understanding ecological connectivity is to genetically characterise newly settled recruits and identify potential source populations by genetic assignments. This approach also overcomes the confounding effects of analysing multiple cohorts simultaneously, and addresses the potential temporal stochasticity of recruitment, which is largely unquantified for reef corals.

Taking advantage of methodological improvements, I assess population connectivity on ecological as well as evolutionary timescales of the model brooding coral species *Pocillopora damicornis* from microsatellite genotype data, and I compare estimates of ecological connectivity with that of the closely related coral *Seriatopora hystrix.* First, I present a novel genetic technique to reliably identify species and recently described lineages within the pocilloporid family, which enables population genetic analyses to be

focused on genetically (and presumably biologically) meaningful units of observation. I then infer recent and historic larval exchange between populations of the two most common lineages of *P. damicornis* (Type α and β) from population genetic patterns of adult coral colonies from 28 sites on the Great Barrier Reef (GBR). And finally I estimate larval dispersal and retention over contemporary timescales by genetically assigning newly settled recruits of *P. damicornis* Type α and β, as well as of *S. hystrix*, to potential source populations.

I found both high genetic similarities over large spatial scales and high genetic differentiation at local spatial scales among populations of *P. damicornis* Type α and β. I explain the high genetic similarity among populations separated by hundreds of kilometres by the potential of brooded larvae to have long pelagic larval duration (PLD) combined with strong longshore currents along the GBR. Further, I hypothesise that genetic differentiation among populations over small spatial scales is a signature of more recent single recruitment events from genetically distinct sources combined with the spatially and temporally stochastic nature of recruitment. I found no evidence for predominantly clonal reproduction within populations, despite the large energy allocation of this species to asexually produced brooded larvae, further undermining the paradigm that brooded larvae settle close to parent colonies shortly after release.

Genetic assignment of recruits to potential source populations confirmed that larval dispersal and retention is site-specific for both lineages of *P. damicornis* studied, as well as for *S. hystrix*, in accordance with the capacity of brooded coral larvae to both settle immediately upon release, and stay in the water column for extended periods of time, and the spatial and temporal differences in current regimes between locales.

Strongly self-recruiting populations of *P. damicornis* Type α were found at exposed sites in the Palm Islands, while predominantly self-recruiting populations of *P. damicornis* Type β and *S. hystrix* were located on sheltered sites in the Palm Islands, and at the NE exposed site of Lizard Island; other populations were found to be largely dependent on exogenous larval replenishment. Differences in habitat preference and dispersal characteristics between *P. damicornis* Type α and β support the recent delineation of lineages within the morphospecies, and demonstrate the timeliness of re-evaluating the biology of several coral species using units of observation identified by techniques of molecular taxonomy.

My results suggest that as a consequence of their complex reproductive strategies and dispersal capacities, pocilloporid species studied here likely form resilient metapopulations on the GBR. Because different habitat types sustain reproductive hotspots of these closely related species, it is reasonable to suggest that whole reefs are included in MPAs, rather than parts of reefs. Genetic connectivity estimates from my study, coupled with realistic high-resolution water circulation models may provide guidelines for suitable spacing of protected areas in inshore, midshelf and outer reef environments of the GBR, that ensure ecologically and demographically relevant connectivity between populations of brooding coral species.

Table of contents

Abstract ..... **i**

Table of contents ..... **iv**

List of Tables ..... **viii**

List of Figures ..... **x**

Chapter 1- **General introduction** **1**

1.1 Connectivity and conservation of coral reefs 2

1.2 Assessing population connectivity in corals 3

1.2.1 The genetic approach 4

1.2.2 The modeling approach 6

1.3 Reproductive strategies and connectivity in corals 7

1.4 Aims of the thesis 9

1.5 Nomenclature 10

Chapter 2 - A rapid genetic assay for the identification of the most common Pocillopora damicornis genetic lineages on the Great Barrier Reef 12

Abstract 13

2.1. Introduction 14

2.2. Materials and methods 16

2.2.1. Ethics Statement 16

2.2.2. Assay development 16

2.2.3. Testing the assay 17

2.3. Results and Discussion 19

Chapter 3 - Revisiting the connectivity puzzle of the common coral Pocillopora damicornis 23

Abstract 24

3.1. Introduction 25

3.2. Materials and Methods 27

3.2.1. Sample collection 27

3.2.2. DNA extraction and microsatellite genotyping 30

3.2.3. Data analyses 31

3.3. Results 34

3.3.1. Density of Type α and β colonies 34

3.3.2. Genotype probability and clone mates 36

3.3.3. Genetic diversity 37

3.3.4. Genetic structure 40

3.3.5. First generation migrants 48

3.3.6. Passive particle dispersal modelling 50

3.4. Discussion 52

3.4.1. Larval characteristics and water circulation patterns explain high connectivity over large spatial scales 54

3.4.2. Lack of clonal population structure suggests that brooded larvae are not retained locally on the GBR 56

3.4.3. Explanations for strong genetic structure on small spatial scales 58

3.5. Conclusions 60

3.6. Supplementary material 63

Chapter 4 - Should I stay or should I go? Long- and short-distance migration in two common lineages of Pocillopora damicornis on the Great Barrier Reef 81

Abstract 82

4.1. Introduction 83

4.2. Materials and Methods 86

4.2.1. Sample collection 86

4.2.2. DNA extraction and microsatellite genotyping 88

4.2.3. Data analyses 89
4.3. Results 91
4.3.1. Recruit numbers and population densities 91
4.3.2. Survival of recruits 96
4.3.3. Clones 96
4.3.4. Exclusions, assignments and clustering 99
4.3.5. Spatio-temporal patterns of recruitment 104
4.4. Discussion 108
4.4.1. Variability in recruitment patterns 108
4.4.2. Temporal stochasticity in recruitment 109
4.4.3. Low proportions of clones, but high levels of self-recruitment 110
4.4.4. Potential for metapopulation resilience and implications for conservation 113
4.5. Conclusions 115
Chapter 5 - Spatial and temporal recruitment patterns of Seriatopora hystrix inferred from genotypic analysis of adults and recruits 117
Abstract 118
5.1. Introduction 119
5.2. Materials and Methods 121
5.2.1. Sample collection 121
5.2.2. DNA extraction, genetic identification and microsatellite genotyping 122
5.2.3. Data analyses 125
5.3. Results 127
5.3.1. Recruit numbers and survivorship 127
5.3.2. Spatial – genetic patterns of recruitment 131
5.3.3. Temporal patterns of recruitment 133
5.3.4. Genetic structure of adults and recruits 136

5.4. Discussion 137

5.4.1. Spatially variable recruitment patterns 137

5.4.2. Recruitment from unknown sources 140

5.4.3. Clonality in S. hystrix 141

5.4.4. Temporal variability of recruitment 142

5.4.5. Implications for conservation 143

5.5. Conclusions 144

Chapter 6 - General discussion: major results and future research 146

6.1. Major results and conclusions of the thesis 147

6.2. General discussion 147

6.2.1. Species identification of coral recruits – a new era for recruitment studies 147

6.2.2. Anything is possible – scale-free patterns of genetic differentiation and connectivity in pocilloporid corals 148

6.2.3. Patterns of connectivity in pocilloporid corals and their implications for MPA design 149

6.3. Future research 151

References 154

List of Tables

Table 2.1. Location and phylogenetic identities of *Pocillopora damicornis* samples from Schmidt-Roach et al. (2012a). All samples were correctly identified by the RFLP PCR assay as Type α, Type β or "other *Pocillopora*". "Type α LHI" specimens (indicated with an *) were identified as "other *Pocillopora*" by the assay. 15

Table 2.2. Characteristics of the newly developed microsatellite primers. 20

Table 2.3. Multiplex groups for microsatellite genotyping. A universal TET-labeled M13-F was added to groups M1 and M2. 20

Table 3.1. Sampling sites and sample sizes. Final sample sizes, after removing sympatric clone mates are shown in brackets. Type β populations that were omitted from population-based analyses are indicated with *. Location of reefs indicated by I= inshore, M = midshelf, and O = outer reefs. Habitat type indicated by E = exposed windward, S = sheltered leeward, L = lagoon 29

Table 3.2. Multiplex groups for microsatellite genotyping. A universal TET-labelled M13-F was added to groups M1 and M2. 30

Table 3.3. Genetic diversity estimates of Type α (a) and Type β (a) populations. AR = allelic richness, LD = number of locus pairs in significant linkage disequilibrium, HWE = number of loci in HWE; G_{is} = inbreeding coefficient, P < 0.01 are in bold; H_e = mean expected heterozygosity over all loci; H_o = mean observed heterozygosity over all loci, HD = exact P-values by the Markov chain method for heterozygote deficiency; HE = exact P-values by the Markov chain method for heterozygote excess; HD_n = Wilcoxon test one-tailed P value for normalized heterozygote deficiency; HE_n = Wilcoxon test one-tailed P value for normalized heterozygote excess, P < 0.01 are in bold. 39

Table 3.4. Estimation of Isolation by Distance by Mantel tests. R_{xy} values indicate the correlation coefficient between genetic difference and linear, as well as log transformed geographical distances between populations/samples of the total dataset and various subsets. Minimum and maximum linear geographic distances between compared sites/samples are shown in kilometres. Significant R values (p<=0.01) are shown in bold, and p values in italics. 44

Supplementary Table 3.1. Descriptive statistics for the 9 microsatellite loci at 25 collection sites of Type α (a) and at 10 collection sites of Type β (b) individuals. N = number of samples, A = number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, G_{is} = inbreeding coefficient, significant values in bold. 63

Supplementary Table 3.2. Values of pairwise genetic differentiation of Type α populations (below diagonal). Values higher than 0.1 are in red, between 0.5 and 0.1 are in orange font. Where P values were calculated, they are shown above diagonal. P values < 0.01 are in italic font, and statistically non-significant (p>0.01) values of genetic differentiation are grayed out. (a) pairwise D_{est} values, (b) pairwise uncorrected F_{st} values, (c) pairwise F_{st} values calculated with ENA correction in FreeNA, (d) pairwise standardised F'_{st} calculated in GenoDive with Meirmans' correction 67

Supplementary Table 3.3. Values of pairwise genetic differentiation of Type β populations (below diagonal). Values higher than 0.1 are in red, between 0.5 and 0.1 are in orange font. Where P values were calculated, they are shown above diagonal. P values < 0.01 are in italic font, and statistically non-significant (p>0.01) values of genetic differentiation are grayed out. (a) pairwise D_{est} values, (b) pairwise uncorrected F_{st} values, (c) pairwise F_{st} values calculated with ENA correction in FreeNA, (d) pairwise standardised F'_{st} calculated in GenoDive with Meirmans' correction 71

Table 4.1. Names and locations of sampling sites. Location of reefs indicated by I= inshore, M = midshelf, and O = outer reefs. Habitat type indicated by E = exposed windward, S = sheltered leeward, L = lagoon 89

Table 4.2. Number of recruits for two lineages (Type α and Type β) of *Pocillopora damicornis* at each of the sampling sites in the central (Palm Is.) and northern (Lizard Is.) GBR, in each sampling period. 92

Table 4.3. Settlement sites and times of clonal recruits of *Pocillopora damicornis* Type α (a) and β (b) lineages. Euclidean distances from their adult clone mates are shown in meters. 98

Table 4.4. Potential origin of recruits based on assignment / exclusion test results. Exclusion threshold from a population was set to 0.1 assignment probability. Recruits not excluded from the local populations are termed "local". "Short-distance migrants" are recruits that were excluded from the local adult population, but were assigned to another adjacent reference population within 10 km. Recruits that were excluded from all reference populations within 10 km of the collection site, but were assigned to distant populations are called "long-distance migrants". Recruits excluded from all known populations are termed "unknown source". 102

Table 5.1. Names and locations of sampling sites of *S. hystrix* adult colonies. Habitat type indicated by E = exposed windward, S = sheltered leeward, L = lagoon 123

Table 5.2. Multiplex groups of primers from Underwood et al. (2006) used for microsatellite genotyping of *S. hystrix* recruits (a) and adults (b). 126

Table 5.3. Number and source of recruits at each of the sampling sites, in each sampling period. SDM = short-distance migrant, LDM = long-distance migrant, Unknown = source unknown, N/A = no genotype data 129

List of Figures

Fig. 2.1. Schematic overview of the diagnostic mitochondrial putative control region. The primer pair Pdam -F and -R amplifies a region that contains two type-specific deletions: a 6-bp deletion in the mtDNA of Type β specimens, and an 8-bp deletion in Type α specimens. The amplicon, when subjected to *Alu1* restriction digestion, gives unique and readily recognizable fragment sizes for Type α, Type β and all other *Pocillopora* species and lineage types. Scissors indicate *Alu1* recognition sites, numbers show the size of the corresponding fragments in bp. 17

Fig. 2.2. Image of 4% TBE agarose gel of the PCR RFLP assay products. A 100 bp ladder is run in the left-hand side lane. 19

Fig. 2.3. STRUCTURE plot (K=3) of 329 *Pocillopora damicornis* (sensu Veron and Pichon, 1976) specimens from Lizard Island. Genetic lineage was determined by the PCR RFLP assay. 21

Fig. 2.4. Results of the Factorial Correspondence Analysis on 329 *Pocillopora damicornis* (sensu Veron and Pichon, 1976) microsatellite genotypes. The first two coordinates explain 100% of the variability. Genetic lineage was determined by the PCR RFLP assay. 22

Fig. 3.1. Map of the study area. Site codes are given in Table 3.1. 28

Fig. 3.2. Estimated density of Type α and β *Pocillopora damicornis* colonies around Lizard Island and the Palm Islands. Colony density was estimated by dividing the number of colonies recorded at each site (denoted by black dots) by the length of the collectors' tracklog (see Methods), and interpolated between sampling sites using the "spline with barrier" method in ArcGIS 10 (ESRI). 35

Fig. 3.3. Cumulative number of clone pairs as a function of distance of clones. Type α = blue dots, Type β = red boxes. 37

Fig. 3.4. Greyscale matrix of D_{est} values of Type α populations. Exposed populations are indicated with an asterisk (*). Actual values are shown in Supplementary Table 3.2a. 41

Fig. 3.5. Results of the spatially explicit Bayesian model-based cluster analysis, as implemented in Geneland, on the Type α dataset – relative membership proportion of populations to each genetic cluster. Model types and number of clusters are indicated on the right hand side of the graphs (Sp=a priori spatial information, NSp= no a priori spatial information, C= correlated allele frequencies, NC= non-correlated allele frequencies). The corresponding number of clusters is indicated with K. Exposed populations are indicated with an asterisk (*). 43

Fig. 3.6. Greyscale matrix of D_{est} values of Type β populations. Exposed populations are indicated with an asterisk (*). Actual values are shown in Supplementary Table 3. 45

Fig. 3.7. Results of the spatially explicit Bayesian model-based cluster analysis, as implemented in Geneland, on the Type α (A) and Type β (B) dataset – relative membership proportion of populations to each genetic cluster. Model types and number of clusters are indicated on the right hand side of the graphs (Sp=a priori spatial information, NSp= no a priori spatial information, C= correlated allele frequencies, NC= non-correlated allele frequencies). The corresponding number of clusters is indicated with K. Exposed populations are indicated with an asterisk (*). 46

Fig. 3.8. Greyscale matrix of D_{est} values of the pooled database of Type α and β populations. 48

Fig. 3.9. Assignment probabilities of 84 Type α (a) and 52 Type β (b) putative first generation migrants (in rows, indicated by the collection sites' code, see Table 3.1) to potential source populations (in columns). The red scale indicates assignment probabilities, and the highest assignment probability for each individual is indicated by a black dot. 49

Fig. 3.10. Examples of simulations of passive particle dispersal with Connie2. Dispersal time 50 days, depth 5 m. 51

Supplementary Fig. 3.1. Photographs of "typical" *P. damicornis* Type α (A), β (B) and "other *Pocillopora*" (C). Morphology of the lineages often overlaps. 73

Supplementary Fig. 3.2. Principal coordinate analysis of pairwise D_{est} values (a), F_{st} values without correction (b), F_{st} values with ENA correction (c), and F_{st} values with Meirmans' correction (d) for Type α populations. The first two coordinates only represent 58.15 %, 56.77 %, 55.38 %, and 61.20 % of the variation of data for D_{est}, non-corrected F_{st}, ENA corrected F_{st}, and Meirmans-corrected F_{st}, values, respectively, and hence fail to reveal a large part of the relationships. Black dots = northern GBR, red squares = central GBR, blue triangles = Southern GBR 74

Supplementary Fig. 3.3. Principal coordinate analysis of pairwise D_{est} values (a), F_{st} values without correction (b), F_{st} values with ENA correction (c), and F_{st} values with Meirmans' correction (d) for Type β populations. The first two coordinates only represent 70.75 %, 70.38 %, 70.36 %, and 82.06 % of the variation of data for D_{est}, non-corrected F_{st}, ENA corrected F_{st}, and Meirmans-corrected F_{st}, values, respectively, and hence fail to reveal a large part of the relationships. Black dots = northern GBR, red squares = central GBR. 76

Supplementary Fig. 3.4. STRUCTURE plot of Type α individuals. Each column represents an individual, with the colours showing proportional cluster membership. a) K=3 identified by the Evanno method, b) K=20 identified by the NSp-C model in Geneland. Exposed populations are indicated with an asterisk (*). 78

Supplementary Fig. 3.5. Significant but weak relationship between pairwise genetic distance and log transformed geographic distance in kms of Type α (a) and β (b) *P. damicornis* specimens. Large and small genetic distances can be observed at both large and small distances. The slope of the regression lines and their significance are driven by the relatively lower numbers of large within-site (i.e. approximately less than 100 m) genetic differences. 79

Supplementary Fig. 3.6. STRUCTURE plot of Type β individuals. Each column represents an individual, with the colours showing proportional cluster membership. a) K=3 identified by the Evanno method, b) K=10 identified by the NSp-C model in Geneland. Exposed populations are indicated with an asterisk (*). 80

Fig. 4.1. Map showing sampling sites in the northern and central Great Barrier Reef (GBR) for the adult and juvenile population genetic studies. Black squares indicate collection sites of adult colony samples, white circles in black squares denote sites where settlement tiles have also been deployed. See Table 4.1 for site names. 87

Fig. 4.2. Correlations between recruit numbers and density estimates of adult colonies of *Pocillopora damicornis* Type α and β at Lizard Island and the Palm Islands. The method used to estimate population densities is denoted as either Q for the quadrat-method or T for the tracklog-method (see Methods). Site names are abbreviated as per Table 4.1. 94

Fig. 4.3. Number and source of Type α and β recruits of *Pocillopora damicornis* (pie diagrams) in relation to population density maps around Lizard Island and the Palm Islands. Colony density was estimated by dividing the number of colonies recorded at each site (denoted by black dots) by the length of the collectors' tracklog (see Methods), and interpolated between sampling sites using the "spline with barrier" method in ArcGIS 10 (ESRI). Pie charts show the proportion of recruits from four source categories. Sizes of the pie charts are proportional to the numbers of recruits sampled at the corresponding site throughout the study period (also shown in brackets below the site names). 95

Fig. 4.4. Size distribution of Type α (black bars) and β (white bars) recruits on year-round tiles, measured by the number of polyps per recruit (on x axis). Dashed line indicates maximum number of recruits observed on regularly sampled tiles at Palm Islands (max 2 mo old recruits). 96

Fig. 4.5. Probability of assignment and proportional membership in *a posteriori* genetic clusters of recruits of *Pocillopora damicornis* Type α (a) and Type β (b) lineages. The gray scale matrix shows the assignment probabilities of each recruit (in rows) to each adult reference population (in columns, names as per Table 4.1). Non-exclusion from the surrounding reference population is indicated with a red dot. The annotation and color codes to the left-hand side show the site, the year and the period of collection. Identical MLGs are color coded in the "Clones" column. To the right-hand side, proportional cluster membership in *a posteriori* genetic clusters of recruits is shown on a barplot (STRUCTURE plot), at K=2, 5 and 25 for Type α, and K=2, 10 and 23 for Type β recruits. 100

Fig. 4.6. Proportional membership in *a posteriori* genetic clusters of adult (reference) samples. Genetic clusters correspond to those in Fig. 4.5. Adult clone mates of recruits are indicated by colored rectangles to the left of each column and color coded as per Fig. 4.5. Population names as per Table 4.1. 103

Fig. 4.7. Stacked bar charts comparing recruitment rates of *Pocillopora damicornis* Type α (a) and β (b) lineages at leeward (left charts) and windward (right charts) sites through time. For each site, the y axis represents the normalized number of recruits per day per 100 $m^{2,}$ while n in brackets behind each site name shows the actual number of recruits collected during the sampling period. Dispersal classes were identified by genetic assignment tests (see Methods). Self-recruiters could not be identified among Type β recruits at NE Pelorus, NE Orpheus and SW Lizard Island because of insufficient numbers of adult colonies for assignment tests. 106

Fig. 5.1. Map of the study area. White squares indicate collection sites of colony samples, black dots indicate recruit sampling sites. The pie charts show the proportion of recruits from four source categories plus those without genotype data. The sizes of pie charts are proportional within a region to the numbers of recruits (shown in brackets) sampled at the corresponding site throughout the study period (May 2009 – May 2010 around Lizard Island; May 2009 – May 2011 around the Palm Islands). Recruits were only sampled at NW Pelorus and NW Orpheus in 2011, therefore data from this year is shown on separate pie charts left of the main pies to make inter-habitat comparisons easier. Note that local recruits were not identified at NE Pelorus, due to the lack of local reference population. 124

Fig. 5.2. Schematic figure of banding patterns of products of the expanded genetic assay from Chapter 2 after electrophoresis on an agarose gel. Numbers show fragment sizes in bp. 125

Fig. 5.3. Correlation between the density of adult *S. hystrix* colonies and recruits at sites around Lizard Island (red squares) and the Palm Islands (blue dots). Site codes are 1 – NE Lizard, 2 – SE Lizard, 3 – NW Lizard, 4 – SW Lizard, 5 – NE Pelorus, 6 – NE Orpheus, 7 – W Pelorus, 8 – NW Orpheus. 128

Fig. 5.4. Size distribution of *Seriatopora hystrix* recruits on year-round tiles in the May 2009 – May 2010 period (dark grey bars) and the May 2010 – May 2011 period (light grey bars). Recruit size is shown on the x axis by the number of polyps per recruit, and the number of recruits within each size-class is shown on the y axis. The dashed line indicates maximum number of recruits observed on regularly sampled tiles at Palm Islands, and therefore represents the maximum estimated size of two-months-old recruits. 131

Fig.5.5. Reverse cumulative number of clones as a function of Euclidean distance between clone mates on a logarithmic scale (a) and on a linear scale (b). Red lines show logarithmic and green lines exponential fitted curves, with respective functions and R^2 values similarly colour-coded. 133

Fig. 5.6. Stacked bar chart of recruitment rate at each sampling site, dispersal distance category, and time-window. For each site, Y axis shows normalized mean number of recruits per day per 100 m^2. Potential dispersal distance classes were identified by genetic assignment tests. The actual numbers of recruits collected during the sampling period (n) are shown in brackets. 135

Fig. 5.7. Variation in recruitment rate between corresponding time-windows in consecutive years at sheltered sites of the Palm Islands. Y axis shows changes between the number of recruits in each time-window in a given year, compared to the previous year. Significance of the change in recruitment rates was tested by paired t-tests. Significant values are indicated by filled markers, non-significant values by hollow markers. Change could not be calculated for NW Orpheus in Aug-Sep 2010 vs. 2009 because the denominator was zero. 136

Fig. 5.8. Mean estimated log probability of the Bayesian clustering results of *Seriatopora hystrix* recruits. The curve reaches a plateau at K=7. 137

Fig. 5.9. Structure plots of *S. hystrix* recruits and adults at K=7. Bayesian clustering was run on the merged genotype database of adults and recruits, therefore clusters (colours) of the two plots are identical. Recruits are grouped hierarchically by site, year, and season of settlement. Estimated natal origin of each recruit is shown by a colour-code consistent with Figs. 5.1 and 5.6. Adult specimens are grouped by site and genetic lineage identity, as identified by P. Warner (unpublished thesis). 139

Chapter 1

General introduction

1.1 Connectivity and conservation of coral reefs

Tropical coral reefs are mosaics of seemingly disconnected habitat patches at almost all spatial scales (Veron, 2000; Hellberg, 2007), however, local populations of the species that inhabit these highly productive and diverse ecosystems often form metapopulations (Kritzer and Sale, 2006). Metapopulations are regional groups of spatially separated, but demographically interconnected subpopulations, with a dynamic system-behaviour that gives resilience to the network (Hanski, 1999). On a genetic level, benefits of connectivity among subpopulations include: (1) individuals of connected populations are more likely to carry higher intra-individual genetic diversity (heterozygosity; Frankham, 2006), which is thought to result in their increased fitness (reviewed in Hansson and Westerberg, 2002); (2) interconnected populations are likely to have higher inter-individual genetic diversity, which fuels natural selection (Frankham et al., 2002); and (3) connectivity increases local effective population size, thereby reducing the risk of genetic erosion through drift (Frankham et al., 2002). On an ecological level, demographic links between local populations may reinforce locally depleted populations from external sources, and in extreme cases, can maintain a dynamic equilibrium between local extirpations and new colonization events (Hanski, 1999). Recognition that Aristotle's classic theory of holism, i.e. "the whole is greater than the sum of its parts", holds also for metapopulation viability, has led to acknowledgement of the importance of creating networks of protected areas to sustain strong connectivity among subpopulations (e.g. Bennett, 1999; Crooks and Sanjayan, 2006).

In the marine realm, designation of marine protected areas (MPAs) has become the most widely adopted strategy for managing and protecting biodiversity (Gubbay, 1995), with an exponentially increasing trend in their numbers (Edgar et al., 2007). Indeed, in tropical coral reef ecosystems, MPAs have been shown not only to increase fish stocks within their boundaries (Williamson et al., 2004) and spillover of target fish species to surrounding areas (Harrison et al., 2012), but also to significantly enhance coral recruitment through a cascade of ecological responses of the ecosystem to decreased anthropogenic pressures (Mumby et al., 2007). To maximize benefits of these complex ecosystem responses and increase resilience of solitary MPAs in the current era of increasing anthropogenic disturbances, including rapid changes in climate, it has been proposed that marine reserves should be spaced in a way that accommodates routine

inter-reef dispersal of species that have a key role in creating or maintaining the coral reef habitat (Palumbi, 2001; Botsford et al., 2009; McCook et al., 2009; Steneck et al., 2009; Almany et al., 2009). Yet in practice, inclusion of aspects of connectivity in the design of marine ecological networks has been hindered by substantial gaps in our knowledge of the metapopulation dynamics, including source-sink dynamics and demographically significant dispersal distances, of many coral reef organisms. For example, the world's largest marine reserve network on the iconic Great Barrier Reef (GBR) was created using extremely sophisticated and complex site-selection algorithms (Lewis et al., 2003), which did not include connectivity.

1.2 Assessing population connectivity in corals

Studying population connectivity of tropical coral reef species is particularly challenging, because the adult life-stage of most reef-dwelling organisms is sessile or sedentary on the spatial scale of reef patches, and thus connections between nodes of their metapopulations are largely maintained via dispersal of planktonic larvae (Pineda et al., 2007; Cowen and Sponaugle, 2009). When larvae are released simultaneously and form large aggregated slicks, their direct tracking provides rough estimates of larval dispersal (Willis and Oliver, 1988, 1990). However, since larvae of most reef species do not form conspicuous slicks, and the direct tracking of small and numerous larvae in a vast and complex fluid environment poses technical difficulties, empirical larval dispersal studies have mainly been based on indirect techniques (for an exception, see Carlon and Olson (1993)) . The development of permanent skeletal structures in an early phase of ontogenesis of fishes permits estimation of larval origins and dispersal tracks from the chemical composition of otoliths (Thorrold et al., 2007). Experimental manipulation of otolith chemistry of fish larvae via injecting rare isotopes of alkaline earth metals into the mother fish prior to spawning (transgenerational isotope labelling) enables further refinement of the method and the application of mark-recapture techniques (Thorrold et al., 2006; Woodcock et al., 2011). However, because of the lack of structures in coral larvae that can be tagged, the application of these chemistry-based techniques to coral larvae is impossible, which limits empirical dispersal studies to the analysis of genetic signatures (Hellberg et al., 2002; van Oppen and Gates, 2006).

1.2.1 The genetic approach

The genetic composition (allele and genotype frequencies) of biological populations reflects processes of selection, random drift, mutation, inbreeding and migration (Hartl et al., 1997). By using neutral genetic markers in genetic analyses, the effect of selection is eliminated, and genetic differentiation of populations, as well as intra-population levels of genetic diversity will carry the signature of present and past population connectivity and effective population sizes (Waples and Gaggiotti, 2006). Most early population genetic studies on connectivity of scleractinian corals were based on allozymes (e.g. Stoddart, 1984; Ayre and Resing, 1986; Ayre and Willis, 1988; Ayre and Dufty, 1994; Stobart and Benzie, 1994; Hellberg, 1994; Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000, 2004; Ridgway et al., 2001; Ridgway, 2005; Whitaker, 2006), because DNA markers proved difficult to develop in corals (reviewed in van Oppen and Gates, 2006). However, the neutrality of allozymes is sometimes dubious (summarized in Hey, 1999). Therefore, making use of advances in DNA techniques, subsequent population genetic studies have used nuclear DNA microsatellite markers (e.g. Magalon et al., 2004, 2005; Underwood et al., 2007, 2009; van Oppen et al., 2008), as well as the polymorphic ITS (internal transcribed spacer) region of the ribosomal DNA (Takabayashi et al., 2003; Zvuloni et al., 2008), although this latter has been criticized because the nuclear ITS region has relatively low mutation rates, and in many instances high levels of intra-individual variation, and therefore has limited use for population genetic approaches (Van Oppen and Gates, 2006 and references therein).

In corals, larval dispersal and hence population connectivity occurs over a continuum of spatial and temporal scales (Paris, 2011), and the different scales have different implications for the metapopulation. A few migrants per generation are enough to genetically homogenise populations (Wright, 1931), but are unlikely to have immediate demographic impact over ecologically significant timescales (over the course of a few generations). Such low intensity connectivity (i.e. evolutionary connectivity) is therefore more important for speciation and processes that determine species ranges over evolutionary timescales (i.e. over the course of thousands of generations) than for prompt restoration of depleted populations, although there is potential for a few

colonisers to establish new populations or re-establish extinct populations (Hanski, 1991). However, demographically significant numbers of migrants have to be exchanged between populations over ecological timescales (i.e. ecological connectivity) for larval dispersal to be significant as a reliable path to recovery after an acute disturbance.

Separating genetic signatures of contemporary *ecological connectivity* from those of *evolutionary connectivity* is particularly challenging in corals, because of a range of biological and statistical issues (Palumbi, 2003; van Oppen and Gates, 2006). Traditional population genetic statistical methods are based on equilibrium models, such as F-statistics (Weir and Cockerham, 1984), which are relatively insensitive to changes in gene flow over contemporary timescales (Manel et al., 2005) and have unrealistic assumptions for most natural populations (Whitlock and McCauley, 1999). Significant improvements to the original F-statistics (Hedrick, 2005; Meirmans, 2006; Jost, 2008) now provide powerful tools to measure genetic differentiation of populations and infer population connectivity over evolutionary timescales. At the same time, new individual-based statistical approaches have been developed that describe sub-population genetic structures (Pritchard et al., 2000; Falush et al., 2007), and enable researchers to genetically assign individuals to potential source populations or to detect first-generation migrants (Rannala and Mountain, 1997; Cornuet et al., 1999; Wilson and Rannala, 2003; Piry et al., 2004). While these improvements in statistics now allow researchers to make reliable inferences to both evolutionary and contemporary immigration rates (reviewed in Manel et al., 2005), biological characteristics of corals create further challenges for assessment of ecological connectivity. Namely, the lifespan of genotypes in corals can be extremely long due to their high potential for asexual reproduction (Hughes and Jackson, 1980; Highsmith, 1982), which may create misleading patterns of genetic structures in adult populations (Botsford et al., 2009). To overcome this difficulty, it is possible to genetically characterise juveniles or newly settled recruits and make comparisons between genetic structures of the adult populations and cohorts of recruits (Zvuloni et al., 2008), however this approach has not been explored in detail until recently.

A fundamentally different individual-based genetic approach is to identify parents of specimens within a population by matching their genotypes (Jones and Ardren, 2003). Indeed, parentage analysis has been successfully used in coral reef fishes to directly assess dispersal of larvae from parents (Jones et al., 2005; Planes et al., 2009; Harrison et al., 2012), but its application in coral larval dispersal analysis is problematic, due to sampling requirements that are hard to satisfy. For reliable parent assignments, this technique requires that almost all potential parents are sampled (H. Harrison, pers. comm.; Jones et al., 2010), however coral gametes are spawned into the water column and may disperse substantially, thus the potential pool of parents may be widely scattered spatially.

1.2.2 The modeling approach

Because coral larvae are transported by ocean currents, the development of increasingly realistic water circulation models (e.g. Cowen et al., 2000; Condie et al., 2005; Lambrechts et al., 2008), combined with improved understanding of the behavioural and physiological characteristics of coral larvae (e.g. Harii et al., 2002; Harrison, 2006; Graham et al., 2008) now enable modeling of their dispersal (e.g. the Connectivity Modeling System, https://www.rsmas.miami.edu/users/cparis/cms/description.html). The major benefits of modeling compared to empirical approaches is that models can be run on larger spatial and temporal scales (e.g. Kool et al., 2011); they can be used to simulate historical, present, and future dispersal events, and can accommodate changes in climate (Munday et al., 2009), as well as variability in the geomorphology of coastlines or topography of reefs (Paris et al., 2002). To assess the credibility and increase the accuracy of models, their predictions need to be cross-validated with results of empirical studies (Galindo et al., 2010). Modelled gene flow showed a very good match to observed genetic patterns in coral populations in the Caribbean (Baums et al., 2006; Galindo et al., 2006; Kool et al., 2010; Foster et al., 2012), encouraging similar interdisciplinary approaches to be tested in other reef systems around the world.

1.3 Reproductive strategies and connectivity in corals

Scleractinian corals have a suit of reproductive strategies that typically result in different dispersal capabilities (Harrison and Wallace, 1990). The two fundamentally different modes of sexual reproduction are (1) releasing gametes into the water column for external fertilization and larval development ("broadcast spawning"), and (2) brooding planulae within coral polyps following internal fertilization ("brooding"; Harrison and Wallace, 1990). Typically, larvae that develop externally from spawned gametes have an obligatory 2.5-5 days of pelagic development before they become competent to settle (Babcock and Heyward, 1986; Miller and Mundy, 2003; Harrison, 2006) and may have competency periods of up to 240 days (Graham et al., 2008). In contrast, brooded planulae are competent to settle immediately when released, and maintain their competency for over 100 days (Richmond, 1987; Harii et al., 2002). Consequently, externally developed coral larvae are thought to provide the most common mechanism for long distance dispersal among coral populations, and indeed several comparative studies have found that gene flow is higher among broadcast spawning species than brooding species (e.g. Nishikawa et al., 2003; Underwood et al., 2009). However, both modeling (Black, 1993; Blanco-Martin, 2000; Figueiredo et al., 2012; Andutta et al., 2012) and genetic studies (e.g. Ayre and Hughes, 2000, 2004; van Oppen et al., 2008, 2011b) have shown that dispersal does not always follow these predicted patterns, because water circulation features can override larval capacities in the final outcome of dispersal.

Further to differences in dispersal, different reproductive methods will have different genetic consequences on the progeny (Table 1.1.). Broadcast spawning typically results in genetically recombined offspring, however self-fertilisation may occur (e.g. Stoddart et al., 1988). On the other hand, brooding can produce asexual offspring, as well as genetically recombined larvae, but brooded larvae can also rise from self-fertilized eggs (e.g. Sherman, 2008). And finally, fragmentation produces clones of the parent colony, however non-lethal somatic mutations may appear and create genetic diversity between fragments (e.g. van Oppen et al., 2011c).

The pocilloporid family harbours the most common brooding corals on the GBR (Veron, 2000), but substantial inter-specific differences in life history strategies within

this family predict a variety of connectivity patterns. The pocilloporid needle coral *Seriatopora hystrix* (Dana, 1846) releases its sexually produced planulae (Ayre and Resing, 1986) several times a year (reviewed in Fadlallah, 1983), therefore it takes advantage of a range of different environmental conditions that likely affect its dispersal and development, such as different current regimes and water temperatures. Accordingly, estimates of connectivity, dispersal and larval retention in *S. hystrix* are complex and often contradictory. Predominantly self-seeding populations were reported from the GBR (Ayre and Dufty, 1994; Ayre and Hughes, 2000) and the Red Sea (Maier et al., 2005), with limited larval dispersal on scales of a few hundred meters to a few tens of kilometres. In contrast, panmixia over ~ 8 km was later described from the same region in the Red Sea (Maier et al., 2009) and evidence for occasional long-distance dispersal was reported from NW Australian reefs (Underwood et al., 2007) and from the GBR (Van Oppen et al., 2008).

Pocillopora damicornis (Linnaeus, 1758) is a peculiar pocilloporid coral species, because its life history strategy encompasses both brooding and spawning reproductive modes; however the implications of this mixed strategy for connectivity have generally not been considered. This species is reported to release brooded larvae throughout the year, with seasonal peaks in planulation that vary geographically (e.g. Harrigan, 1972; Harriott, 1983; Richmond and Jokiel, 1984; Villanueva et al., 2008). Planulae were found to be exclusively asexually produced at various sites around Australia (Stoddart, 1983; Ayre and Miller, 2004; Schmidt-Roach et al., 2012), creating highly clonal populations, particularly in south-western Australia (Stoddart, 1984). Conversely, populations of *P. damicornis* are primarily sexually maintained on the GBR, a paradox that has taunted researchers for over a decade (Ayre et al., 1997; Ayre and Miller, 2004; Sherman et al., 2005). Clonal populations were also found in Kenya (Souter et al., 2009) and Southern Taiwan (Yeoh and Dai, 2010), however in this latter location, 26 % and 6 % of larvae in two single broods, respectively, were found to be of sexual origin (Yeoh and Dai, 2010). Conversely, populations of *P. damicornis* are primarily sexually maintained on the GBR, a paradox that has taunted researchers for over a decade (Ayre et al., 1997; Ayre and Miller, 2004; Sherman et al., 2005). Until recently, spawning has only been inferred from the sudden disappearance of mature gametes in histological samples of *P. damicornis* (Stoddart and Black, 1985; Glynn et al., 1991; Ward, 1992).

A recent observation of spawning has unveiled that eggs are negatively buoyant (Schmidt-Roach et al., 2012b), which suggests low dispersal capabilities of externally produced larvae. Further to the complexity of its reproductive strategies, the nomical species *P. damicornis* has been found to have very high intraspecific diversity (Flot et al., 2008a; Souter, 2010; Schmidt-Roach et al., 2012a), with five distinct lineages on the GBR that potentially represent cryptic species (Schmidt-Roach et al., 2012a). Therefore it is reasonable to suspect that earlier studies that described strong genetic subdivision on small spatial scales in populations of *P. damicornis* on the GBR and remarkable genetic similarity at the scale of hundreds of kilometres (Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000, 2004), have been confounded by the inclusion of several potential cryptic species in analyses. Because there have been substantial advances in DNA techniques applicable to coral genetic studies, statistical methods of population genetics, and molecular taxonomy since the last assessment of population connectivity in *P. damicornis*, it is timely to re-visit this issue and provide conservation managers with much needed information on ecological connectivity in lineages of this model coral species.

1.4 Aims of the thesis

The overarching aim of this thesis is to improve current understanding of population connectivity in corals and ultimately contribute to refining the design of MPA networks in the tropical coral reef environment. In particular, my goal is to obtain estimates of ecological connectivity and its spatio-temporal stochasticity for two common pocilloporid coral species on the GBR, *Seriatopora hystrix* and *Pocillopora damicornis*, taking advantage of recent improvements in DNA techniques applicable to coral genetic studies and statistical methods, as well as discoveries in molecular coral taxonomy.

In Chapter 2, I present a novel genetic assay that quickly and cost-efficiently identifies the two most common lineages of the *P. damicornis* nominal species on the GBR. This study provides the tool to focus connectivity research in the subsequent two chapters on genetically meaningful units of observation.

In Chapter 3, I describe population genetic patterns and subpopulation genetic structures in adult populations of the two most common lineages of *P. damicornis* on the GBR, and make inferences about evolutionary and ecological connectivity. Results of this chapter are comparable to previous studies, because they describe connectivity from population genetic patterns of adult specimens, but are novel in the techniques used and in that I differentiate among lineages of the *P. damicornis* morphospecies.

In Chapter 4, I genetically characterize newly settled recruits of *P. damicornis*, and determine their potential natal source population by assigning them to adult reference populations from Chapter 3. From these estimates, I draw conclusions about contemporary connectivity, as well as spatial and temporal variability of recruitment in this species.

In Chapter 5, I first present a modification of the genetic assay described in Chapter 2, which allows identification of *S. hystrix* recruits from among other pocilloporid recruits. Armed with this new assay, I repeat the methodology applied in Chapter 4, this time on newly settled *S. hystrix* recruits. This chapter is a result of a collaboration with the parallel Ph.D. project of P. Warner, and the reference adult genotype dataset was kindly provided by the collaborator.

In Chapter 6, I synthesize key findings from the preceding chapters into a cohesive discussion, with an emphasis on the potential application and implications of the results in further studies and in practical conservation management. I also identify topics for future research that could possibly unveil some of the remaining grey areas of connectivity in the study species.

1.5 Nomenclature

Throughout this thesis I use the term “lineage” to refer to the intraspecific genetic diversity in the *P. damicornis* morphospecies (Souter, 2010; Schmidt-Roach et al., 2012a). While new species have not been described within *P. damicornis* formally yet, Schmidt-Roach et al. (2012a) have shown that P. *damicornis* Types α and β are true evolutionary or phylogenetic lineages and also evolutionarily significant units (ESUs), as these two taxa show reciprocal monophyly in mtDNA and significant genetic

differentiation in nDNA. In addition there are significant biological differences between the two types (personal observation, Schmidt-Roach et al. unpublished). By using the term “lineage” my thesis is also consistent with the literature on the subject.

Chapter 2

A rapid genetic assay for the identification of the most common *Pocillopora damicornis* genetic lineages on the Great Barrier Reef

This chapter is inserted without Acknowledgements as in press in PLoS ONE: Torda G, Schmidt-Roach S, Peplow LM, Lundgren P, van Oppen MJH (2012) A rapid genetic assay for the identification of the most common *Pocillopora damicornis* genetic lineages on the Great Barrier Reef. *PLoS ONE* (in press)

All the data was collected and analysed by G. Torda, who also wrote the chapter and manuscript after intellectual contributions from all co-authors.

Abstract

Pocillopora damicornis (Linnaeus, 1758; Scleractinia, Pocilloporidae) has recently been found to comprise at least five distinct genetic lineages in Eastern Australia, some of which likely represent cryptic species. Due to similar and plastic gross morphology of these lineages, field identification is often difficult. Here we present a quick, cost effective genetic assay as well as three novel microsatellite markers that distinguish the two most common lineages found on the Great Barrier Reef. The assay is based on PCR amplification of two regions within the mitochondrial putative control region, which show consistent and easily identifiable fragment size differences for the two genetic lineages after *Alu1* restriction enzyme digestion of the amplicons.

2.1. Introduction

Many scleractinian coral species show high morphological variability that has been related to differing environmental conditions. Reciprocal transplant experiments have demonstrated that the same genet may exhibit different growth forms in response to light and water movement intensities (Willis, 1985; Bruno and Edmunds, 1997). However, some putative eco-morphs within single species represent distinct genetic lineages (Stobart and Benzie, 1994; Medina et al., 1999; Carlon and Budd, 2002; Combosch et al., 2008; Flot et al., 2008a; Nunes et al., 2008; Forsman et al., 2009; Souter, 2010).

Pocillopora damicornis (Linnaeus, 1758) is a key model scleractinian coral species displaying a wide spectrum of morphotypes throughout its range (Veron and Pichon, 1976), some of which occur in sympatry. The biology of this species, especially its reproductive modes, seasonality and lunar periodicity have often been found to be unexplainably variable, sometimes contradicting, among and even within studies (e.g. Harriott, 1983; Richmond and Jokiel, 1984; Stoddart and Black, 1985; Ward, 1992; Tanner, 1996). Recently, four independent studies have discovered sympatric, but seemingly reproductively isolated genetic lineages within the morphospecies *P. damicornis*: Flot et al. (2008b) report five distinct mitochondrial lineages from Hawaii, Pinzón and LaJeunesse (2011) found three genetic lineages in the Tropical East Pacific, Souter (2010) identified two from the coasts of Tanzania and Kenya, and Schmidt-Roach et al. (2012a) describe five lineages from the coast of Eastern Australia. The latter study showed that the genetic lineages of *P. damicornis* (sensu Veron and Pichon, 1976) differ in their mode of reproduction, as well as in seasonal and lunar reproductive peaks. In light of these new results, the complex biology of the morphospecies *P. damicornis* should be re-visited, using the newly identified genetic lineages as the units of observation. Although the genetic lineages seem to be associated with phenotypic characteristics, phenotypic plasticity and cryptic appearance complicate confident identification, especially for the untrained eye. Currently, the only practical technique to differentiate among these genetic lineages is sequencing mitochondrial regions characteristic for each lineage (Schmidt-Roach et al., 2012a). However, the relatively high costs and time-consuming nature of this procedure make their routine application to large sample sets unrealistic.

This paper presents a quick and relatively low-cost genetic assay to reliably identify the two most common, and best resolved genetic lineages of *P. damicornis* from the Great Barrier Reef (GBR), Types α and β (2012a). Our assay targets the mtDNA putative control region (as described in Flot and Tillier, 2007) by RFLP analysis of PCR amplicons. We tested the performance of the assay by comparing its results against two datasets: (i) 145 coral samples with known lineage identity based on a multi-locus (both nuclear and mtDNA) sequence analysis (2012a; Table 1), and (ii) 329 samples of *P. damicornis* (identified morphologically sensu Veron and Pichon, 1976), collected around Lizard Island, central GBR, and characterised by nine polymorphic microsatellite loci, three of which were developed as part of this study.

Table 2.1. Location and phylogenetic identities of *Pocillopora damicornis* samples from Schmidt-Roach et al. (2012a). All samples were correctly identified by the RFLP PCR assay as Type α, Type β or "other *Pocillopora*". "Type α LHI" specimens (indicated with an *) were identified as "other *Pocillopora*" by the assay.

Location	Outgroup	Type α	Type β	Type γ	*Pocillopora verrucosa*	Type δ	Type ε	**Total**
Great Detached Reef			3	1	1		1	**6**
Great Keppel Island		19						**19**
Long Reef			3	1	2			**6**
Lord Howe Island		1+4*						**5**
Myrmidon Reef	1		1					**2**
Night Reef		3	3					**6**
Orpheus/Pelorus Island		3	19	4	10			**36**
One Tree Island		6						**6**
Rib Reef		2	3	1				**6**
Solitary Islands						3		**3**
Tydeman Reef			3		3			**6**
Wallace Islet Reef		21	3					**24**
Wilkie Reef		2	16	2				**20**
Total	**1**	**61**	**54**	**9**	**16**	**3**	**1**	**145**

2.2. Materials and methods

2.2.1. Ethics Statement

All necessary permits were obtained for the described field studies. Specimens for this study were collected under permit numbers G08/28215.1 and G09/30237.1, issued by the Australian Government's Great Barrier Reef Marine Park Authority. The locations of sample collection are not privately-owned, and no endangered or protected species were collected.

2.2.2. Assay development

We searched the genome of pocilloporid species for conserved lineage-specific indels. Following unsuccessful trials on nuclear genomes, we aligned the putative control region (i.e., the region between the *atp8* and *cox1* genes (Flot and Tillier, 2007) of the mitochondrial genome) of pocilloporid species, including the newly identified genetic lineages that are present on the GBR. The alignment of GenBank sequences NC_009797.1, NC_009798.1, NC_010244.2, NC_010245.2, NC_011162.1, NC_011160.1, JX624790 – JX625114 in BioEdit v7.0.1 (Hall, 1999) using ClustalW Multiple Alignment (Thompson et al., 1994) showed that an eight-bp deletion is characteristic for *P. damicornis* Type α and a six-bp deletion is unique for Type β, *sensu* Schmidt-Roach et al. (2012a). The design of primers that directly bind to these lineage-specific indels was unsuccessful, therefore, using the web-based program Primer3 (Rozen and Skaletsky, 2000) we developed primers that target the region containing both indels, with a total length of 705 (Type α), 707 (Type β), and 713 bp (all other *Pocillopora* spp. and genetic lineages, hereafter "other *Pocillopora*"). Primer sequences are Pdam-F 5'-AAG AAG ATT CGG GCT CGT TT-3' and Pdam-R 5'-CGC CTC CTC TAC CAA GAC AG-3'. These primer sequences do not occur in the mtDNA genome of the pocilloporid genera *Seriatopora* and *Stylophora*. The detection of such small amplicon size differences is challenging. Therefore, to facilitate rapid and reliable identification, i.e. to enable the use of simple agarose gels with reasonable electrophoresis time without compromising identification accuracy, we included a digestion step with *Alu1*. This restriction enzyme digest generates a unique banding pattern for Type α (84, 116 and 389 bp fragments), Type β (92, 110, 116 and 389 bp

fragments) and "other *Pocillopora*" (92, 116 and 389 bp fragments; Fig. 2.1), that is recognisable on a high density agarose gel.

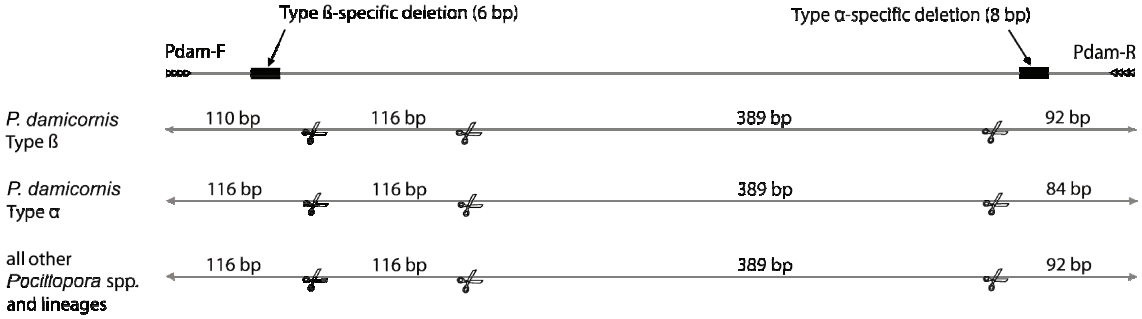


Fig. 2.1. Schematic overview of the diagnostic mitochondrial putative control region. The primer pair Pdam -F and -R amplifies a region that contains two type-specific deletions: a 6-bp deletion in the mtDNA of Type β specimens, and an 8-bp deletion in Type α specimens. The amplicon, when subjected to *Alu1* restriction digestion, gives unique and readily recognizable fragment sizes for Type α, Type β and all other *Pocillopora* species and lineage types. Scissors indicate *Alu1* recognition sites, numbers show the size of the corresponding fragments in bp.

2.2.3. Testing the assay

The assay was first tested on the 145 DNA samples that were used in the phylogenetic study of Schmidt-Roach et al. (2012a; Table 2.1). PCR reactions were run in 10 µl volumes using a Qiagen Multiplex PCR Kit (Qiagen®), following the manufacturer's protocol, with 2 pmol of both primers, and approximately 40 ng of the template DNA. Thermal cycling protocol was 15 min at 95°C, 30x (30 s at 95°C; 90 s at 60°C and 60 s at 72°C) and 10 min at 60°C. PCR products were subjected to Alu1 restriction enzyme digestion without purification for 1 hour at 37°C, using 5 µl of PCR product with 0.25 µl (2.5 U) of Alu1 enzyme and 1 µl of 10x NE Buffer 4 (BioLabs Inc.) in a 10 µl reaction. Five µl of the digested products were run on 4 % TBE agarose gels (15 x 20.5 cm in size) for 4 hrs at 150 Volts. Banding patterns were scored manually. The resulting lineage identities of samples were compared to the published phylogenetic memberships (Schmidt-Roach et al., 2012a).

We also compared the assay's performance to an independent 329 *P. damicornis* (identified morphologically sensu Veron and Pichon, 1976) multilocus microsatellite genotypes. These samples were collected at Lizard Island, northern GBR, and stored in absolute ethanol, until extracting DNA by a modified protocol of the salt precipitation method described in Wilson et al. (2002). Microsatellite primers developed by Magalon

et al. (2004) and Starger et al. (2008) were tested for amplification and polymorphism on a random subset of 50 samples. One marker from Magalon et al. (2004) and five from Starger et al. (2008) were found to be polymorphic in our samples. To increase the discriminative power of genotyping, we developed additional microsatellite markers, as follows. Twelve *P. damicornis* colonies were collected at Orpheus Island, central GBR, and were transferred into aquaria at the Australian Institute of Marine Science. After an acclimatization time of four days, colonies were bleached with 10 $\mu g\ l^{-1}$ of the herbicide diuron (Negri et al., 2005) over two weeks to remove most *Symbiodinium* cells from the coral tissue. Bleached fragments of each colony were preserved in absolute ethanol. DNA was extracted using the Qiagen DNEasy Tissue Kit and checked for *Symbiodinium* contamination by amplification of the ITS region, according to Coleman et al. (1994). None of the twelve samples showed amplification for *Symbiodinium* and were used for microsatellite isolation following Glenn and Schable (2005). After the creation of an enriched microsatellite library, primers for 15 microsatellites were designed using Primer3, and these loci were tested for polymorphism. Three marker sets were identified to amplify polymorphic microsatellite regions (Table 2), and were pooled with the selected markers from Magalon et al. (2004) and Starger et al. (2008) in three multiplex and one simplex PCR reactions (Table 3).

All 329 *P. damicornis* DNA samples were genotyped at the nine microsatellite loci, following the Qiagen Multiplex PCR Kit (Qiagen®) protocol, using 0.5 pmol of the M13-tailed F primers, and 2 pmols of all other primers (Table 3). Thermal cycling was similar to the assay's protocol, adjusting the annealing temperatures as per Table 3. PCR products were separated on a MegaBACE 1000 DNA Analysis System. Electropherograms were analyzed using MegaBACE Fragment Profiler v1.2 (Amersham Biosciences). All automatic scoring was checked manually.

The Bayesian clustering method implemented in STRUCTURE v2.3.3 (Pritchard et al., 2000) was run on the multilocus genotype dataset for K=3 to test whether the genotypes cluster according to their lineage identity, using the admixture model without locprior, and independent allele frequencies without initial population information, with a burn-in of 100,000 and 100,000 MCMC replications after the burn-in. Additionally, a Factorial Correspondence Analysis (FCA) was carried out in Genetix 4.05.2 (Belkhir et al., 1996) on the multilocus genotypes. The first two coordinates were plotted for visual analysis. The PCR RFLP assay was run on the same 329 DNA samples.

2.3. Results and Discussion

Consistent with our expectations, three easily distinguishable banding patterns were seen on the agarose gels after running the assay on any *Pocillopora* DNA sample (Fig. 2.2).

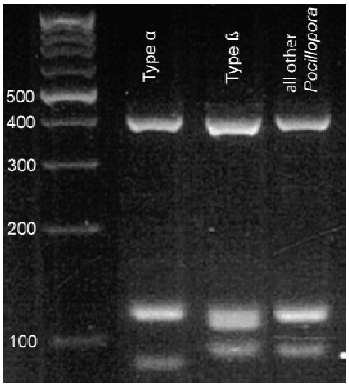


Fig. 2.2**.** Image of 4% TBE agarose gel of the PCR RFLP assay products. A 100 bp ladder is run in the left-hand side lane.

The assay correctly identified all GBR samples from the study of Schmidt-Roach et al. (2012a) as either Type α, β or "other *Pocillopora*", and classified the Type α LHI samples as "other *Pocillopora*" (Table 1). The taxonomic status of Type α LHI specimens is uncertain, but some evidence shows that they represent a distinct genetic lineage (Schmidt-Roach et al., 2012a), therefore the assay correctly identified these as "other *Pocillopora*". Since the GBR samples originate from 10 different reefs, spanning more than 12° of latitude, these results provide high confidence of the appropriateness of the assay as an identification tool for the whole of the GBR. Testing the assay on populations outside the East coast of Australia was beyond the scope of this study, therefore we recommend sequencing a representative number of specimens in any non-GBR population to determine whether the type-specific deletions are present, prior to adopting the presented assay as an identification tool.

Table 2.2. Characteristics of the newly developed microsatellite primers.

Locus	F sequence	R sequence	Motif	Amplicon size range (bp)	Tm
Pd4	ACGCACACAAACCAACAAAC	TAATTCCATCAACTCAAAGGGG	(AAAC)5	130-190	60°C
Pd11	TCGTTTGAAGGGAAATGCTC	GGCATGCTATGTATGCGAGA	(CA)7 T (AC)13	120-180	60°C
Pd13	TGTTCCTCTCTTTCTCTCTTCCA	CATTTATGTTCCTTTCACGGC	(TCTT)5	130-194	60°C

Table 2.3. Multiplex groups for microsatellite genotyping. A universal TET-labeled M13-F was added to groups M1 and M2.

Group	Locus	5' MOD	Mean size	running Tm	Source
M1	**Pd3_004**	TET (M13)	180	53°C	Starger et al. 2008
	PV7	HEX	220		Magalon et al. 2004
M2	**Pd4**	TET (M13)	148	60°C	new primer
	Pd11	FAM	157		new primer
	Pd3_002	HEX	200		Starger et al. 2008
M3	**Pd13**	HEX	153	60°C	new primer
	Pd3_008	TET	180		Starger et al. 2008
	Pd2_007	FAM	250		Starger et al. 2008
S	**Pd3_009**	FAM	350	52°C	Starger et al. 2008

The PCR RFLP assay identified 205 Type α, 93 Type β, and 31 "other *Pocillopora*" specimens among the 329 samples from Lizard Island. The Bayesian model clustered specimens according to their mtDNA lineage identity (Figure 3). Similarly, the FCA of the multilocus microsatellite genotypes showed three well distinguished clusters that correspond to the genetic lineage identity, assigned by the assay (Figure 4). The congruence between the nuclear microsatellite and mtDNA data strengthens the hypothesis that these lineages are genetically and hence reproductively isolated.

Fig. 2.3. STRUCTURE plot (K=3) of 329 *Pocillopora damicornis* (sensu Veron and Pichon, 1976) specimens from Lizard Island. Genetic lineage was determined by the PCR RFLP assay.

The genetic assay presented here provides a simple means to ensure that future studies avoid sample misidentification in *P. damicornis*. Furthermore, while the recruits of pocilloporid species are readily distinguished from the recruits of other scleractinian families by their characteristic skeletal structures (Babcock et al., 2003), species identification of recruits merely by morphology is very unreliable. The primers of this assay were designed to only amplify *Pocillopora* spp. DNA, and not the DNA from other pocilloporid genera. The assay is therefore also suitable to reliably identify Type α and Type β *P. damicornis* from among pocilloporid recruits, which will open doors to genetic lineage-specific recruitment studies.

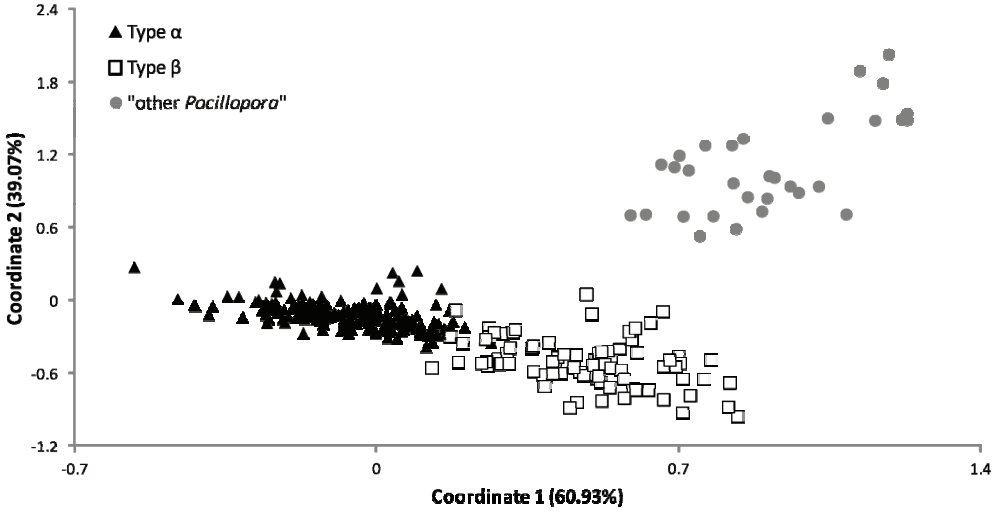


Fig. 2.4. Results of the Factorial Correspondence Analysis on 329 *Pocillopora damicornis* (sensu Veron and Pichon, 1976) microsatellite genotypes. The first two coordinates explain 100% of the variability. Genetic lineage was determined by the PCR RFLP assay.

Chapter 3

Revisiting the connectivity puzzle of the common coral *Pocillopora damicornis*

Abstract

Understanding levels of connectivity among scleractinian coral populations on various temporal and spatial scales is critical not only for the management of coral populations themselves, but also for that of the entire coral reef ecosystem. *Pocillopora damicornis* is one of the most widespread and common hermatypic corals in the Indo-Pacific, but connectivity of its populations is not well understood, mainly because its reproductive biology and taxonomy have been unresolved until recently. In this chapter I use multilocus microsatellite genotypes to assess the spatial genetic structure of two putative cryptic species (Type α and β lineages) of *P. damicornis* that have been described recently from the GBR, and infer rates of connectivity at various spatial scales and among habitat types spanning 3 latitudinal sectors of the GBR. Similar to previous studies, I found both high genetic similarities over large spatial scales and strong genetic differentiation at local scales in both lineages. Intra-population genetic structures and evidence for recent immigration suggest that genetic differentiation over small spatial scales is likely a consequence of stochastic re-seeding of populations from different genetic pools. I explain high genetic similarity among populations over hundreds of kilometres by life history characteristics of these corals, namely long competency periods of brooded planulae and multiple planulation events each year, combined with strong longshore currents that govern larval dispersal along the GBR. High geneflow over the scale of hundreds of km on the GBR suggests that the two lineages of *P. damicornis* are likely to be able to maintain high levels of genetic diversity, especially on outer shelf reefs. Similarly to previous studies on populations of *P. damicornis* on the GBR, I found no evidence in the genetic composition of adult populations for predominantly clonal reproduction, further undermining the paradigm that brooded (asexually-produced) larvae settle close to parent colonies shortly after release.

3.1. Introduction

Tropical coral reefs are inherently patchy ecosystems, thus sessile marine organisms like corals rely on a dispersive larval stage to maintain connectivity among populations. Genetic tools have been widely and successfully applied to infer the extent of connectivity between reefs for many marine species (reviewed in Hedgecock et al., 2007; Weersing and Toonen, 2009), but there have been comparatively few studies of population connectivity based on genetic markers for corals. The physical structure and most of the primary production of coral reef ecosystems are supplied by scleractinian corals (e.g. Hatcher, 1990). Hence, understanding levels of connectivity among coral populations is important, not only for the management of coral populations themselves, but also for that of the myriad of vertebrates and invertebrates that rely on them for shelter, food and habitat.

Pocillopora damicornis (Linnaeus, 1758) is a widespread and common scleractinian coral on the Great Barrier Reef (GBR) (Veron, 2000), but despite it being one of the most extensively studied coral species, its reproductive biology, population genetic structure, and the extent and direction of inter-reef connectivity are not well understood. The species is thought to have a mixed reproductive strategy, involving both the monthly release of brooded larvae (e.g. Harrigan, 1972) that are asexually produced (Stoddart, 1983), as well as the release of gametes for external fertilization at unknown periods of time (Stoddart and Black, 1985; Ward, 1992; Schmidt-Roach et al., 2012b). Release of brooded larvae is typically synchronised to a specific lunar phase, but the lunar phase differs among reef regions (Harrigan, 1972; Muir, 1984; Richmond and Jokiel, 1984; Tanner, 1996; Fan et al., 2002). South-western Australian populations of *P. damicornis* were found to be predominantly clonal (Stoddart, 1984), consistent with a high allocation of energy into brooded clonal larvae, and in line with predictions of the “strawberry-coral model” (Williams, 1975). This model posits that species with both sexual and asexual reproduction typically use sexual events to maintain high levels of genetic diversity and disperse over large spatial scales, and asexual reproduction to colonise and exploit local habitats where the adult genotype has been successful. In contrast to the clonal population structures found on SW Australian reefs, the majority of populations studied on the GBR are of sexual origin, despite larvae being asexually produced (summarized in Ayre and Miller, 2004; Sherman et al., 2005). This raises

questions about why an organism would invest in asexual, brooded larvae, an energetically expensive mode of reproduction, when there is little evidence that such offspring contribute significantly to the next generation (Ayre and Miller, 2004). Due to strong genetic subdivision on small spatial scales in populations of *P. damicornis* on the GBR, distant reefs often show greater genetic similarity than adjacent sites within a reef (Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000, 2004) – a pattern difficult to interpret.

Recent evidence that *P. damicornis* comprises several sympatric, but genetically isolated lineages (Flot et al., 2008a; Souter, 2010; Schmidt-Roach et al., 2012a), raises concerns about whether earlier findings of genetically subdivided reefs (Ayre et al., 1997; Ayre and Hughes, 2000) were misinterpreted because several potential cryptic species were unknowingly included in population samples. Following the description of five lineages of *P. damicornis* on the GBR (Schmidt-Roach et al., 2012a) and the subsequent development of a rapid genetic assay capable of distinguishing the two most common lineages (Chapter 2), it is now possible to focus research efforts on genetically more meaningful units. In combination, contradictory population genetic structures, emerging evidence of cryptic speciation within the current morphospecies *P. damicornis*, and advances in DNA techniques applicable to coral genetic studies make it timely to revisit the connectivity and population genetic puzzle of this model scleractinian coral species.

Less than a decade ago, difficulties in developing highly polymorphic microsatellites markers for corals limited population genetic studies to the use of allozymes (Stoddart, 1984; Ayre and Resing, 1986; Ayre and Willis, 1988; Ayre and Dufty, 1994; Stobart and Benzie, 1994; Hellberg, 1994; Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000, 2004; Ridgway et al., 2001; Carlon and Budd, 2002; Ridgway, 2005; Whitaker, 2006). Because there is doubt about whether all allozymes are truly neutral markers (e.g. summarized in Hey, 1999), the advent of microsatellite development for coral species enables and warrants further exploration of coral population connectivity.

Here I use multilocus microsatellite genotypes to assess the spatial genetic structure of the two most common lineages of *P. damicornis* on the GBR. My nested sampling design and comprehensive analyses enable comparisons of genetic structure for these two lineages across various spatial scales and among habitat types spanning 3 latitudinal sectors of the GBR.

3.2. Materials and Methods

3.2.1. Sample collection

One fragment approximately 2 cm long was collected from each of 1040 colonies of *P. damicornis* (sensu Veron, 2000) at 28 sites in the northern, central and southern GBR, spanning from Lizard Island in the north (-14.6°) to Chinaman Reef (-22.0°) in the south (Fig. 3.1). Each colony was photographed and, based on the digital time stamp for each photograph, the software Robogeo 5.6.11 (Pretek Inc.) was used to extract each colony's coordinates from the diver's track log. The track log was obtained by towing a handheld Garmin Etrex GPS unit in a waterproof container on the surface. Between 6 and 99 individuals were sampled per site (Table 3.1). Collections were made haphazardly on the upper reef slope along a zigzag transect approximately 200 m long, between 2 m and 10 m of depth. Colonies within one m of a colony already sampled were avoided. Coral branches were fixed in absolute ethanol. This sampling design allowed analyses at four spatial scales: within sites (1-100 meters), between adjacent sites (a few km), between distant sites within a sector (10s of km), and between sectors (100s of km). It also enabled comparisons between different habitat and reef types, such as exposed front and sheltered back reefs, and inshore, mid-shelf and outer-shelf reefs (Fig. 3.1).

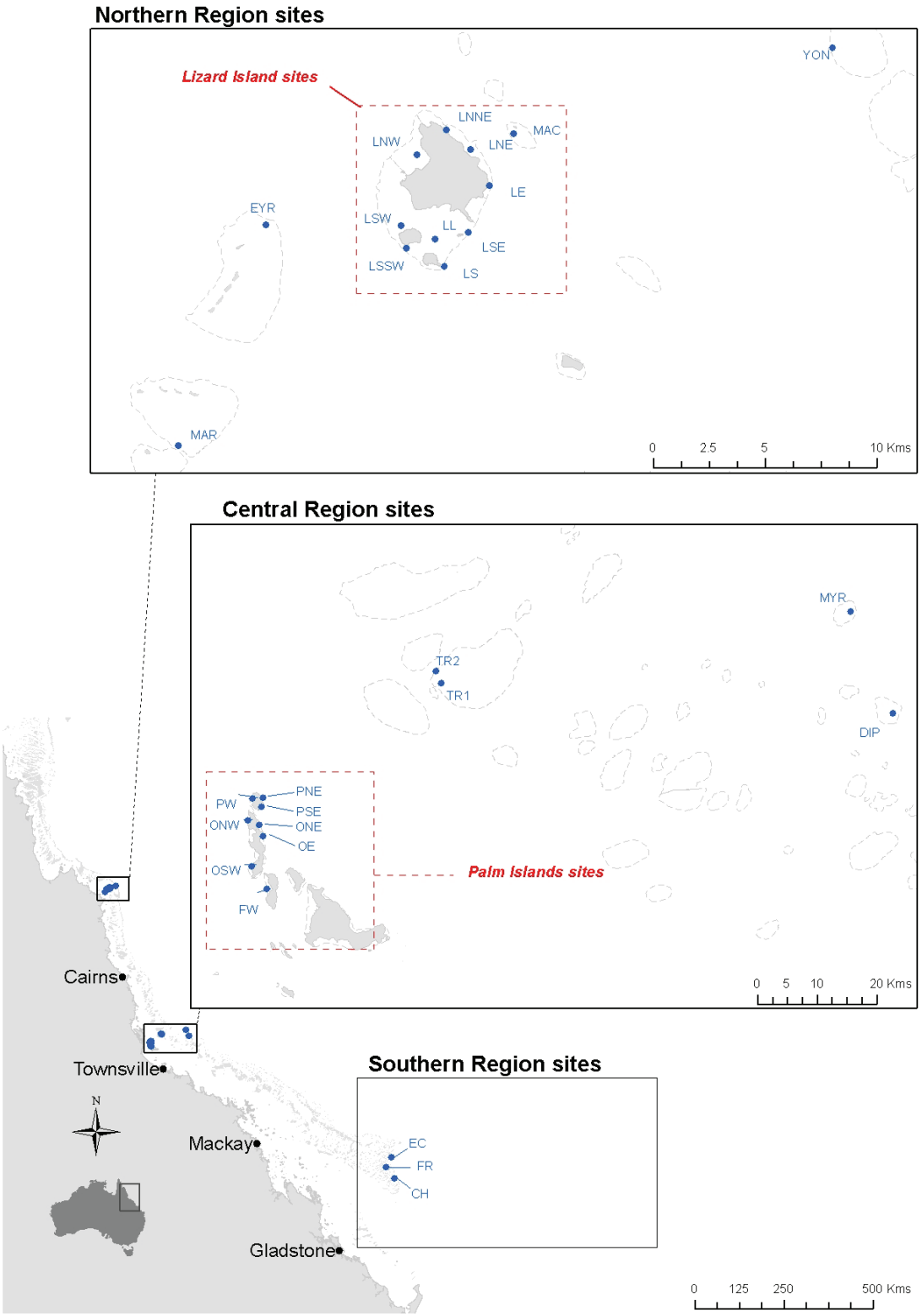


Fig. 3.1. Map of the study area. Site codes are given in Table 3.1.

		Site name	Code	WGS '84 coordinates Longitude	Latitude	Location	Habitat	Sample size Type α	Type β	other *Pocillopora*	unknown
Northern Region		Martin Reef	MAR	145.353	-14.777	I	S	12 (12)	4 (4)*		
		Eyrie Reef	EYR	145.388	-14.688	I	L	26 (26)	4 (4)*		
	Lizard Island sites	South-Southwest Lizard	LSSW	145.445	-14.697	M	S	28 (26)	2 (2)*		1
		Southwest Lizard Island	LSW	145.443	-14.688	M	S	58 (51)	5 (4)*	2	1
		Northwest Lizard Island	LNW	145.449	-14.659	M	S	31 (27)	45 (40)	21	
		North-Northeast Lizard	LNNE	145.461	-14.649	M	E	65 (52)	16 (16)	4	2
		Northeast Lizard Island	LNE	145.471	-14.658	M	E	15 (15)	3 (3)*	2	
		East Lizard Island	LE	145.478	-14.672	M	E	23 (23)	10 (10)		
		Southeast Lizard Island	LSE	145.470	-14.691	M	E	14 (14)	15 (14)	20	
		Lizard Island Lagoon	LL	145.456	-14.694	M	L		8 (7)	2	
		South Lizard Island	LS	145.460	-14.705	M	E	15 (14)	3 (3)*		1
		MacGillivray Reef	MAC	145.488	-14.651	M	S	23 (21)			
		Yonge Reef	YON	145.617	-14.616	O	S	6 (6)	18 (18)		1
Central Region	Palm Islands sites	West Fantome Island	FW	146.510	-18.684	I	S		14 (14)		
		Southwest Orpheus Island	OSW	146.487	-18.649	I	S		13 (13)		
		Northwest Orpheus Island	ONW	146.481	-18.580	I	S	13 (13)	23 (23)	1	2
		West Pelorus Island	PW	146.488	-18.547	I	S	18 (17)	36 (36)	1	3
		Northeast Pelorus Island	PNE	146.504	-18.546	I	E	78 (75)	4 (4)*		
		Southeast Pelorus Island	PSE	146.502	-18.560	I	E	31 (28)			
		Northeast Orpheus Island	ONE	146.498	-18.587	I	E	41 (36)	2 (2)*	2	
		East Orpheus Island	OE	146.504	-18.603	I	E	43 (34)	1 (1)*		
		Trunk Reef 1	TR1	146.765	-18.354	M	S	14 (14)	2 (2)*	1	
		Trunk Reef 2	TR2	146.664	-18.125	M	L	47 (46)			
		Dip Reef	DIP	147.457	-18.418	O	S	35 (35)			
		Myrmidone Reef	MYR	147.392	-18.263	O	S	6 (6)			
Southern Region		Chinaman Reef	CH	152.654	-22.001	O	S	42 (42)			
		East Cay Reef	EC	152.563	-21.471	O	S	30 (30)			
		Frigatte Reef	FR	152.427	-21.732	O	S	31 (30)			
		Total						745 (693)	228 (220)	56	11

Table 3.1. Sampling sites and sample sizes. Final sample sizes, after removing sympatric clone mates are shown in brackets. Type β populations that were omitted from population-based analyses are indicated with *. Location of reefs indicated by I= inshore, M = midshelf, and O = outer reefs. Habitat type indicated by E = exposed windward, S = sheltered leeward, L = lagoon

3.2.2. DNA extraction and microsatellite genotyping

DNA was extracted using a slightly modified protocol of the salt precipitation method described in Wilson et al. (2002). Samples were genotyped using nine microsatellite markers (Table 3.2), following the multiplex groups, primers and protocols described in Chapter 2. Samples from four sites, DIP, CH, EC and FR, were not genotyped for marker Pd4.

To determine the lineage identity of each sample (Supplementary Fig. 3.1), a rapid genetic assay (see Chapter 2) was used. The vast majority of samples (72%; n=745) were identified as Type α, 228 (22%) as Type β (sensu Schmidt-Roach et al., 2012a), 56 (5%) as "other *Pocillopora*", and 11 (1%) did not give reliable results (Table 3.1). All subsequent analyses were carried out on Type α and Type β samples separately, omitting "other *Pocillopora*" and unidentified samples which potentially include the poorly resolved lineage Type γ and *P. verrucosa* (Schmidt-Roach et al., 2012a). For ease of reading, I refer to populations of these lineages by (α) and (β) following the populations' respective codes (e.g. ONE(α) denotes the α lineage at the NE Orpheus site).

Table 3.2. Multiplex groups for microsatellite genotyping. A universal TET-labeled M13-F was added to groups M1 and M2.

Group	Locus	5' MOD	Mean size	running Tm	Source
M1	**Pd3_004**	TET (M13)	180	53°C	Starger et al. 2008
	PV7	HEX	220		Magalon et al. 2004
M2	**Pd4**	TET (M13)	148	60°C	Chapter 2
	Pd11	FAM	157		Chapter 2
	Pd3_002	HEX	200		Starger et al. 2008
M3	**Pd13**	HEX	153	60°C	Chapter 2
	Pd3_008	TET	180		Starger et al. 2008
	Pd2_007	FAM	250		Starger et al. 2008
S	**Pd3_009**	FAM	350	52°C	Starger et al. 2008

The densities of adult coral colonies around Lizard and the Palm Islands were estimated using the software package GPS TrackMaker (Geo Studio Technology Ltd.), by dividing the number of colonies at each sampling location by the length of the divers' corresponding GPS tracklogs (henceforth "tracklog-method"). Since the effective search radius of the divers varied from site to site depending on visibility, I did not attempt to determine the area that the divers covered, hence actual density units (e.g. colonies/m2) are not represented by this metric. I interpolated density values of surveyed sites in ArcGIS 10 (Environmental Systems Resource Institute) by the "Spline with barriers" method. This method used island contours as barriers for the reef area around the Lizard Island group and for three of the Palm Islands: Pelorus, Orpheus and Fantome Islands.

3.2.3. Data analyses

To assess the discriminative power of sets of loci, Genotype Probability (GP) for each sample and each locus was calculated in GENALEX 6.4 (Peakall and Smouse, 2006). Repeated multilocus genotypes (MLG) were identified in the dataset, and were considered to be clone mates if the product of GP for all locus combinations was < 0.001. Allopatric clone mates (for this study defined as originating from different sampling sites) were kept, while all but one copy of sympatric clone mates were removed before subsequent analyses.

For population-based genetic analyses, I considered the sampling sites as putative populations, and I refer to them hereafter as "populations". Ten Type β populations with a sample size lower than six were omitted, leaving ten populations for these analyses (Table 3.1).

Deviations of populations from Hardy–Weinberg Equilibrium (HWE) and genotypic linkage disequilibrium (LD) were tested in Genepop web version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) using the log likelihood ratio statistic (G-test). Descriptive statistics were obtained in GENALEX. The F_{is} analogue G_{is} values were calculated in GenoDive (Meirmans and Van Tienderen, 2004). Allelic richness was calculated in FSTAT v2.9.3.2 (Goudet, 2001) for each locus and population.

Populations that lacked data for a locus (DIP, CH, EC and FR for locus Pd4) were excluded from analyses based on all nine microsatellite loci, and instead analysed separately for the eight loci for which data were available. Allelic richness was compared between populations with Kruskal-Wallis tests in the statistical software R (R Development Core Team 2011). Expected equilibrium gene diversity was computed from the observed number of alleles under the assumption of a constant-size population (Luikart et al., 1998) for each population and for each locus in the software package Bottleneck 1.2.02 (Cornuet and Luikart, 1996; Piry et al., 1999) and tested for relative heterozygosity excess and deficit using the two-phased model of mutation (TPM). Allele frequency distributions were graphed and compared to a normal L-shaped distribution, as expected under mutation-drift equilibrium.

Various methods for estimating genetic differentiation among populations were compared. These included (i) uncorrected pairwise F_{st} values by the “weighted" analysis of variance method (Cockerham, 1973; Weir and Cockerham, 1984), as implemented in Genepop; (ii) the standardised pairwise F'_{st}, estimated using an AMOVA (Meirmans, 2006) in GenoDive; (iii) pairwise F_{st} values corrected for null alleles (ENA correction), computed in FreeNA (Chapuis and Estoup, 2007); (iv) and Jost’s actual measure of genetic differentiation D_{est} (Jost, 2008), calculated in SMOGD 1.2.5 (Crawford, 2010). To account for unbalanced sample sizes, the significance of uncorrected F_{st} values was assessed by a Fisher exact test (Goudet, 1995) in Genepop with the default Markov chain parameters. Significance levels of D_{est} values were determined by a permutation test, randomizing alleles over all compared populations, using R code from Alberto et al. (2011). I have also tested genetic differentiation in the pooled dataset of Type α and β specimens by D_{est} in order to obtain results that are comparable to those of previous studies that ignored the existence of lineages in the *P. damicornis* morphospecies (e.g. Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000).

To test for isolation by distance (IBD), measures of genetic differentiation were correlated against linear and logarithmic geographic distance between sites using Mantel tests with 999 random permutations in GENALEX. Complementary to this conventional population-based method, IBD was also tested using pairwise genetic and

geographic distances between individual samples, in Alleles in Space (Miller, 2005), with 1,000 permutations.

To detect putative first generation migrants, the probability that each individual belongs to each reference population was computed in GeneClass2 (Piry et al., 2004) using the criteria and probability computation algorithm of Rannala and Mountain (1997), with 10,000 simulated genotypes. I followed the two-step analysis described by the authors of the software, excluding an individual from its reference population if the probability of exclusion was greater than 0.99 ($\alpha <= 0.01$), and nominating another reference population as a potential source for the excluded individual where assignment probabilities were greater than 0.1.

Furthermore, to describe the genetic structuring within all samples, multilocus genotypes were clustered by the spatially explicit Bayesian clustering method implemented in Geneland 3.3.0 (Guillot et al., 2005), with 100,000 iterations and a thinning rate of 100. This model with a priori spatial information (Sp) enforces spatial genetic integrity of individuals that are close together in space, and hence yields rather homogeneous cluster memberships for sampling sites, when samples are collected in a highly aggregated, nested fashion, as in this study. Therefore this analysis provides an easy-to-interpret visualisation of the dominant genetic structuring of populations over the study area. When a priori spatial information is not provided to the analysis (NSp), this spatial integrity is not enforced, and sampling sites show complex, heterogeneous composition. These maps are harder to interpret visually, but give more spatial-genetic resolution, and a more realistic cluster-composition of populations. Additionally, when a correlated allele frequencies model (C) is used, subtle local genetic deviations are enhanced, and more clusters are created than the non-correlated allele frequencies model (NC). The use of all four combinations of models (Sp-C, Sp-NC, NSp-NC and NSp-C) to explore the dataset provides increasing complexity of results and facilitates the detection of differing levels of genetic structure, from the spatially and genetically most dominant structure to those that are distinguished by only subtle differences.

For comparison, the widely used fully Bayesian model-based clustering method implemented in STRUCTURE v2.3.3 (Pritchard et al., 2000) was run without

population information, under the admixture model and correlated allele frequencies, with a burn-in of 100,000 and another 100,000 MCMC replications after the burn-in. Five independent chains were run for each K from K = 1 to K = 26. I used STRUCTURE HARVESTER (Earl and vonHoldt, 2012) to find the uppermost level of structure by plotting ΔK calculated as $\Delta K = m|L''(K)|/s[L(K)]$, over each value of K (Evanno et al., 2005).

To assist in the interpretation of the results of the genetic analyses by simulating potential dispersal capacity of brooded larvae, virtual Lagrangian particle transport was modelled in the 0.025° x 0.025°-cell circulation model of the GBR in Connie 2.0 (*CSIRO Connectivity Interface*, http://www.csiro.au/connie2/). Collection sites were selected as both sources and sinks for dispersal of passive particles at a depth of 5 m over a dispersal period of 1, 15, 50 and 100 days. The assumption of absolute passivity of particles and the use of arbitrary release dates make the results indicative of the transport capacity of water currents, and do not reflect actual dispersal of recruits to and from the study populations.

3.3. Results

3.3.1. Density of Type α and β colonies

Adult colonies of *P. damicornis* Type α were an order of magnitude more abundant on the exposed sides of the Palm Islands than on leeward sides (Fig. 3.2). At Lizard Island, there was no evidence of a windward-leeward abundance pattern. Instead, high abundance of Type α adult colonies was found at both the exposed NNE Lizard Island site and the relatively sheltered SW Lizard Island site, while both the exposed SE and sheltered NW sides of the island had comparably low densities (Fig. 3.2).

In contrast to the pattern found for Type α populations in the Palm Islands, densities of Type β colonies were substantially higher on leeward sides than on windward sides of the Palm Islands (Fig. 3.2). As for Type α, abundances of Type β colonies did not follow a windward-leeward pattern around Lizard Island, but were greatest at NW and NNE sites, (Fig. 3.2).

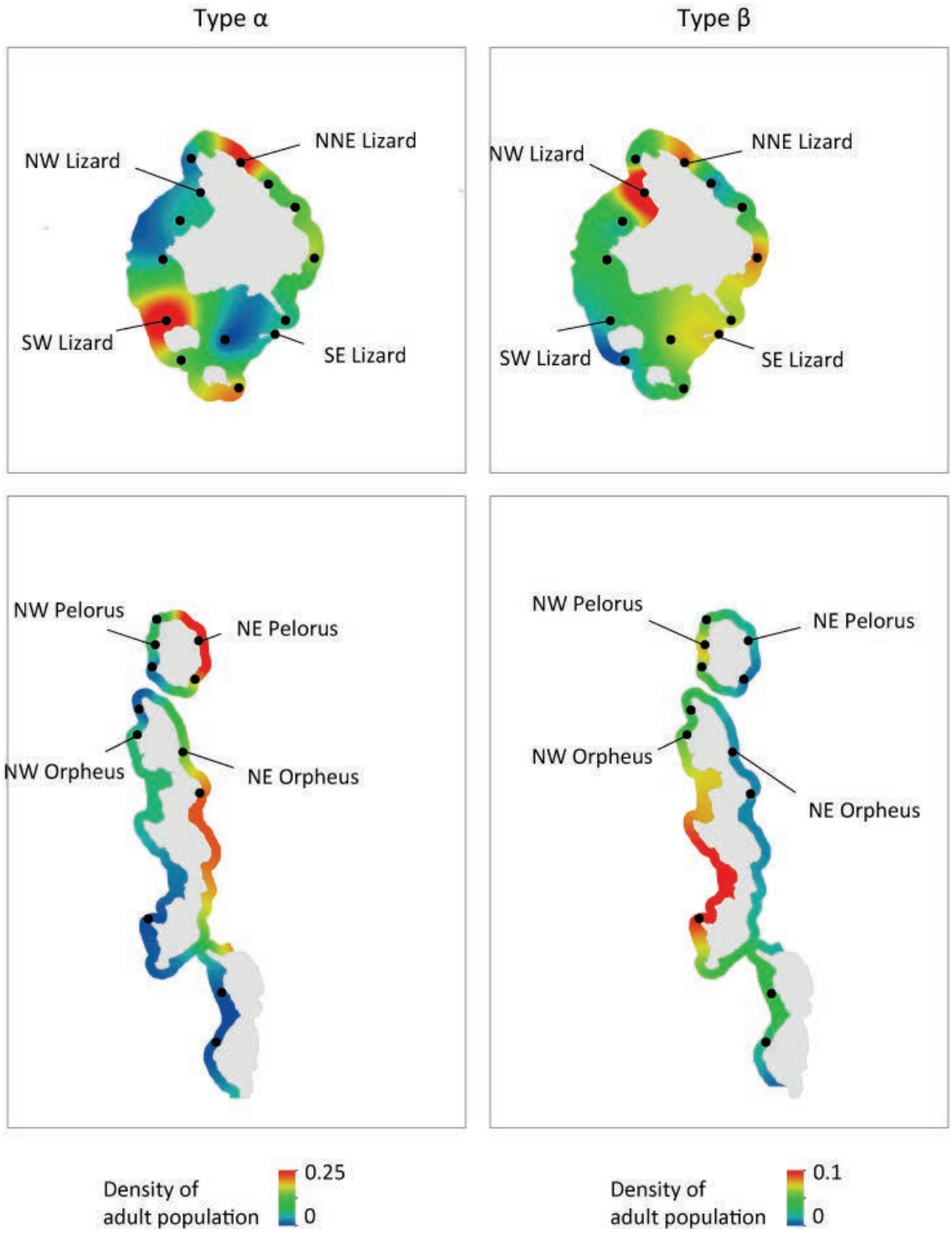


Fig. 3.2. Estimated density of Type α and β *Pocillopora damicornis* colonies around Lizard Island and the Palm Islands. Colony density was estimated by dividing the number of colonies recorded at each site (denoted by black dots) by the length of the collectors' tracklog (see Methods), and interpolated between sampling sites using the "spline with barrier" method in ArcGIS 10 (ESRI).

3.3.2. Genotype probability and clone mates

Type α

In total, 102 repeated MLGs were identified within population samples of the α lineage (13.7 % of all α samples) with genotype probability (GP) less than 0.001, without apparent spatial aggregation within any sampling site or sector. Clone mates were found to be separated by distances that ranged from less than the accuracy of the GPS (~ 5 m) to 444 km (Fig. 3.3). Five pairs of allopatric clone mates (PNE-YON, LSSW-LSW, PNE-PSE, LNE-LSSW, PNE-PSE), and one allopatric clone triplet (OE-PNE-PSE) were detected. Allopatric MLG clones were kept, while all but one copy of sympatric clone mates were removed before subsequent analyses, reducing the sample size to 693 coral fragments (Table 3.1), that belong to 681 unique MLG.

Type β

Thirteen identical MLGs (5.7 %) were detected within population samples of the β lineage, all having GP values less than 0.001 for all 9 microsatellite loci. All but one pair occurred sympatrically in lagoonal or sheltered sites at Lizard Island, within a maximum distance of 230 meters (Fig. 3.3). After removing all but one copy of these sympatric clone mates, 220 samples were left in the dataset. However, due to low sample sizes (< 6 samples) remaining in some cases, 10 sites were excluded from further analyses, leaving a total of 191 samples distributed over 10 sites in the dataset for the β lineage (Table 3.1), that belong to 191 unique MLG.

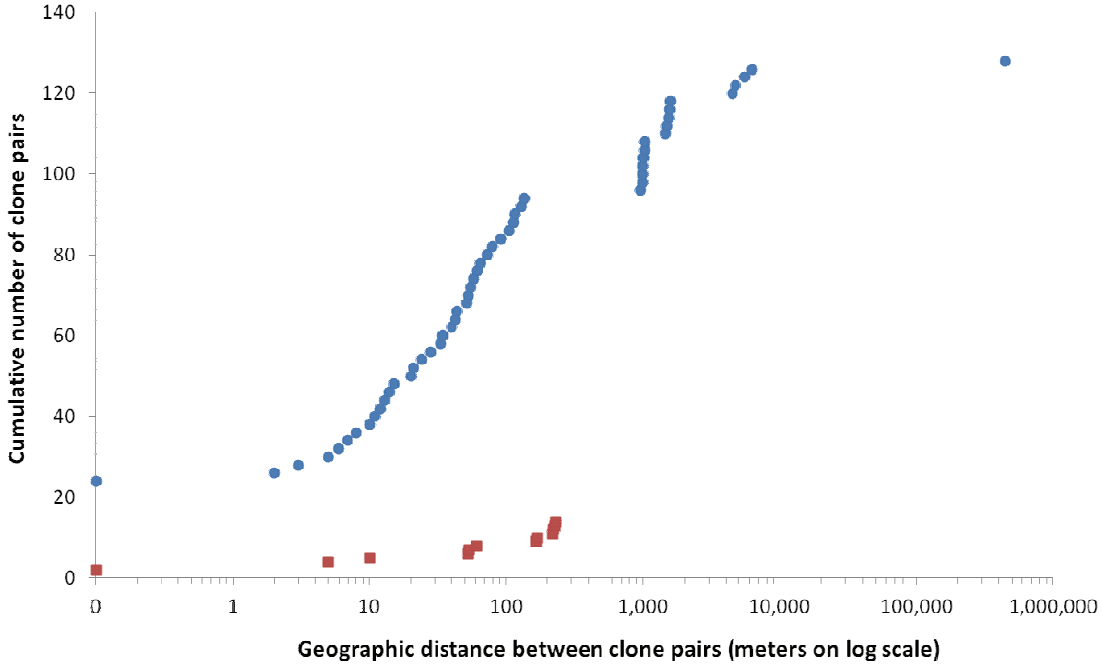


Fig. 3.3. Cumulative number of clone pairs as a function of distance of clones. Type α = blue dots, Type β = red boxes.

3.3.3. Genetic diversity

Type α

All loci were polymorphic in all Type α populations of *P. damicornis* (DIP, CH, EC and FR samples were not genotyped for Pd4). Number of alleles per locus (A) ranged from 2 to 15 and expected heterozygosity (He) ranged from 0.043 to 0.818 (Supplementary Table 3.1a).

Allelic richness (AR) was not significantly different among α populations, when 9 loci were analysed for 21 populations, excluding the DIP, CH, EC and FR populations (Kruskal-Wallis test p=0.8919), or when 8 loci were analysed for all 25 populations (p=0.9824). MAR, LE, LS, YON and PSE populations were in HWE for all 9 loci, while the lowest number of loci in HWE was 5 in the LSE, PW, PNE and DIP populations. Mean G_{is} over all loci was significantly different from zero in 9 out of 25 populations (Table 3.3a). Significant ($p < 0.01$) LD was present in 25 out of 727 valid locus pair comparisons (3.4 %) (Table 3.3a). No locus pair was in significant LD in all populations. Cases where data were missing for a locus or where no contingency table was produced due to low sample numbers were considered non-valid comparisons.

All but one population had a normal L-shaped allele frequency distribution, consistent with mutation–drift equilibrium. The sample size of one population showing deviation from the L-shaped distribution, MYR, was too low (N=6) for this analysis to give a reliable result. At $p < 0.01$, no populations had a significant heterozygote excess, but ONW, PW, PNE, DIP, CH, EC, and FR populations showed significant heterozygote deficiency. Under the assumption of mutation-drift equilibrium, none of the populations showed significant heterozygote excess relative to the respective allele numbers (Wilcoxon sign-rank one-tailed test probability $0.367 < p < 0.999$), indicating the absence of recent population bottleneck events at any site. PNE and ONE populations showed significant relative heterozygote deficiency ($p < 0.01$) (Table 3.3a).

Type β

Similarly, all loci were polymorphic in all 10 Type β populations, with 2 to 11 alleles, giving 0.07 to 0.84 He per locus (Supplementary Table 3.1b). The maximum number of loci in HWE was 7 (LL and FW populations), and the lowest was 4 (LNNE, OSW and PW populations). When estimated for all loci combined, all but two populations departed significantly from HWE (Table 3.3b). Allelic richness was not significantly different between populations ($p=0.4277$).

Significant LD was present in 16 out of 285 (5.6 %) valid comparisons, but no locus pair was in LD in all populations. The sample size of the only population that showed deviation from the normal L-shaped allele frequency distributions, LL, was too low (N=7) for this test to be reliable. Only the FW population showed significant heterozygote excess, and all but the FW and OSW populations showed significant heterozygote deficiency. Under the mutation-drift equilibrium assumption and relative to the allelic richness, none of the populations had significant heterozygote excess ($0.180 < p < 0.990$) nor deficiency ($0.014 < p < 0.850$), suggesting the lack of recent population bottlenecks or admixtures (Table 3.3b).

Table 3.3. Genetic diversity estimates of Type α (a) and Type β (a) populations. AR = allelic richness, LD = number of locus pairs in significant linkage disequilibrium, HWE = number of loci in HWE; G_{is} = inbreeding coefficient, P < 0.01 are in bold; H_e = mean expected heterozygosity over all loci; H_o = mean observed heterozygosity over all loci, HD = exact P-values by the Markov chain method for heterozygote deficiency; HE = exact P-values by the Markov chain method for heterozygote excess; HD_n = Wilcoxon test one-tailed P value for normalized heterozygote deficiency; HE_n = Wilcoxon test one-tailed P value for normalized heterozygote excess, P < 0.01 are in bold.

(a)

	AR	LD	HWE	G_{is}	H_e	H_o	HD	HE	HD_n	HE_n
MAR	2.5	0	9	-0.124	0.435	0.510	0.914	0.086	0.500	0.545
EYR	2.4	0	8	0.028	0.368	0.366	0.206	0.794	0.014	0.990
LSSW	2.5	0	8	-0.076	0.436	0.479	0.250	0.750	0.019	0.986
LSW	2.4	3	8	-0.054	0.427	0.455	0.761	0.239	0.125	0.898
LNW	2.5	2	7	-0.171	0.443	0.527	0.955	0.046	0.500	0.545
LNNE	2.9	3	7	**0.027**	0.495	0.487	0.037	0.963	0.019	0.986
LNE	2.7	1	6	-0.023	0.416	0.441	0.034	0.966	0.014	0.990
LE	2.5	0	9	-0.114	0.418	0.475	0.311	0.689	0.180	0.850
LSE	2.6	0	5	-0.071	0.451	0.501	0.450	0.550	0.213	0.820
LS	2.6	1	9	-0.128	0.445	0.519	0.537	0.463	0.285	0.752
MAC	2.4	0	8	-0.055	0.380	0.411	0.411	0.589	0.102	0.918
YON	2.3	0	9	-0.168	0.403	0.509	0.804	0.201	0.545	0.500
ONW	3.1	0	8	**0.242**	0.531	0.430	**0.001**	1.000	0.545	0.500
PW	3.2	1	5	**0.237**	0.551	0.441	**0.000**	1.000	0.410	0.633
PNE	2.6	4	5	**0.056**	0.472	0.449	**0.000**	1.000	**0.003**	0.998
PSE	2.2	1	9	-0.026	0.384	0.401	0.534	0.466	0.180	0.850
ONE	2.5	3	7	**0.003**	0.426	0.431	0.232	0.768	**0.002**	0.999
OE	2.3	0	8	-0.165	0.387	0.457	0.984	0.016	0.019	0.986
TR1	2.5	1	7	0.019	0.458	0.467	0.097	0.903	0.150	0.875
TR2	2.5	2	6	-0.182	0.475	0.567	0.638	0.362	0.367	0.674
DIP	2.7	0	5	**0.019**	0.462	0.461	**0.000**	1.000	0.037	0.973
MYR	2.7	0	8	-0.111	0.465	0.567	0.759	0.241	0.213	0.820
CH	2.7	2	6	**0.157**	0.424	0.363	**0.001**	1.000	0.037	0.973
EC	2.7	0	6	**0.144**	0.431	0.377	**0.002**	0.999	0.191	0.844
FR	2.5	1	6	**0.226**	0.421	0.333	**0.000**	1.000	0.191	0.844

(b)

	AR	LD	HWE	G_{is}	H_e	H_o	HD	HE	HD_n	HE_n
LNW	4	4	5	**0.157**	0.590	0.504	**0.000**	1.000	0.213	0.820
LNNE	3.9	1	4	**0.37**	0.584	0.386	**0.000**	1.000	0.082	0.936
LE	3.8	0	5	**0.216**	0.535	0.449	**0.001**	0.999	0.014	0.990
LSE	3.7	1	6	**0.214**	0.530	0.437	**0.000**	1.000	0.410	0.633
LL	3.5	0	7	**0.252**	0.532	0.439	**0.001**	0.999	0.410	0.633
YON	4.1	0	6	**0.136**	0.567	0.507	**0.002**	0.999	0.014	0.990
FW	2.8	0	7	-0.315	0.508	0.690	0.995	**0.005**	0.850	0.180
OSW	3.1	1	4	-0.048	0.527	0.577	0.023	0.977	0.752	0.285
ONW	3.6	7	5	**0.225**	0.562	0.451	**0.000**	1.000	0.545	0.500
PW	3.8	2	4	**0.192**	0.596	0.492	**0.000**	1.000	0.213	0.820

3.3.4. Genetic structure

The four measures of genetic differentiation (F_{st}, ENA corrected F_{st}, Meirmans' F'_{st} and Jost's D_{est}) showed good correlation (Type α: $0.90 < R < 0.99$ and Type β: $0.83 < R < 0.99$ at $p < 0.001$) and gave very similar overall patterns of genetic structuring (Supplementary Figs. 3.2 and 3.3, Supplementary Tables 3.2 and 3.3). Because D_{est} is not sensitive to genetic diversity, and is based on the finite island model (Jost, 2008), and hence accounts for both migration and mutation rates, I describe the magnitude of population differentiation by this parameter (Figs. 3.4 and 3.6, Supplementary Tables 3.2a and 3.3a). Its interpretation is also more straightforward than that of other measures of genetic differentiation because it gives the mean proportion of pairwise-private alleles in each population.

Type α

In general, Type α populations in the Northern GBR were genetically less divergent from each other than populations in the Central GBR. Thirty-eight out of 66 pairwise comparisons (58%) among populations of the Northern GBR were non-significant (Fig. 3.4, Supplementary Table 3.2). The majority of the significant differences were attributable to four populations (LNE, LE, LS and YON), three of which were exposed habitats. All significant values were below 0.05 (Fig. 3.4, Supplementary Table 3.2), showing weak to moderate genetic differentiation.

Greater genetic structuring was detected within the Central GBR, where only eight out of 45 (18%) pairwise comparisons were non-significant (Fig. 3.4, Supplementary Table 3.2). Most notably, gene flow seemed to be highly restricted between leeward and windward sites (Fig. 3.3, Supplementary Table 3.2). In fact, the leeward site PW was strongly divergent from all other sites except ONW, including northern and southern GBR sites as well. Genetic differentiation among the windward sites (PNE, PSE, ONE and OE) was either non-significant or significant but low. The midshelf reef populations TR1 and TR2 were weakly divergent from windward inshore reef populations, highly different from leeward inshore reef populations, and not significantly different from each other. There was no significant genetic differentiation between the outer reef populations DIP and MYR, and the latter also showed no differentiation from the exposed inshore OE and midshelf TR1 populations. Other than

the substantial genetic differentiation of the leeward sites, all pairwise D_{est} values in the central GBR were below 0.05 (Fig. 3.4, Supplementary Table 3.2).

The three southern GBR populations ranged from being similar to moderately divergent (Fig. 3.4, Supplementary Table 3.2).

Genetic differentiation was often less across sectors, i.e. between some Northern, Central and Southern GBR populations, than among adjacent populations within a sector. The populations that showed high genetic similarity within their respective sectors were usually also similar among sectors, e.g. windward Palm Island sites to the least divergent Lizard Island sites (Fig. 3.4, Supplementary Table 3.2). Non-significant pairwise values were found between sites separated by > 1100 km (Fig. 3.4, Supplementary Table 3.2).

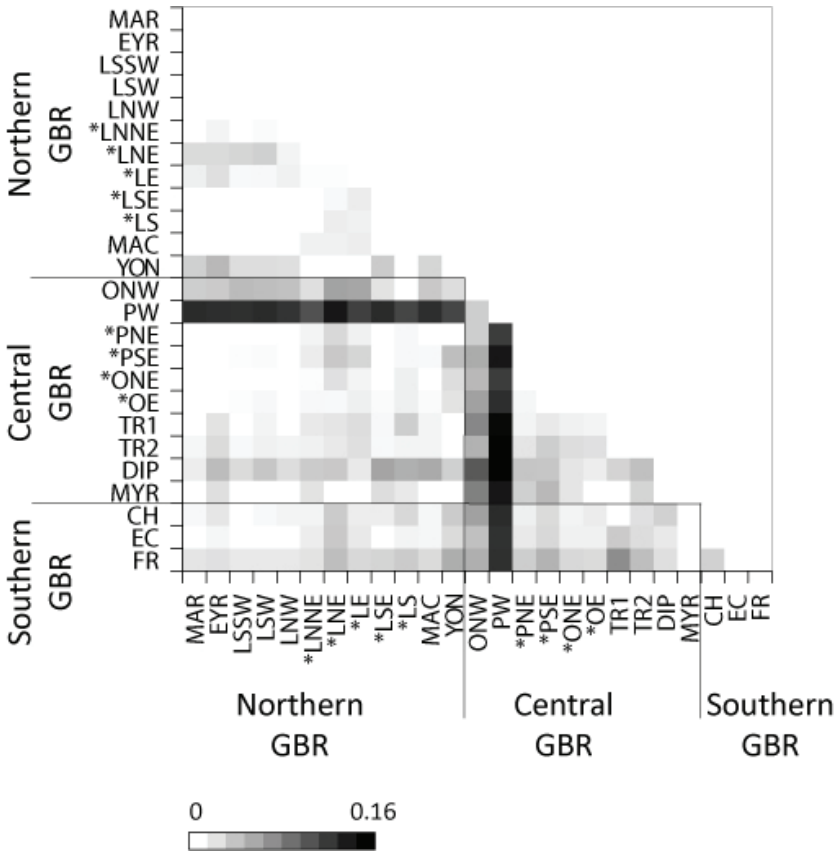


Fig. 3.4. Greyscale matrix of D_{est} values of Type α populations. Exposed populations are indicated with an asterisk (*). Actual values are shown in Supplementary Table 3.2a.

Results of the spatially explicit Bayesian clustering confirmed the above patterns, and revealed further layers of genetic structure. The spatial, non-correlated allele frequency model (Sp-NC) gave 3 clusters that demonstrated a fundamental divide between the southern GBR (plus the outer reefs of the central GBR) and the northern and central GBR (Fig. 3.5). It also highlighted the strong deviation of ONW and PW from all other populations. The spatial, correlated frequency model revealed the divergence of the most deviant sites, such as LNNE, LNE and LE in the northern GBR, or CH in the southern GBR, and clearly demonstrated a restricted cross-shelf geneflow in the central GBR, as well as between exposed and sheltered sites of Orpheus and Pelorus Islands. According to the non-spatial, non-correlated allele frequency model (NSp-NC), there are signs of genetic similarities between sites of the Northern and Central GBR (Fig. 3.5). The most complex pattern was detected using the non-spatial, uncorrelated allele frequencies model (NSp-C) (Fig. 3.4). Similar to patterns found for D_{est} values, genetic deviation was detected between some populations from adjacent sites, while substantial similarities were detected between other populations at distant sites. The majority of the 20 genetic clusters had members from different geographic zones, and reef types (Fig. 3.5).

The Evanno method (Evanno et al., 2005) on the fully Bayesian cluster analysis in STRUCTURE gave three clusters as the most likely level of K, and showed similar patterns of genetic structure as the NSp-C model (Supplementary fig. 3.4a). Furthermore, it demonstrated that most individuals had highly mixed origins, and that the most divergent sites hosted individuals that belonged to genetic clusters unique to that site, or very rare at other sites (Supplementary fig. 3.4b).

A Mantel test of pairwise D_{est} values and linear and logarithmic geographic distances among *populations* revealed significant Isolation by Distance (IBD) only among outer reef sites (Table 3.4). Correlation of the pairwise genetic and geographic distances between *individuals* showed significant, but weak IBD over the whole study area, as well as in the Central GBR, and among outer reef samples. There was also weak, but significant IBD in the total dataset and all regional subsets when geographic distance was log-transformed, except the Northern GBR, Lizard Island and inshore reef subsets (Table 3.4, Supplementary fig. 3.5).

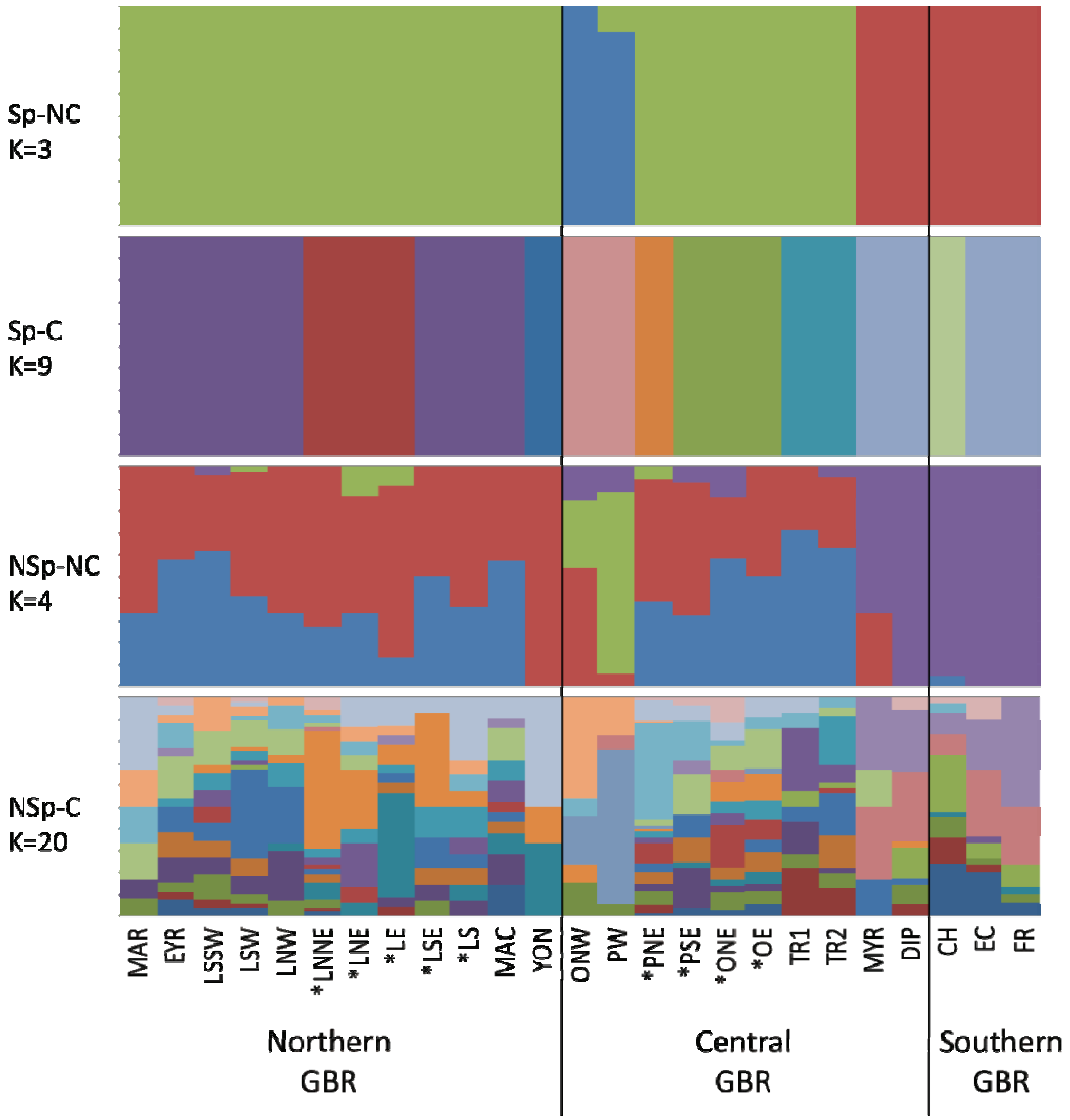


Fig. 3.5. Results of the spatially explicit Bayesian model-based cluster analysis, as implemented in Geneland, on the Type α dataset – relative membership proportion of populations to each genetic cluster. Model types and number of clusters are indicated on the right hand side of the graphs (Sp=a priori spatial information, NSp= no a priori spatial information, C= correlated allele frequencies, NC= non-correlated allele frequencies). The corresponding number of clusters is indicated with K. Exposed populations are indicated with an asterisk (*).

Table 3.4. Estimation of Isolation by Distance by Mantel tests. R_{xy} values indicate the correlation coefficient between genetic difference and linear, as well as log transformed geographical distances between populations/samples of the total dataset and various subsets. Minimum and maximum linear geographic distances between compared sites/samples are shown in kilometres. Significant R values (p<=0.01) are shown in bold, and p values in italics.

Between populations

Type α	linear distance		log distance		Minimum	Maximum
	R_{xy}	P	R_{xy}	P	linear distance (km)	
Total dataset	0.016	0.235	0.105	0.138	1.0	1116.1
Northern populations	0.433	0.030	0.351	0.052	1.0	33.5
Central populations	0.082	0.246	0.047	0.319	1.5	104.6
Lizard Island populations	0.023	0.435	0.071	0.255	1.0	6.9
Palms Island populations	0.016	0.415	-0.015	0.540	1.5	6.6
Inshore reef populations	-0.195	0.064	-0.206	0.117	1.5	449.2
Midshelf reef populations	0.418	0.060	0.422	0.031	1.0	435.0
Outer reef populations	**0.562**	*0.009*	0.494	0.015	18.4	1105.6

Type β	linear distance		log distance		Minimum	Maximum
	R_{xy}	P	R_{xy}	P	linear distance (km)	
Total dataset	**0.478**	*0.001*	**0.493**	*0.001*	1.5	460.2
Northern populations	0.076	0.311	0.042	0.348	1.5	19.3
Lizard Island populations	-0.296	0.140	-0.241	0.253	1.5	4.9
Palms Island populations	0.852	0.072	0.850	0.086	3.5	15.2

Between individuals

Type α	linear distance		log distance		Minimum	Maximum
	R_{xy}	P	R_{xy}	P	linear distance (km)	
Total dataset	**0.262**	*0.001*	**0.222**	*0.001*	0	1116.4
Northern region individuals	-0.004	0.640	0.002	0.467	0	33.6
Central region individuals	**0.279**	0.001	**0.215**	*0.001*	0	104.8
Lizard Island individuals	-0.004	0.621	0.002	0.465	0	7.0
Palms Island individuals	0.034	0.089	**0.080**	*0.004*	0	7.4
Inshore reef individuals	-0.046	0.847	0.002	0.448	0	449.6
Midshelf reef individuals	0.078	0.011	**0.073**	*0.001*	0	435.5
Outer reef individuals	**0.103**	*0.003*	**0.131**	*0.001*	0	1105.7

Type β	linear distance		log distance		Minimum	Maximum
	R_{xy}	P	R_{xy}	P	linear distance (km)	
Total dataset	**0.206**	*0.001*	**0.217**	*0.001*	0	461.1
Northern region individuals	0.022	0.313	0.046	0.071	0	33.6
Lizard Island individuals	0.010	0.386	0.036	0.130	0	6.3
Palms Island individuals	**0.164**	*0.001*	**0.201**	*0.001*	0	17.1

Type β

The overall pattern of genetic structure in Type β populations was very similar to that of Type α populations. Both high genetic similarity and strong divergence could be observed on scales of a few km to > 400 km. Among populations of the Northern GBR, nine out of 15 pairwise comparisons (60%) were non-significant and all other values were below 0.05 (Fig. 3.6, Supplementary Table 3.3). In this sector, the most divergent sites were LE and LNNE, accounting for five out of six significant comparisons in this sector. In the Central GBR, only one out of six pairwise comparisons (17%) was non-significant (PW-ONW). FW and OSW were genetically highly divergent from all populations, including each other, as well as populations of the Northern GBR. Both ONW and PW showed less differentiation from populations of the Northern GBR and from the adjacent FW and OSW populations (Fig. 3.6, Supplementary Table 3.3).

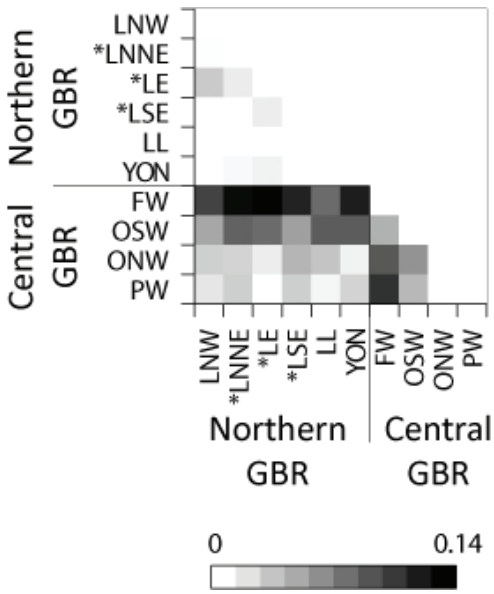


Fig. 3.6. Greyscale matrix of D_{est} values of Type β populations. Exposed populations are indicated with an asterisk (*). Actual values are shown in Supplementary Table 3.

The Sp-NC model cluster analysis showed a divide among the Northern and Central GBR, however the NSp-NC model showed that the differentiation is not absolute, with some degree of admixture (Fig. 3.7). Clustering with the SP-C model identified the most divergent populations, FW and OSW. Results of the NSp-C model clustering showed a very complex picture, with the same differentiation pattern as observed by pairwise D_{est}. However, the high similarity that was detected between LE and PW populations from pairwise D_{est} values was not obvious from the cluster memberships (Fig. 3.7).

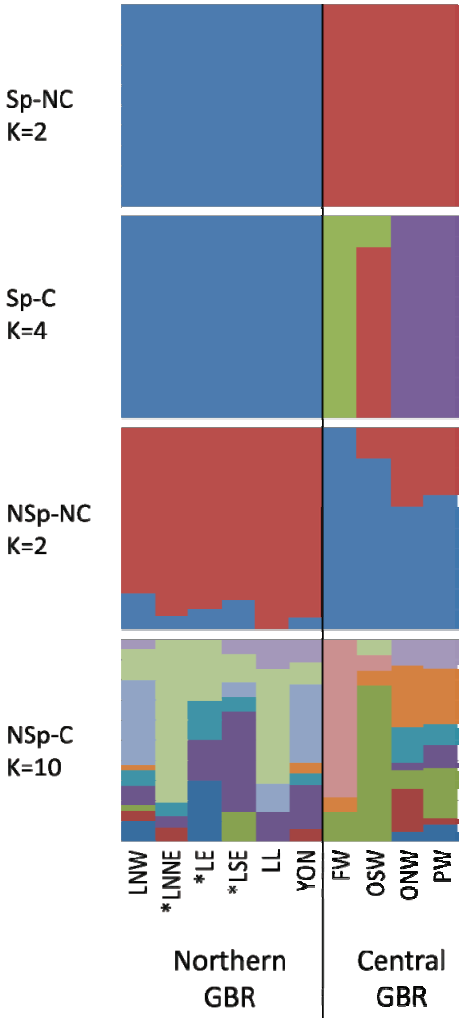


Fig. 3.7. Results of the spatially explicit Bayesian model-based cluster analysis, as implemented in Geneland, on the Type α (A) and Type β (B) dataset – relative membership proportion of populations to each genetic cluster. Model types and number of clusters are indicated on the right hand side of the graphs (Sp=a priori spatial information, NSp= no a priori spatial information, C= correlated allele frequencies, NC= non-correlated allele frequencies). The corresponding number of clusters is indicated with K. Exposed populations are indicated with an asterisk (*).

The Evanno-method identified three genetic clusters as the most likely value of K, with clusters membership shared between and within sectors, except for the marked divergence of FW and OSW populations from other populations (Supplementary Fig. 3.6). The STRUCTURE plot also shows that individuals had admixed genotypes at K=3, and that populations within a sector had more similar compositions than populations between sectors (Supplementary Fig. 3.6).

Significant D_{est}-based IBD was found in the total Type β dataset, but not in any subset of the data (Table 3.5). Individual-based Mantel tests gave weaker correlation of pairwise genetic and geographic distances (linear and log-transformed) over the whole dataset. Individuals from the Palm Islands also showed significant, but weak IBD over both linear and log transformed geographical distances (Table 3.5).

Pooled dataset of Type α and β

Generic patterns of genetic differentiation in the pooled dataset of Type α and β samples were very similar to those of Type α and Type β specimens separately, in the sense that high genetic similarity and strong divergence could be observed on all spatial scales (Fig 3.7). However, while the overall pattern is similar, the actual pairwise values of genetic differentiation of populations are often substantially different than values calculated in analyses of the separated genetic lineages. For example, when pooled, the highest genetic differentiation is detected between FR and FW, as well as FR and OSW. This is because the FR population is made up of only Type α specimens, while the FW and OSW populations are Type β (Table 3.1).

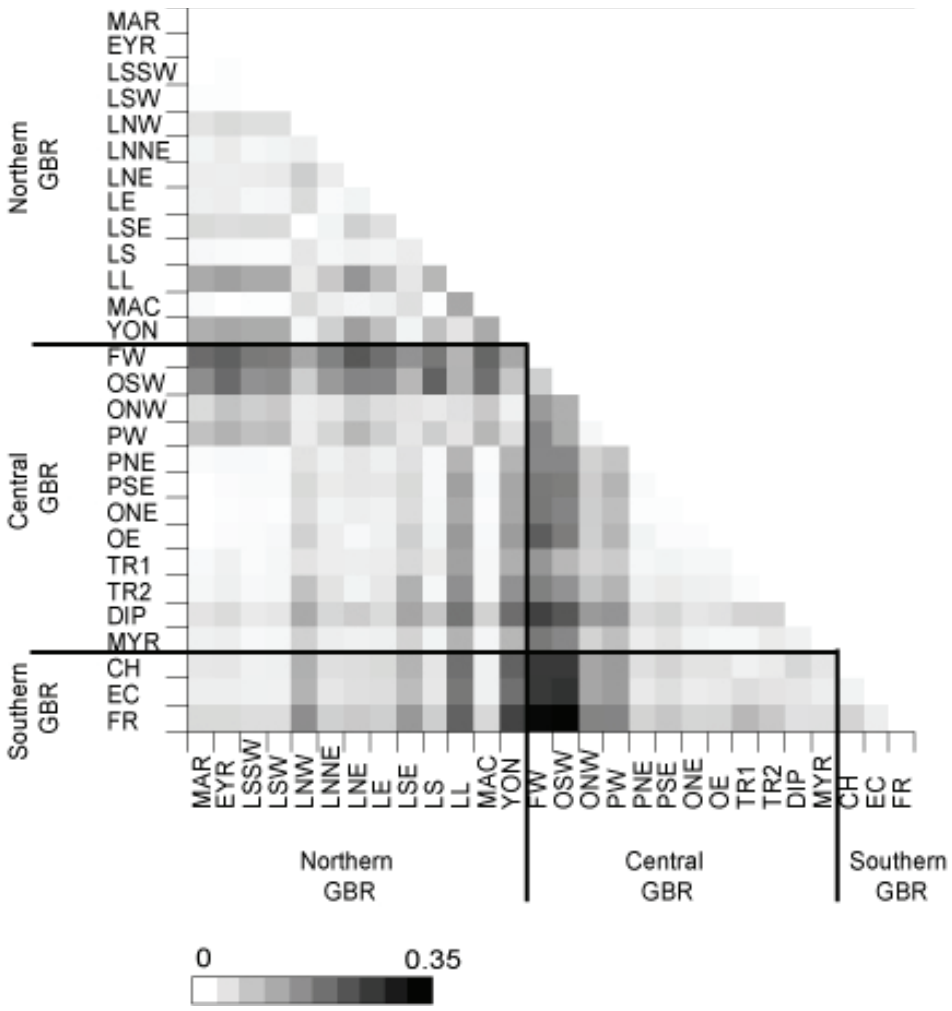


Fig. 3.8. Greyscale matrix of D_{est} values of the pooled database of Type α and β populations.

3.3.5. First generation migrants

Exclusion tests identified 84 putative first-generation migrants in populations of *P. damicornis* Type α, and 52 in Type β populations, corresponding to ~12 % and 27 % of corals sampled, respectively (Fig. 3.9). Out of the 84 Type α putative F1 migrants, 58 could not be assigned to any reference population with the threshold probability for assignment set at 0.1. The vast majority of these non-assigned migrants were collected at PW, PNE and ONE sites. The individuals that exceeded the 0.1 probability threshold were usually simultaneously assigned to several potential source populations with high probabilities (Fig. 3.9a), concordant with expectations of low genetic differentiation among the related reference populations.

Among the 52 Type β putative F1 migrants, 17 were not assigned to potential source populations (Fig. 3.9b). Almost all collection sites had one or more such non-assigned individuals. Peak assignment probabilities were found for Lizard Island populations in all but three cases (Fig. 3.9b).

(a)

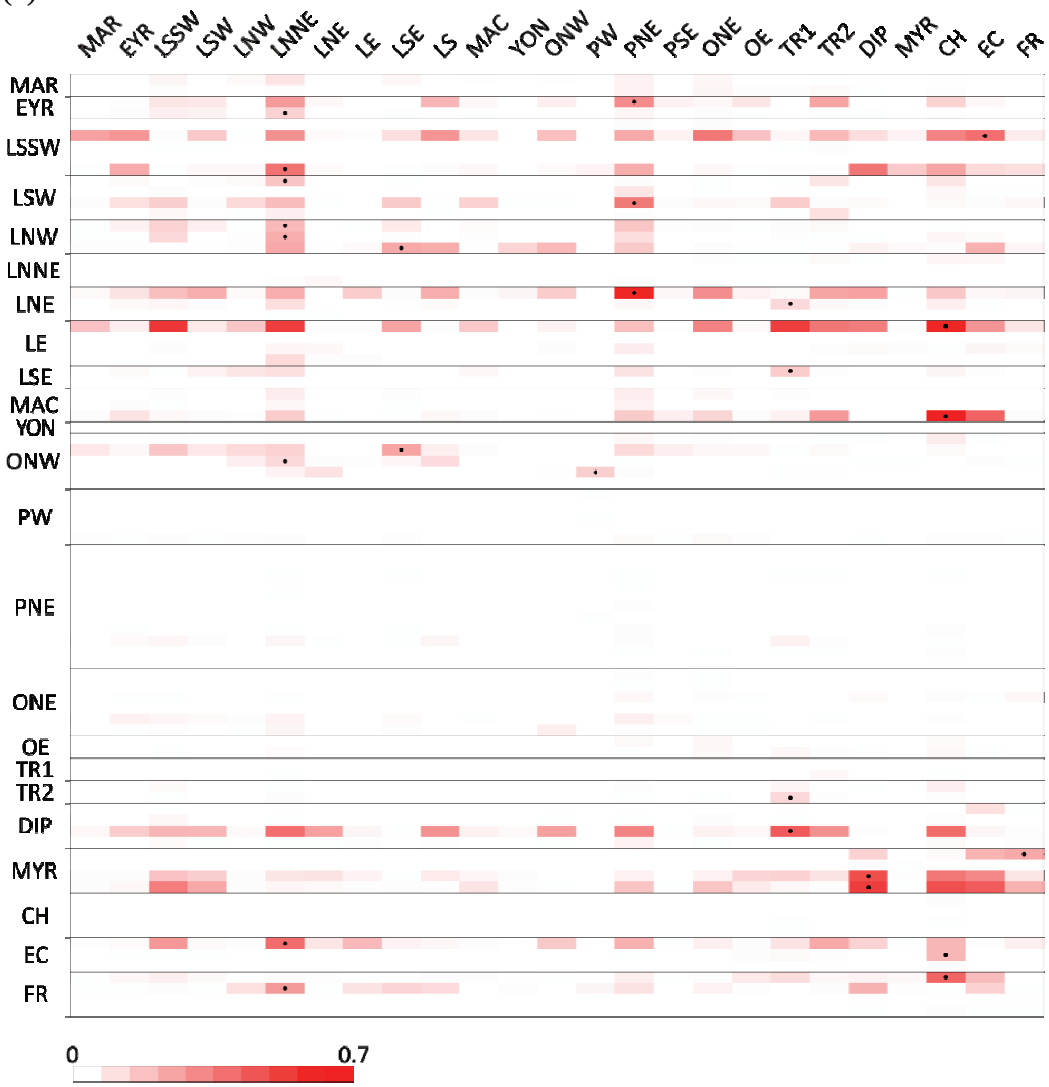


Fig. 3.9. Assignment probabilities of 84 Type α (a) and 52 Type β (b) putative first generation migrants (in rows, indicated by the collection sites' code, see Table 3.1) to potential source populations (in columns). The red scale indicates assignment probabilities, and the highest assignment probability for each individual is indicated by a black dot.

(b)

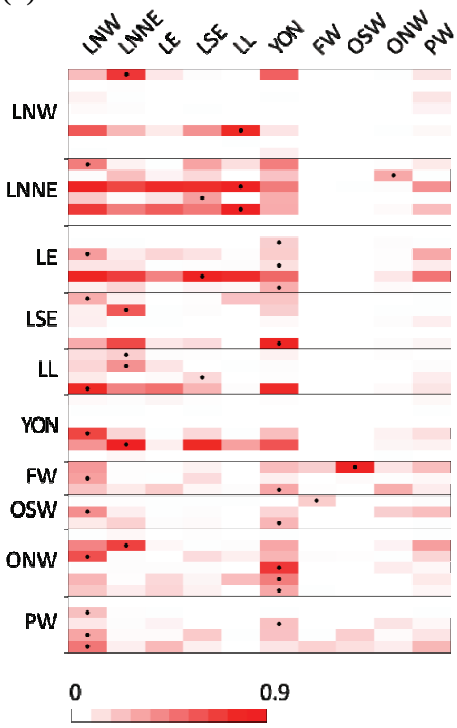


Figure 3.9. continued

3.3.6. Passive particle dispersal modelling

Model runs in Connie 2.0 showed that, on the arbitrary release dates and with passive larval behaviour, 50 days was long enough for particles from the Lizard Island group to drift as far south as the Swains group of the Southern GBR (Fig. 3.10). Reverse dispersal modelling showed that reefs in the Northern Sector of the GBR could potentially receive particles from SE Papua New Guinea within 50 days. Dispersal from the Palm Islands showed substantial temporal variability. Currents retained particles around the Palm Island group, or transported them a few tens of km north or south from the release site, but kept them inshore, at most release dates. However, occasionally particles were conveyed to the stronger south-easterly currents flowing along the outer shelf reefs of the GBR and transported for hundreds of km (Fig. 3.10).

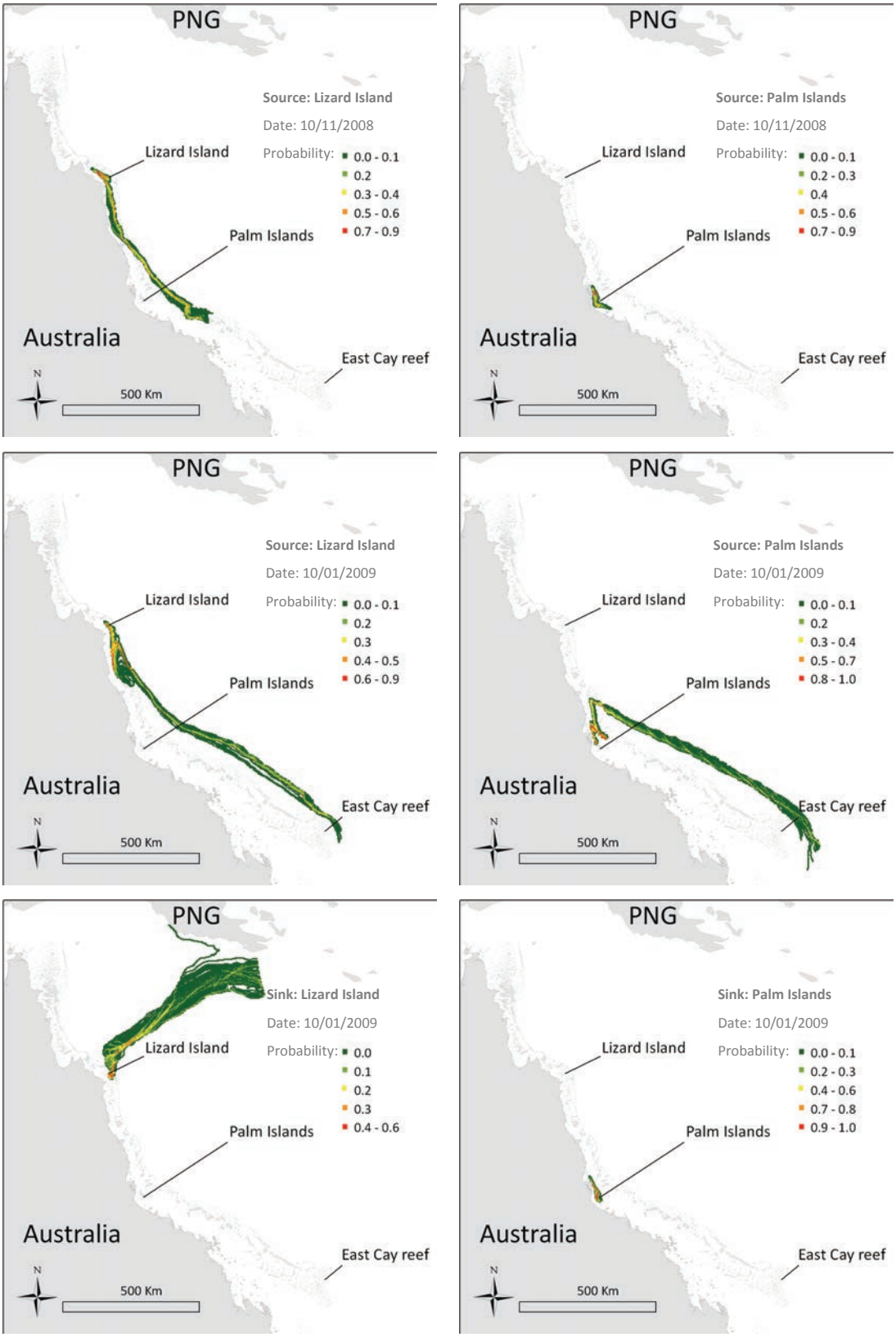


Fig. 3.10. Examples of simulations of passive particle dispersal with Connie2. Dispersal time 50 days, depth 5 m.

3.4. Discussion

This study shows that the population genetic structures of two lineages of *P. damicornis* on the GBR are spatially uncorrelated and patterns of connectivity are scale-independent across distances ranging from metres up to ~1100 km. Estimates of genetic differentiation revealed that some adjacent populations (<10 km apart) are moderately to highly distinct, while other adjacent populations show low genetic differentiation or are genetically indistinct. Stronger genetic structures over small spatial scales occurred most frequently among inshore reefs of the Central GBR. Similarly, at large spatial scales (from hundreds of km up to ~ 1100 km), cases of both high genetic similarity and strong genetic divergence were observed between pairs of populations. Such inconsistency in patterns of connectivity among populations is not unprecedented for species of pocilloporid corals. Ayre et al. (1997) and Adjeroud and Tsuchiya (1999) both reported less genetic differentiation between distant reefs than between collection sites within reef complexes for populations of *P. damicornis* on the GBR and Japan, respectively. For the pocilloporid coral *Stylophora pistillata*, Takabayashi et al. (2003) reported panmixia among populations separated by thousands of kilometres in the Western Pacific, whereas Zvuloni et al. (2008) found significant genetic differences among populations of this species over ~10 km in the Red Sea. The recent discovery that *P. damicornis* comprises several lineages (Flot et al., 2008a; Souter, 2010; Schmidt-Roach et al., 2012a) has raised concerns about whether results of earlier genetic studies on both this species and other pocilloporid species, were confounded by the inclusion of potential cryptic species in the analyses. I focused on molecular operational taxonomic units (Blaxter et al., 2005) in this study, but nevertheless, I also found that population genetic structures encompass patterns of genetic similarity and genetic divergence at multiple spatial scales in two lineages of *P. damicornis,* confirming the variable patterns of population connectivity reported in other studies (Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000, 2004). When the lineage identity of the specimens was ignored, genetic differentiation of the pooled "populations" showed the same overall patterns as when Type α and β lineages were analysed separately. This is possible because the lineages are represented in the pooled "populations" with variable proportions, therefore "populations" with predominantly Type α samples show more genetic similarity to each other than to populations where Type β samples dominated, regardless of their geographic position. The fact that

previous studies on genetic differentiation and connectivity of *P. damicornis* on the GBR (Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000, 2004) obtained similar results to the ones of this study might be due to the above described bias. Alternatively, it is possible that these authors sampled specimens predominantly from one single genetic lineage if study sites were similar in habitat characteristics, because the genetic lineages seem to have differing habitat preferences (see abundance maps, Fig 3.2).

Weak values for Isolation by Distance (IBD) metrics for populations and individuals of both lineages of *P. damicornis* further support my conclusion that distant populations can be closely connected, and spatially close populations can be genetically differentiated. While an increasing number of studies point to the inappropriateness of Euclidean distances to explain population connectivity in marine organisms (e.g. White et al., 2010; Alberto et al., 2011; Andrew et al., 2012), and promote the replacement of IBD analyses with Isolation by Resistance (IBR), when carefully interpreted, IBD is still a valid analysis. For sessile organisms with a pelagic larval phase, such as corals, dispersal is mainly driven by ocean currents. IBD is therefore expected to occur among populations where dominant, year-round currents form genetic barriers between populations, or where the geographic distances between sites are so vast that they cannot be sufficiently bridged even by strong currents. The veracity of this metric for corals is supported by the significant IBD values that I detected in the central GBR among inner- and mid-shelf populations, which is consistent with limited cross-shelf geneflow as a consequence of predominantly longshore current patterns in the sector, as demonstrated by particle dispersal simulations in this study (Fig. 3.8) and by Dight et al. (1990). The lack of significant IBD among populations in the northern GBR corresponds to differences in current regimes between the two sectors. Here the longshore currents during the Trade wind season (March – September) are interrupted by frequent current reversals and cross-shelf water motion during the October – February non-Trade wind season (Frith et al., 1986). Overall, IBD in the total dataset, particularly among outer reef sites, albeit significant, is very weak, especially considering the vast distances that separate some of these sites.

Correlation plots clearly demonstrate that pairwise genetic distances have similar ranges on all spatial scales, indicating that complex mechanisms underlie gene flow in this species. The apparent paradox of genetic similarity over large geographic scales but substantial differentiation over a few km is also supported by results of the Bayesian cluster analyses. Highly mixed and largely consistent proportional membership of genetic clusters for most individuals in both lineages suggests that relatively high gene flow on the scale of the GBR is the norm. Populations that showed moderate or high differentiation from most other populations are stark deviations from the norm, and some or most of their individuals comprise unique or otherwise less frequently occurring genetic clusters. These patterns raise important questions about how strong genetic breaks form and persist on such small spatial scales, why they do not form at all locales, and how gene flow concurrently occurs over scales greater than 1000 km in this morphospecies.

3.4.1. Larval characteristics and water circulation patterns explain high connectivity over large spatial scales

Pocillopora damicornis is one of the few coral species known to have mixed modes of reproduction (i.e. broadcast spawning and brooding), which has important implications for its patterns of dispersal and population connectivity. This species releases vast numbers of brooded larvae every lunar cycle (e.g. Harrigan, 1972; Muir, 1984; Richmond and Jokiel, 1984; Tanner, 1996), and has been suggested to spawn gametes for external fertilization at unknown frequencies in Western Australia (Stoddart and Black, 1985; Ward, 1992). However, in Taiwan it has been found to release asexual and sexual larvae within single broods (Yeoh and Dai, 2010). Schmidt-Roach et al. (2012a) showed that both lineages studied here are brooders of asexual larvae. Such brooded coral larvae are competent and prone to settle immediately or shortly after release (Atoda, 1947; Harrigan, 1972; Harii et al., 2002), and concordantly, genetic evidence of short dispersal distances of brooded larvae exists for natural populations of *P. damicornis* in south-western Australia (Stoddart, 1984). At the same time, brooded larvae of *P. damicornis* also maintain their capacity to successfully metamorphose, settle and establish themselves after spending extended periods of time in the pelagic larval phase. Richmond (1987) found that 5% of larvae survived and 3% were still

competent to settle after 103 d, and Harii et al. (2002) kept 50% of larvae alive for 100 d, 21.3% of which settled successfully. Thus substantial proportions of brooded larvae also have the capacity for long-distance migration, highlighting mechanisms for both dispersal and retention of brooded larvae.

Typically, larvae that develop externally from broadcast spawning are thought to provide the most common mechanism for long distance dispersal among coral populations, given the obligatory 2-5 days of pelagic development required before such larvae become competent to settle (Babcock and Heyward, 1986; Miller and Mundy, 2003; Harrison, 2006) and their comparatively long competency periods, which range from 23 days (Harrison et al., 1984) to 240 days (Graham et al., 2008). Direct tracking studies indicate that larvae of spawning corals can disperse over tens of km (Willis and Oliver, 1988, 1990), and potentially even further (Wilson and Harrison, 1998). However, spawned larvae can also be retained locally, depending on larval behaviour, timing of peak larval settlement, and local water circulation patterns (Wolanski et al., 1989; Miller and Mundy, 2003; Andutta et al., 2012). In addition, observations that spawned eggs of *P. damicornis* are negatively buoyant provide another mechanism that would limit dispersal distances of developing larvae (Schmidt-Roach et al., 2012b). Thus, in the case of *P. damicornis* broadcast spawning is more likely to produce retained larvae than vagabonds.

Modelling studies of larval dispersal have demonstrated that local circulation phenomena may play a large role in the final outcome of dispersal events (e.g. Siegel et al., 2008). A pilot study of hydrodynamic currents in Orpheus Island's Pioneer Bay, near one of the collection sites in my study, demonstrated that local currents are able to retain floating satellite transmitters within well-defined spatial boundaries over the course of several weeks (V. Lukoschek pers. comm.). Such local circulation patterns undoubtedly contribute to the strong genetic breaks I observed on small spatial scales, such as the genetic differentiation found between leeward and windward Palm Island sites. However, although local circulation patterns may facilitate larval retention in some cases (see also Andutta et al., 2012), in other circumstances, local circulation patterns may disperse local larval cohorts away from a site, enabling prevailing currents to transport larvae over significant distances (e.g. Willis and Oliver, 1988; Treml et al.,

2007). Given that colonies of *P. damicornis* release larvae monthly, and that current regimes are variable throughout the year, it is unlikely that any one current pattern would prevail consistently and with similar efficiency over every single reproductive event. Considering that a few migrants per generation can obscure genetic differentiation between populations (e.g. One Migrant Per Generation Rule (OMPG),Wright, 1931), circulation patterns and larval characteristics may explain the weak genetic differentiation found among some populations of *P. damicornis*, e.g. among populations of the northern GBR, and provide a plausible explanation for genetic similarities over hundreds of km. Modelling results of passive particle dispersal simulations to and from the sites sampled in this study clearly demonstrate that dispersal over hundreds of km is feasible within 50 days, for example, between SE Papua New Guinea and Lizard Island, as well as from Lizard Island to the Swains Group in the southern GBR. The long competency periods of larvae and the existence of such blue highways therefore explain the low genetic differentiation between some Northern, Central and Southern GBR sites in this study. They also provide a potential explanation for the high number of putative first generation migrants with unidentified origins; such migrants could have arrived from very different genetic pools, e.g. from south Papua New Guinea.

3.4.2. Lack of clonal population structure suggests that brooded larvae are not retained locally on the GBR

Strong evidence that brooded larvae of *P. damicornis* on the GBR are asexually produced (Stoddart, 1983; Ward, 1992; Ayre and Miller, 2004; Schmidt-Roach et al., 2012a), combined with predictions that the behaviour of brooded larvae results in the majority being locally retained, suggest that population genetic structures of this species should be highly clonal. While such clonal populations were found in south-western Australia (Stoddart, 1984), previous studies of population genetic structures of *P. damicornis* on the GBR (Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000; Ayre and Miller, 2004; Sherman et al., 2005) and south of the GBR at Lord Howe Island (Miller and Ayre, 2004) have not found signatures of predominantly asexual reproduction. The proportions of clones found in the present study (13.7 % and 5.7 % for Type α and β, respectively) are in line with these latter results. However, it must be

noted that the true proportions of clones in my study populations are slightly underestimated, because a small number of colonies within ~1 m of colonies sampled were excluded from collections to avoid clones by fragmentation. Although asexual reproduction through fragmentation may be important in the formation of local populations under specific conditions (Adjeroud and Tsuchiya, 1999), my study focussed on mechanisms of population connectivity through dispersive larval stages.

Discrepancies between the predicted consequences of asexual, brooded larvae and the generally aclonal population structures found suggest that the brooded larvae of *P. damicornis* do not play a major role in local recruitment, despite their intrinsic potential to do so and their high output. Such patterns suggest that asexual larvae are not retained locally, but they are also consistent with several other alternative explanations. As hypothesised by Ayre and Miller (2004), diverse post-settlement selection and intense competition among clonal juveniles may take place on sub-reef scales. However, the authors themselves considered it unlikely that these processes alone could maintain such uniformly high levels of diversity across a wide range of sites and sectors. Another potential explanation is that the water column is likely to contain several cohorts of recruits from many different reefs and reproductive events, which would provide a diverse source of larval recruits to a site. If brooded larvae are largely dispersed away from their natal site by currents, differences in reef density between sectors could explain the clonal populations found on south-western Australian reefs but not on the GBR and its satellite reefs, because the chances of receiving recruits from other reefs on the west coast are much lower than on the east coast. Accordingly, the low density of reefs in western Australia are likely to yield a low density and diversity of coral recruits in the water column, and proportionally increase the importance of the few locally settling recruits to build or re-build a population following major disturbance events.

Considering the vast numbers of clonal larvae that are produced every month by populations of *P. damicornis*, the low contribution of asexual larvae to local recruitment is poorly explained by potential dispersal through local currents alone. Given the likelihood that currents may be weaker or even absent during reproductive events in some years or at some sites, brooded larvae would be expected to settle in high numbers around parent colonies. It is possible that high local larval predation could also

contribute to the low clonal population structures observed. Although synchronous release of large numbers of larvae is likely to satiate predators and increase overall chances for larval survival (Harrison et al., 1984; Jokiel et al., 1985), this strategy may fail if the size of the predator population is disproportionately large compared to the number of larvae released. *Pocillopora damicornis* is one of only a few brooding corals that release larvae throughout the year, thus the potential to swamp predators is low, especially where the population density is below a critical threshold. Small-sized or juvenile reef fish associated with *P. damicornis*, such as *Chromis* and *Dascyllus* spp., are potential predators that are locally abundant. In summary, the lack of clonal structure found for populations of *P. damicornis* in my study suggests that retention rates of asexual larvae are low, and raises further questions about how genetic breaks can occur on small spatial scales.

3.4.3. Explanations for strong genetic structure on small spatial scales

The low values found for the majority of significant pairwise D_{est} estimates suggest that small or moderate genetic differentiation is common among populations of both lineages of *P. damicornis*. At the same time, some sites were highly distinct, either from all others (PW(α), FW(β)) or from most others (ONW(α)). The individuals collected from the most divergent sites form unique or otherwise very infrequent genetic clusters according to the Bayesian cluster analyses. While larval behaviour and water circulation patterns may explain the widespread coexistence of weakly diverged populations, they fail to explain the strong genetic breaks found in these cases. It is improbable that physical factors retaining larvae and/or gametes act consistently over several generations and reproductive events, and even low numbers of migrants per generation would break down such genetic barriers (Wright, 1931).

Post-settlement selection, as suggested by Zvuloni et al. (2008) for the related pocilloporid coral *Stylophora pistillata*, does not conform with the theory that microsatellites are generally neutral genetic markers. Furthermore, in my study, as well as that of Ayre et al. (1997), environmental and habitat characteristics are very similar between highly diverged adjacent sites, while sometimes being quite different among

spatially separated, but genetically similar sites. Accordingly, I discuss two alternative explanations for the existence of stark genetic breaks among the populations of *P. damicornis* I studied: (i) stochasticity of recruitment events and re-seeding from different genetic pools following mass mortality, and (ii) reproductive isolation or hybridization.

Both modelling (Siegel et al., 2008) and field studies (Willis and Oliver, 1988, 1990) have shown that local water circulation features, such as eddies, boundary mixing and fronts between water parcels, may sweep newly released larvae together into ‘‘packets,’’ which can travel vast distances and stay together throughout their pelagic life stage. It is therefore possible that sections of the same reef, separated by as little as a few km, receive different “packets” of recruits following mass mortality, and recover from genetically very distinct sources. As all of my study sites have been impacted by major disturbance events, including severe thermal bleaching and cyclones, over the past decades (Sweatman et al., 2011), stochastic recruitment pulses may contribute to the patterns observed. Sharp population declines, followed by re-colonisation from a limited number of larvae could, in theory, reduce both the number of alleles (allelic richness, AR) and gene diversity (e.g. expected heterozygosity, H_e). However, the *P. damicornis* populations examined showed no such signs of population bottlenecks or founder events. Complex life history traits may provide this species, as well as corals in general, with the capacity to create and maintain genetic diversity in their populations. Such traits include overlapping generations, indeterminate genotypic lifespans, potentially immortal genotypes (Hughes and Jackson, 1980; Highsmith, 1982; Botsford et al., 2009), hybridization (Kenyon, 1994; van Oppen et al., 2002; Willis et al., 2006; Combosch et al., 2008) and frequent somatic mutations (van Oppen et al. 2011). Recovery from surviving remnants of the original colonies at a site (Diaz-Pulido et al. 2009; van Oppen et al. 2011) combined with genetically distinct “packets” of recruits arriving stochastically could explain the strong genetic breaks observed between some adjacent sites, as well as the relative heterozygote deficiency that was detected in several populations in this study. In the Palm Islands, where strong, small scale genetic breaks were found, the complexity and temporal variability of water circulation patterns (Luick et al., 2007; Steinberg, 2007; Hogan et al., 2010) is likely to facilitate stochastic recruitment pulses. These sites were also heavily impacted by a

severe bleaching event in 1998, which was followed by extensive coral mortality (Marshall and Baird, 2000; Berkelmans et al., 2004). This area has been known to host genetically very distinct populations of other coral species as well. Souter et al. (2010) and van Oppen et al. (2011b) found strong genetic structures over small spatial scales in *Acropora* spp. and reached similar conclusions about the origin of the observed genetic patterns. Another pocilloporid coral, *Seriatopora hystrix* was found to have genetically highly distinct populations in ONW (Van Oppen et al., 2008), however since it was the only population sampled in the area in that study, it is unclear whether it's distinctness is due to spatial isolation from the other populations sampled, as suggested by the authors, or a result of random recruitment following major perturbations.

Another potential explanation for the existence of a unique genetic cluster of individuals within a matrix of otherwise well interconnected populations is the possibility that individuals in these clusters successfully hybridized with other closely related *Pocillopora* species or *P. damicornis* lineages and their offspring proliferated locally. Hybridization is described from a wide range of scleractinian coral species, and is considered to play a major role in their diversification (Willis et al., 2006). If the morphology of such hybrids fits the description of *P. damicornis* (sensu Veron, 2000), they could have been collected for this study, and if the lineage-specific recognition site of the mitochondrial DNA (Chapter 2) remained intact following hybridization due to maternal inheritance of mitochondria, they would have slipped through the filter that I applied to ensure the genetic purity of units of observation in this study. However, to test these hypotheses, thorough sequence analyses, including nuclear loci would be needed, which is beyond the scope of this study.

3.5. Conclusions

My study reveals spatially complex genetic structure in populations of the two most common lineages of *P. damicornis* on the GBR. High genetic similarity among populations over hundreds of kilometres can be explained by life history characteristics of these corals combined with hydrodynamic circulation patterns that govern larval dispersal. Conversely, genetic differentiation among populations over small spatial scales can be explained by the spatially and temporally stochastic nature of successful

recruitment events, mainly driven by cycles of disturbance (Connell, 1978) and turbulent water flow (Siegel et al., 2008). I found no evidence for predominantly clonal reproduction, despite the large energy allocation of this species into asexually produced brooded larvae, further undermining the paradigm that brooded larvae settle close to parent colonies shortly after release.

I argue that strong genetic breaks observed between some adjacent populations cannot be adequately explained by local retention of brooded larvae, because water circulation patterns would need to act consistently throughout the year and over several consecutive years or even decades, considering the monthly recruitment events (e.g. Harrigan, 1972; Richmond and Jokiel, 1984) and overlapping generations characteristic of this species. As alternative hypotheses, I propose re-seeding of populations from different genetic pools due to the stochastic nature of recruitment, and hybridization coupled with local proliferation of hybrids.

Although measures of genetic differentiation are often low among populations of lineages within the *P. damicornis* complex over large geographic domains of the GBR, in both this and previous studies, one should exercise caution when interpreting such measures as indicating high contemporary migration rates. A serious limitation of population genetic investigations on adult coral colonies is that the ages of genotypes studied are impossible to determine, and can range from a few years to decades, or even centuries – in fact, coral genotypes may be virtually immortal due to their high potential for asexual reproduction (Hughes and Jackson, 1980; Highsmith, 1982; Botsford et al., 2009). The contemporary population structure of these organisms is an accumulated result of connectivity processes, perturbations and re-colonisation events that have occurred over undetermined periods of time. Nevertheless, evidence of high connectivity at the majority of sites suggests that dispersal and disturbance events on ecological timescales even out to create a matrix of well interconnected populations over multiple generations, and since MPAs are created to conserve populations (and metapopulations) of species over long timescales, estimates of connectivity from this and similar studies should be incorporated into conservation planning. The variability of population genetic structures among geographic regions and among habitat types within

regions indicates that dispersal mechanisms are also spatially highly variable, a fact that should be reflected in MPA design.

In my study, high gene flow over the scale of hundreds of km on the GBR maintains high genetic diversity and facilitates the spread of adaptive mutations in the two lineages of *P. damicornis*, suggesting that they have good potential for adaptation to climatic change and other anthropogenic stressors. This is especially true for populations of outer shelf reefs, which are well connected by strong longshelf currents. However, to better understand dispersal distances of ecologically significant numbers of larvae, I suggest that traditional population genetic studies are complemented by genetic studies on recent recruitment and by Lagrangian particle dispersal modelling using fine-scale circulation models.

3.6. Supplementary material

Supplementary Table 3.1. Descriptive statistics for the 9 microsatellite loci at 25 collection sites of Type α (a) and at 10 collection sites of Type β (b) individuals. N = number of samples, A = number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, G_{is} = inbreeding coefficient, significant values in bold.

(a)

Population		Pd11	Pd13	Pd2007	Pd3002	Pd3004	Pd3008	Pd3009	Pd4	PV7
MAR	**N**	8	12	11	12	11	11	10	12	12
	A	3	2	6	3	2	3	2	3	4
	H_o	0.500	0.583	0.545	0.833	0.727	0.364	0.200	0.167	0.667
	H_e	0.398	0.413	0.636	0.559	0.463	0.475	0.180	0.156	0.635
	G_{is}	-0.191	-0.375	0.189	-0.457	-0.538	0.279	-0.059	-0.023	-0.006
EYR	**N**	26	26	25	24	26	25	23	26	26
	A	4	3	9	3	2	3	5	3	4
	H_o	0.385	0.154	0.680	0.292	0.346	0.360	0.304	0.154	0.615
	H_e	0.527	0.208	0.625	0.258	0.286	0.333	0.274	0.145	0.661
	G_{is}	0.289	0.278	-0.068	-0.11	-0.19	-0.061	-0.088	-0.042	0.088
LSSW	**N**	20	26	26	19	22	26	26	25	25
	A	6	4	7	4	2	4	6	4	4
	H_o	0.750	0.231	0.654	0.579	0.727	0.500	0.269	0.120	0.480
	H_e	0.553	0.323	0.619	0.454	0.463	0.447	0.308	0.115	0.646
	G_{is}	-0.335	0.304	-0.037	-0.249	-0.556	-0.098	0.144	-0.021	**0.275**
LSW	**N**	44	48	39	43	49	48	48	45	47
	A	5	2	8	3	3	4	4	4	4
	H_o	0.523	0.250	0.718	0.395	0.551	0.417	0.271	0.267	0.702
	H_e	0.535	0.249	0.701	0.368	0.439	0.415	0.244	0.238	0.655
	G_{is}	0.035	0.007	-0.011	-0.063	-0.246	0.006	-0.101	-0.108	-0.062
LNW	**N**	26	27	25	25	27	27	24	27	26
	A	6	2	7	4	2	2	3	3	4
	H_o	0.538	0.444	0.760	0.600	0.741	0.333	0.333	0.259	0.731
	H_e	0.473	0.346	0.705	0.450	0.466	0.366	0.284	0.233	0.661
	G_{is}	-0.118	-0.268	-0.058	-0.314	**-0.576**	0.107	-0.154	-0.096	-0.087
LNNE	**N**	50	47	43	46	49	46	51	50	50
	A	8	5	14	4	4	4	4	4	5
	H_o	0.680	0.191	0.767	0.587	0.592	0.391	0.392	0.260	0.520
	H_e	0.606	0.230	0.776	0.506	0.531	0.382	0.467	0.234	0.724
	G_{is}	-0.113	**0.176**	0.023	-0.149	-0.103	-0.014	0.169	-0.101	**0.291**
LNE	**N**	15	15	15	12	11	15	14	15	15
	A	6	3	7	4	3	2	4	2	4
	H_o	0.400	0.200	0.533	0.500	0.818	0.067	0.786	0.067	0.600
	H_e	0.478	0.184	0.662	0.406	0.574	0.064	0.610	0.064	0.704
	G_{is}	0.196	-0.05	0.228	-0.189	-0.385	0	-0.254	0	0.182
LE	**N**	19	23	23	16	23	23	22	23	22
	A	4	2	6	4	2	4	4	3	4
	H_o	0.579	0.043	0.652	0.688	0.870	0.130	0.545	0.087	0.682
	H_e	0.438	0.043	0.716	0.518	0.491	0.268	0.485	0.084	0.716
	G_{is}	-0.298	0	0.111	-0.299	**-0.76**	**0.53**	-0.103	-0.011	0.071

Supplementary Table 3.1 continued

Population		Pd11	Pd13	Pd2007	Pd3002	Pd3004	Pd3008	Pd3009	Pd4	PV7
LSE	**N**	13	14	11	9	14	14	14	14	14
	A	5	3	7	2	2	3	3	3	3
	H_o	0.615	0.500	0.727	0.667	0.357	0.429	0.286	0.286	0.643
	H_e	0.515	0.426	0.719	0.444	0.293	0.349	0.357	0.304	0.651
	G_{is}	-0.157	-0.138	0.036	-0.455	-0.182	-0.191	0.235	0.096	0.049
LS	**N**	14	14	14	9	14	14	12	14	14
	A	5	2	6	3	2	3	4	3	3
	H_o	0.571	0.071	0.500	0.889	0.429	0.286	0.500	0.500	0.929
	H_e	0.605	0.069	0.699	0.586	0.337	0.255	0.406	0.401	0.651
	G_{is}	0.092	-0.404	-0.08	-0.471	-0.238	**0.125**	-0.189	-0.213	-0.397
MAC	**N**	21	21	19	21	21	20	21	19	20
	A	5	3	7	3	2	2	3	2	3
	H_o	0.381	0.286	0.579	0.381	0.429	0.150	0.429	0.263	0.800
	H_e	0.485	0.251	0.666	0.325	0.337	0.139	0.357	0.229	0.634
	G_{is}	0.238	-0.116	0.157	-0.147	-0.25	-0.056	-0.176	-0.125	-0.238
YON	**N**	6	6	4	4	6	6	6	6	6
	A	3	2	3	3	2	2	2	2	3
	H_o	0.667	0.167	1.000	0.750	0.500	0.167	0.667	0.167	0.500
	H_e	0.569	0.153	0.594	0.531	0.486	0.153	0.444	0.153	0.542
	G_{is}	-0.081	0	-0.6	-0.286	0.063	0	-0.429	0	0.167
ONW	**N**	10	13	8	9	12	13	10	11	11
	A	8	2	6	4	2	3	2	4	5
	H_o	0.400	0.231	0.375	0.333	0.583	0.462	0.300	0.636	0.545
	H_e	0.770	0.204	0.758	0.741	0.413	0.370	0.255	0.533	0.731
	G_{is}	**0.52**	-0.091	**0.553**	**0.59**	-0.375	-0.21	-0.125	-0.148	**0.298**
PW	**N**	11	17	16	10	12	17	16	13	15
	A	6	3	9	4	4	3	5	3	4
	H_o	0.273	0.059	0.438	0.800	0.500	0.235	0.438	0.692	0.533
	H_e	0.764	0.164	0.809	0.615	0.653	0.213	0.416	0.601	0.720
	G_{is}	**0.67**	0.66	**0.484**	-0.252	**0.275**	-0.076	-0.019	-0.113	**0.291**
PNE	**N**	66	73	68	69	75	70	65	68	75
	A	12	5	15	9	5	6	9	6	3
	H_o	0.515	0.301	0.588	0.319	0.573	0.371	0.323	0.559	0.493
	H_e	0.598	0.341	0.692	0.484	0.441	0.329	0.316	0.470	0.578
	G_{is}	**0.147**	0.123	**0.157**	**0.348**	**-0.295**	-0.122	-0.016	-0.181	0.153
PSE	**N**	28	28	26	26	28	27	25	25	28
	A	3	4	5	3	2	3	4	3	3
	H_o	0.464	0.286	0.462	0.654	0.393	0.333	0.160	0.360	0.500
	H_e	0.493	0.311	0.527	0.471	0.357	0.393	0.151	0.306	0.448
	G_{is}	0.076	0.098	0.144	-0.371	-0.084	0.17	-0.038	-0.155	-0.097
ONE	**N**	32	33	32	29	33	32	36	30	36
	A	7	5	13	3	4	4	6	5	4
	H_o	0.313	0.242	0.875	0.345	0.545	0.313	0.333	0.467	0.444
	H_e	0.517	0.342	0.702	0.300	0.452	0.319	0.292	0.387	0.520
	G_{is}	**0.408**	**0.305**	-0.232	-0.131	-0.193	**0.036**	-0.126	-0.191	0.159

Supplementary Table 3.1. continued

Population		Pd11	Pd13	Pd2007	Pd3002	Pd3004	Pd3008	Pd3009	Pd4	PV7
OE	**N**	32	33	33	29	32	32	30	34	33
	A	3	4	7	3	4	5	5	3	4
	H_o	0.438	0.273	0.576	0.552	0.906	0.250	0.333	0.118	0.667
	H_e	0.372	0.242	0.575	0.428	0.526	0.252	0.337	0.164	0.586
	G_{is}	-0.162	-0.11	0.014	-0.273	**-0.716**	0.024	**0.027**	0.298	-0.122
TR1	**N**	13	14	14	12	11	14	13	14	14
	A	5	3	6	4	2	2	5	3	3
	H_o	0.538	0.357	0.571	0.417	1.000	0.214	0.462	0.357	0.286
	H_e	0.642	0.390	0.536	0.354	0.500	0.477	0.438	0.304	0.482
	G_{is}	0.2	0.122	-0.03	-0.134	**-1**	0.576	-0.014	-0.14	0.438
TR2	**N**	43	46	42	37	42	46	40	43	46
	A	6	5	8	4	2	3	4	3	4
	H_o	0.581	0.239	0.690	0.865	0.857	0.630	0.375	0.233	0.630
	H_e	0.539	0.218	0.681	0.523	0.490	0.511	0.430	0.208	0.673
	G_{is}	-0.067	-0.084	-0.003	**-0.645**	**-0.745**	-0.224	**0.141**	-0.107	0.074
DIP	**N**	33	35	32	31	33	33	24	0	33
	A	7	3	7	4	2	5	5	0	5
	H_o	0.455	0.400	0.313	0.548	1.000	0.606	0.375	0.000	0.455
	H_e	0.488	0.333	0.579	0.479	0.500	0.641	0.579	0.000	0.561
	G_{is}	0.083	-0.186	**0.472**	-0.128	**-1**	0.07	**0.371**	NA	**0.205**
MYR	**N**	6	6	5	6	6	6	4	4	6
	A	3	2	4	3	2	3	4	2	3
	H_o	0.500	0.333	0.600	0.167	1.000	0.667	0.500	0.500	0.833
	H_e	0.403	0.278	0.640	0.403	0.500	0.486	0.563	0.375	0.542
	G_{is}	-0.154	-0.111	0.172	0.643	-1	-0.29	0.25	-0.2	-0.471
CH	**N**	38	42	39	35	42	41	41	0	42
	A	8	6	9	6	4	4	7	0	4
	H_o	0.474	0.286	0.692	0.514	0.476	0.268	0.244	0.000	0.310
	H_e	0.475	0.277	0.797	0.462	0.573	0.450	0.247	0.000	0.533
	G_{is}	0.016	-0.02	**0.144**	-0.099	0.181	**0.414**	0.023	NA	**0.429**
EC	**N**	30	30	24	22	29	28	30	1	30
	A	5	4	8	5	3	4	3	1	4
	H_o	0.200	0.367	0.750	0.500	0.448	0.429	0.300	0.000	0.400
	H_e	0.318	0.364	0.818	0.539	0.474	0.436	0.263	0.000	0.671
	G_{is}	0.385	0.009	**0.104**	**0.096**	0.071	0.034	-0.125	NA	**0.418**
FR	**N**	30	30	25	26	29	29	23	0	30
	A	6	4	6	4	3	4	5	0	4
	H_o	0.267	0.167	0.440	0.538	0.621	0.414	0.087	0.000	0.467
	H_e	0.321	0.157	0.709	0.532	0.511	0.353	0.526	0.000	0.676
	G_{is}	0.186	-0.047	**0.397**	0.007	-0.197	-0.157	**0.841**	NA	**0.325**

Supplementary Table 3.1. continued

(b)

Population		Pd11	Pd13	Pd2007	Pd3002	Pd3004	Pd3008	Pd3009	Pd4	PV7
LNW	**N**	37	40	39	34	37	40	35	39	40
	A	10	8	8	7	5	4	8	5	4
	H_o	0.459	0.700	0.538	0.412	0.432	0.550	0.914	0.359	0.175
	H_e	0.842	0.722	0.771	0.475	0.386	0.460	0.827	0.620	0.204
	G_{is}	**0.465**	0.043	**0.314**	**0.148**	-0.108	-0.184	-0.091	**0.431**	0.156
LNNE	**N**	13	16	16	15	16	16	15	16	16
	A	6	6	9	5	4	4	7	4	3
	H_o	0.154	0.625	0.563	0.267	0.438	0.313	0.800	0.125	0.188
	H_e	0.775	0.723	0.775	0.584	0.510	0.363	0.713	0.590	0.225
	G_{is}	**0.815**	0.167	0.304	**0.568**	0.173	0.171	-0.087	**0.800**	0.196
LE	**N**	8	10	10	9	10	10	8	10	10
	A	6	4	6	6	2	5	5	4	4
	H_o	0.375	0.400	0.300	0.667	0.600	0.600	0.500	0.300	0.300
	H_e	0.750	0.410	0.730	0.642	0.480	0.480	0.500	0.345	0.475
	G_{is}	**0.548**	-0.500	-0.029	0.020	-0.200	-0.143	0.067	0.182	0.413
LSE	**N**	12	14	14	10	14	14	13	13	14
	A	6	5	7	4	4	3	8	4	2
	H_o	0.417	0.643	0.714	0.300	0.357	0.143	0.692	0.385	0.286
	H_e	0.778	0.679	0.783	0.345	0.411	0.135	0.802	0.595	0.245
	G_{is}	**0.498**	0.089	0.125	**0.182**	0.167	-0.020	0.176	**0.388**	-0.130
LL	**N**	6	7	6	7	7	7	7	7	7
	A	3	3	4	5	3	3	5	3	3
	H_o	0.000	0.286	0.667	0.143	0.571	0.571	0.857	0.571	0.286
	H_e	0.500	0.357	0.694	0.684	0.520	0.439	0.765	0.571	0.255
	G_{is}	**1**	0.273	0.13	**0.818**	-0.021	-0.231	-0.043	0.077	-0.043
YON	**N**	16	17	17	16	18	18	18	17	18
	A	8	7	8	7	4	4	7	4	3
	H_o	0.188	0.706	0.588	0.438	0.333	0.556	0.944	0.588	0.222
	H_e	0.820	0.694	0.623	0.613	0.292	0.519	0.779	0.471	0.290
	G_{is}	**0.784**	0.013	0.086	**0.316**	-0.115	-0.043	-0.184	-0.221	0.261
FW	**N**	13	14	11	9	14	14	10	6	14
	A	6	3	4	4	2	2	4	3	2
	H_o	0.769	1.000	0.636	0.222	0.571	0.929	0.800	1.000	0.071
	H_e	0.757	0.561	0.678	0.512	0.408	0.497	0.675	0.569	0.069
	G_{is}	0.024	**-0.767**	-0.61	**0.605**	-0.368	**-0.857**	-0.134	-0.714	0.000
OSW	**N**	13	13	11	12	13	13	11	6	13
	A	6	5	5	2	3	2	4	3	2
	H_o	0.385	0.769	0.182	0.000	1.000	1.000	0.818	0.500	0.538
	H_e	0.657	0.672	0.599	0.278	0.536	0.500	0.707	0.403	0.393
	G_{is}	**0.447**	-0.106	**0.720**	**1.000**	**-0.857**	**-1.000**	-0.111	-0.154	-0.333
ONW	**N**	16	22	15	17	22	23	17	20	23
	A	8	5	5	4	3	4	6	4	4
	H_o	0.500	0.636	0.200	0.412	0.864	0.565	0.412	0.300	0.174
	H_e	0.844	0.608	0.749	0.590	0.515	0.451	0.727	0.409	0.164
	G_{is}	**0.434**	-0.023	**0.749**	**0.329**	**-0.663**	-0.233	**0.458**	**0.290**	-0.041
PW	**N**	26	31	22	30	35	36	29	23	36
	A	11	6	7	5	2	4	8	4	6
	H_o	0.500	0.613	0.455	0.200	0.800	0.528	0.655	0.261	0.417
	H_e	0.808	0.670	0.787	0.599	0.480	0.413	0.722	0.428	0.453
	G_{is}	**0.398**	**0.102**	**0.441**	**0.676**	**-0.659**	-0.265	**0.11**	**0.409**	0.095

(a)

		Northern GBR												Central GBR										Southern GBR		
		MAR	EYR	LSSW	LSW	LNW	LNNE	LNE	LE	LSE	LS	MAC	YON	ONW	PW	PNE	PSE	ONE	OE	TR1	TR2	DIP	MYR	CH	EC	FR
Northern GBR	MAR		0.230	0.592	0.183	0.972	0.085	*0.000*	*0.001*	0.534	0.055	0.040	*0.003*	*0.001*	*0.000*	0.203	0.367	0.347	0.592	0.026	*0.001*	*0.002*	0.013	*0.006*	0.038	*0.001*
	EYR	0.000		0.085	0.188	0.212	*0.000*	*0.000*	*0.000*	0.300	0.021	0.365	*0.000*	*0.000*	*0.000*	0.022	0.043	0.149	0.021	*0.000*	*0.000*	*0.000*	*0.003*	*0.000*	*0.003*	*0.000*
	LSSW	0.000	0.002		0.995	0.375	0.019	*0.000*	*0.005*	0.540	0.058	0.183	*0.004*	*0.000*	*0.000*	0.013	*0.009*	0.064	*0.009*	0.071	*0.000*	*0.000*	0.051	0.015	0.012	*0.000*
	LSW	0.001	0.000	0.000		0.298	*0.000*	*0.000*	*0.000*	0.382	0.025	0.070	*0.004*	*0.000*	*0.000*	0.012	*0.000*	0.056	*0.000*	*0.003*	*0.000*	*0.000*	0.024	*0.000*	*0.008*	*0.000*
	LNW	0.000	0.000	0.000	0.000		0.060	*0.001*	*0.000*	0.716	0.013	0.022	*0.007*	*0.000*	*0.000*	0.029	0.045	0.082	0.177	0.012	*0.000*	*0.000*	0.013	*0.001*	0.019	*0.000*
	LNNE	0.002	0.013	0.004	0.008	0.002		0.012	*0.004*	0.270	0.037	*0.000*	0.030	*0.000*	*0.000*	*0.000*	*0.000*	*0.002*	*0.000*	*0.001*	*0.000*	*0.000*	*0.004*	*0.000*	*0.000*	*0.000*
	LNE	0.027	0.027	0.029	0.033	0.013	0.005		*0.009*	*0.008*	*0.003*	*0.002*	0.050	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.006*	*0.000*	*0.000*	*0.000*	0.011	*0.000*	*0.000*	*0.000*
	LE	0.015	0.024	0.009	0.010	0.014	0.007	0.007		*0.001*	*0.003*	*0.001*	0.064	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.001*	*0.000*	*0.000*	0.010	*0.000*	*0.000*	*0.000*
	LSE	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.017		0.698	0.590	*0.003*	*0.003*	*0.000*	0.070	0.043	0.065	*0.009*	*0.008*	*0.006*	*0.000*	*0.007*	*0.002*	0.020	*0.000*
	LS	0.004	0.005	0.003	0.005	0.007	0.004	0.016	0.014	0.000		0.180	0.239	0.015	*0.000*	*0.001*	*0.004*	*0.002*	*0.002*	*0.000*	*0.001*	*0.000*	*0.008*	*0.000*	*0.001*	*0.000*
	MAC	0.005	0.000	0.001	0.002	0.005	0.014	0.014	0.016	0.000	0.001		*0.003*	*0.000*	*0.000*	0.015	*0.005*	0.038	*0.004*	*0.004*	*0.000*	*0.000*	0.011	*0.002*	*0.000*	*0.000*
	YON	0.033	0.046	0.025	0.025	0.024	0.010	0.008	0.006	0.035	0.001	0.029		*0.009*	*0.000*	0.049	*0.000*	*0.009*	*0.005*	0.011	0.011	*0.000*	0.010	*0.001*	*0.003*	*0.000*
Central GBR	ONW	0.034	0.036	0.045	0.043	0.041	0.024	0.060	0.058	0.022	0.011	0.037	0.025		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	PW	**0.133**	**0.131**	**0.130**	**0.133**	**0.127**	**0.110**	**0.144**	**0.120**	**0.133**	**0.117**	**0.131**	**0.116**	0.034		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	PNE	0.000	0.004	0.004	0.003	0.002	0.012	0.028	0.014	0.002	0.010	0.004	0.008	0.034	**0.122**		*0.003*	0.148	*0.000*	*0.000*	*0.000*	*0.000*	*0.002*	*0.000*	*0.000*	*0.000*
	PSE	0.000	0.003	0.006	0.008	0.002	0.017	0.037	0.029	0.004	0.010	0.009	0.043	0.055	**0.144**	0.005		0.055	0.033	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	ONE	0.000	0.001	0.002	0.002	0.001	0.007	0.023	0.013	0.003	0.015	0.003	0.025	0.046	**0.122**	0.000	0.002		0.065	*0.001*	*0.000*	*0.000*	*0.003*	*0.001*	*0.000*	*0.000*
	OE	0.000	0.004	0.006	0.010	0.000	0.010	0.009	0.015	0.008	0.015	0.008	0.022	0.061	**0.131**	0.011	0.003	0.002		*0.003*	*0.000*	*0.000*	0.019	*0.000*	*0.000*	*0.000*
	TR1	0.007	0.022	0.003	0.013	0.008	0.018	0.021	0.025	0.010	0.034	0.013	0.023	0.077	**0.154**	0.013	0.019	0.014	0.012		0.034	*0.000*	0.030	0.031	*0.000*	*0.000*
	TR2	0.011	0.026	0.009	0.014	0.012	0.014	0.018	0.024	0.009	0.012	0.013	0.016	0.051	**0.159**	0.021	0.034	0.025	0.023	0.004		*0.000*	*0.001*	*0.000*	*0.000*	*0.000*
	DIP	0.016	0.044	0.026	0.038	0.025	0.035	0.037	0.019	0.059	0.052	0.056	0.033	**0.105**	**0.159**	0.039	0.038	0.021	0.016	0.031	0.042		0.218	*0.000*	*0.000*	*0.000*
	MYR	0.020	0.024	0.007	0.012	0.016	0.022	0.017	0.021	0.025	0.019	0.015	0.026	0.081	**0.144**	0.033	0.046	0.021	0.012	0.013	0.030	0.001		0.040	0.142	0.014
Southern GBR	CH	0.010	0.020	0.006	0.009	0.012	0.013	0.035	0.016	0.016	0.028	0.011	0.035	0.061	**0.132**	0.015	0.026	0.012	0.017	0.005	0.023	0.032	0.008		0.048	*0.000*
	EC	0.006	0.011	0.005	0.005	0.004	0.016	0.037	0.019	0.006	0.013	0.011	0.026	0.042	**0.129**	0.019	0.028	0.014	0.014	0.036	0.025	0.018	0.002	0.002		0.015
	FR	0.021	0.023	0.018	0.019	0.019	0.022	0.043	0.028	0.030	0.035	0.026	0.054	0.053	**0.130**	0.034	0.050	0.028	0.026	0.074	0.043	0.024	0.013	0.032	0.005	

Supplementary Table 3.2. Values of pairwise genetic differentiation of Type α populations (below diagonal). Values higher than 0.1 are in red, between 0.5 and 0.1 are in orange font. Where P values were calculated, they are shown above diagonal. P values < 0.01 are in italic font, and statistically non-significant (p>0.01) values of genetic differentiation are grayed out. (a) pairwise D_{est} values, (b) pairwise uncorrected F_{st} values, (c) pairwise F_{st} values calculated with ENA correction in FreeNA, (d) pairwise standardised F'_{st} calculated in GenoDive with

(b)

		Northern GBR												Central GBR										Southern GBR		
		MAR	EYR	LSSW	LSW	LNW	LNNE	LNE	LE	LSE	LS	MAC	YON	ONW	PW	PNE	PSE	ONE	OE	TR1	TR2	DIP	MYR	CH	EC	FR
Northern GBR	MAR		0.090	0.983	0.060	0.417	0.196	*0.000*	*0.000*	0.346	0.022	*0.002*	*0.002*	0.010	*0.000*	0.131	0.043	0.210	0.404	0.025	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	EYR	0.018		0.269	0.270	0.010	*0.007*	*0.000*	*0.000*	0.231	0.020	0.364	*0.000*	*0.000*	*0.000*	*0.004*	0.033	*0.004*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	LSSW	0.000	0.010		0.848	0.455	0.027	*0.000*	*0.000*	0.398	0.012	0.029	*0.004*	*0.000*	*0.000*	*0.000*	*0.001*	0.030	0.036	0.253	*0.000*	*0.000*	*0.000*	*0.001*	*0.000*	*0.000*
	LSW	0.009	0.005	0.000		0.089	*0.000*	*0.000*	*0.000*	0.112	*0.006*	0.014	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.003*	*0.004*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	LNW	0.000	0.012	0.000	0.001		0.064	*0.000*	*0.000*	0.508	*0.000*	*0.002*	*0.001*	*0.000*	*0.000*	*0.007*	0.010	*0.002*	*0.001*	*0.005*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	LNNE	0.012	0.025	0.013	0.017	0.006		0.018	*0.000*	0.204	0.023	*0.001*	0.013	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.001*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	LNE	0.069	0.072	0.060	0.063	0.046	0.015		*0.005*	*0.000*	*0.000*	*0.000*	0.080	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.001*	*0.000*	*0.000*	*0.000*
	LE	0.062	0.063	0.044	0.045	0.047	0.015	0.026		*0.000*	*0.000*	*0.000*	0.239	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	LSE	0.002	0.008	0.003	0.002	0.000	0.008	0.056	0.059		0.416	0.183	0.016	*0.000*	*0.000*	0.079	0.017	0.010	*0.000*	0.010	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	LS	0.036	0.025	0.032	0.024	0.025	0.014	0.051	0.051	0.000		0.033	0.237	0.037	*0.000*	*0.008*	*0.000*	*0.003*	*0.000*	*0.001*	*0.000*	*0.000*	*0.002*	*0.000*	*0.000*	*0.000*
	MAC	0.036	0.000	0.014	0.009	0.019	0.024	0.052	0.054	0.000	0.011		*0.002*	*0.000*	*0.000*	*0.000*	*0.000*	*0.004*	*0.000*	*0.002*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	YON	0.076	0.084	0.071	0.063	0.056	0.019	0.031	0.032	0.054	0.022	0.070		0.017	*0.000*	0.039	*0.000*	*0.006*	*0.000*	0.019	*0.000*	*0.000*	0.086	*0.001*	*0.009*	*0.001*
Central GBR	ONW	0.046	0.084	0.068	0.072	0.064	0.042	0.092	0.073	0.038	0.015	0.077	0.032		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.002*	*0.000*	*0.000*	*0.000*
	PW	**0.194**	**0.235**	**0.208**	**0.214**	**0.201**	**0.169**	**0.211**	**0.192**	**0.186**	**0.160**	**0.222**	**0.160**	0.051		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	PNE	0.015	0.022	0.032	0.021	0.014	0.028	0.071	0.061	0.020	0.028	0.029	0.033	0.050	**0.179**		0.036	0.029	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	PSE	0.006	0.022	0.041	0.035	0.018	0.049	**0.116**	**0.104**	0.038	0.056	0.059	**0.105**	0.091	**0.235**	0.015		0.059	*0.002*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	ONE	0.012	0.013	0.024	0.016	0.012	0.033	0.079	0.071	0.030	0.042	0.031	0.069	0.085	**0.207**	0.006	0.008		0.192	*0.002*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	OE	0.004	0.027	0.024	0.025	0.007	0.027	0.047	0.062	0.040	0.047	0.037	0.076	0.099	**0.236**	0.029	0.023	0.012		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	TR1	0.042	0.065	0.016	0.030	0.035	0.044	0.068	0.088	0.032	0.057	0.045	0.092	0.095	**0.219**	0.070	**0.102**	0.073	0.067		0.047	*0.000*	*0.002*	0.138	*0.000*	*0.000*
	TR2	0.040	0.068	0.029	0.038	0.040	0.036	0.077	0.062	0.051	0.056	0.066	0.069	0.084	**0.205**	0.059	0.081	0.071	0.068	0.024		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	DIP	0.050	0.078	0.039	0.049	0.057	0.056	0.092	0.069	0.082	0.097	0.089	0.086	**0.108**	**0.179**	0.065	0.089	0.049	0.066	0.086	0.073		0.781	*0.000*	*0.000*	*0.000*
	MYR	0.078	0.093	0.050	0.052	0.063	0.051	0.079	0.082	0.070	0.070	0.066	0.092	0.086	**0.190**	0.075	**0.128**	0.065	0.082	0.065	0.089	0.009		0.016	0.386	0.052
Southern GBR	CH	0.043	0.052	0.014	0.023	0.036	0.041	0.068	0.060	0.035	0.043	0.027	0.091	0.076	**0.168**	0.071	**0.105**	0.070	0.061	0.016	0.037	0.074	0.024		*0.004*	*0.000*
	EC	0.042	0.047	0.033	0.030	0.031	0.035	0.075	0.063	0.039	0.050	0.040	0.090	0.070	**0.151**	0.042	0.076	0.044	0.052	0.076	0.060	0.036	0.031	0.016		0.029
	FR	0.081	0.098	0.070	0.083	0.079	0.069	0.090	0.071	0.080	0.076	0.094	0.086	0.085	**0.156**	0.087	**0.119**	0.092	0.091	**0.128**	**0.103**	0.056	0.047	0.082	0.023	

(c)

		Northern GBR												Central GBR										Southern GBR		
		MAR	EYR	LSSW	LSW	LNW	LNNE	LNE	LE	LSE	LS	MAC	YON	ONW	PW	PNE	PSE	ONE	OE	TR1	TR2	DIP	MYR	CH	EC	FR
Northern GBR	MAR																									
	EYR	0.022																								
	LSSW	0.000	0.012																							
	LSW	0.010	0.007	0.000																						
	LNW	0.000	0.015	0.000	0.001																					
	LNNE	0.016	0.027	0.011	0.019	0.008																				
	LNE	0.077	0.077	0.058	0.064	0.049	0.015																			
	LE	0.061	0.068	0.040	0.044	0.047	0.015	0.031																		
	LSE	0.005	0.010	0.004	0.004	0.000	0.009	0.057	0.059																	
	LS	0.038	0.029	0.033	0.022	0.026	0.015	0.051	0.053	0.000																
	MAC	0.041	0.003	0.016	0.008	0.020	0.025	0.054	0.058	0.000	0.010															
	YON	0.077	0.087	0.070	0.063	0.057	0.024	0.035	0.036	0.055	0.028	0.067														
Central GBR	ONW	0.059	0.086	0.071	0.074	0.070	0.045	**0.101**	0.078	0.050	0.024	0.080	0.056													
	PW	**0.205**	**0.239**	**0.208**	**0.216**	**0.205**	**0.167**	**0.217**	**0.200**	**0.193**	**0.167**	**0.226**	**0.177**	0.057												
	PNE	0.018	0.026	0.033	0.023	0.015	0.028	0.073	0.060	0.020	0.024	0.030	0.036	0.044	**0.168**											
	PSE	0.006	0.024	0.043	0.035	0.017	0.052	**0.122**	**0.102**	0.040	0.057	0.062	**0.105**	0.098	**0.240**	0.017										
	ONE	0.015	0.012	0.025	0.016	0.013	0.033	0.082	0.071	0.031	0.040	0.030	0.069	0.081	**0.203**	0.008	0.010									
	OE	0.006	0.032	0.027	0.025	0.008	0.029	0.052	0.064	0.040	0.045	0.039	0.075	**0.103**	**0.236**	0.029	0.023	0.014								
	TR1	0.039	0.061	0.016	0.030	0.033	0.041	0.073	0.081	0.036	0.060	0.048	0.094	0.099	**0.218**	0.066	0.095	0.065	0.064							
	TR2	0.042	0.071	0.030	0.039	0.040	0.037	0.077	0.056	0.052	0.056	0.067	0.070	0.091	**0.206**	0.060	0.083	0.072	0.069	0.024						
	DIP	0.053	0.081	0.040	0.050	0.055	0.051	0.089	0.061	0.082	0.093	0.090	0.090	**0.107**	**0.177**	0.064	0.090	0.052	0.066	0.076	0.068					
	MYR	0.073	**0.100**	0.052	0.053	0.061	0.051	0.084	0.078	0.069	0.068	0.073	0.091	0.090	**0.196**	0.074	**0.124**	0.073	0.080	0.069	0.085	0.014				
Southern GBR	CH	0.041	0.055	0.016	0.023	0.036	0.040	0.071	0.054	0.042	0.045	0.034	0.096	0.077	**0.163**	0.067	**0.101**	0.066	0.061	0.021	0.037	0.063	0.028			
	EC	0.046	0.050	0.033	0.030	0.032	0.032	0.076	0.058	0.044	0.049	0.044	0.092	0.064	**0.142**	0.039	0.076	0.045	0.055	0.074	0.061	0.036	0.040	0.014		
	FR	0.098	**0.113**	0.078	0.093	0.089	0.069	0.093	0.074	0.088	0.083	**0.106**	0.100	0.092	**0.163**	0.087	**0.134**	**0.105**	**0.102**	**0.136**	**0.107**	0.055	0.058	0.089	0.035	

(d)

		Northern GBR												Central GBR										Southern GBR		
		MAR	EYR	LSSW	LSW	LNW	LNNE	LNE	LE	LSE	LS	MAC	YON	ONW	PW	PNE	PSE	ONE	OE	TR1	TR2	DIP	MYR	CH	EC	FR
Northern GBR	MAR																									
	EYR	0.000																								
	LSSW	0.000	0.000																							
	LSW	0.000	0.000	0.000																						
	LNW	0.000	0.008	0.000	0.012																					
	LNNE	0.000	0.026	0.000	0.014	0.000																				
	LNE	0.055	0.096	0.047	0.072	0.053	0.000																			
	LE	0.042	0.075	0.028	0.046	0.055	0.005	0.000																		
	LSE	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.038																	
	LS	0.000	0.020	0.000	0.010	0.015	0.000	0.035	0.028	0.000																
	MAC	0.020	0.000	0.000	0.000	0.017	0.020	0.061	0.060	0.000	0.000															
	YON	0.016	0.092	0.024	0.036	0.052	0.000	0.000	0.000	0.000	0.000	0.063														
Central GBR	ONW	0.000	0.095	0.046	0.076	0.065	0.032	0.075	0.051	0.000	0.000	0.074	0.000													
	PW	**0.261**	**0.387**	**0.318**	**0.364**	**0.353**	**0.312**	**0.316**	**0.286**	**0.275**	**0.218**	**0.363**	**0.129**	0.000												
	PNE	0.000	0.021	0.033	0.022	0.011	0.038	0.097	0.078	0.005	0.020	0.032	0.001	0.054	**0.323**											
	PSE	0.000	0.019	0.038	0.037	0.014	0.063	**0.156**	**0.133**	0.025	0.057	0.074	**0.116**	0.099	**0.379**	0.011										
	ONE	0.000	0.000	0.005	0.005	0.000	0.034	0.081	0.073	0.001	0.025	0.020	0.021	0.082	**0.330**	0.000	0.000									
	OE	0.000	0.025	0.010	0.023	0.000	0.028	0.039	0.066	0.024	0.037	0.038	0.060	**0.111**	**0.376**	0.033	0.018	0.000								
	TR1	0.013	0.085	0.000	0.021	0.037	0.048	0.062	0.099	0.009	0.054	0.052	0.077	0.090	**0.350**	0.093	**0.138**	0.074	0.074							
	TR2	0.027	0.095	0.027	0.048	0.053	0.051	0.099	0.075	0.059	0.061	0.089	0.051	0.094	**0.349**	0.087	**0.114**	0.095	0.092	0.012						
	DIP	0.036	**0.110**	0.040	0.067	0.082	0.086	**0.121**	0.088	**0.107**	**0.126**	**0.132**	0.080	**0.147**	**0.323**	0.100	**0.133**	0.060	0.088	**0.112**	**0.112**					
	MYR	0.009	**0.105**	0.004	0.024	0.062	0.025	0.048	0.057	0.011	0.017	0.052	0.000	0.000	**0.228**	0.069	**0.150**	0.016	0.073	0.030	0.089	0.000				
Southern GBR	CH	0.038	0.073	0.005	0.026	0.052	0.061	0.094	0.079	0.035	0.048	0.032	**0.102**	**0.100**	**0.300**	**0.108**	**0.162**	0.093	0.086	0.005	0.054	**0.117**	0.006			
	EC	0.000	0.034	0.000	0.013	0.011	0.026	0.059	0.045	0.000	0.015	0.019	0.000	0.039	**0.238**	0.045	0.086	0.029	0.044	0.067	0.072	0.041	0.000	0.009		
	FR	0.090	**0.146**	**0.105**	**0.128**	**0.123**	**0.112**	**0.132**	**0.106**	**0.115**	**0.107**	**0.137**	0.090	0.098	**0.267**	**0.139**	**0.180**	**0.136**	**0.135**	**0.197**	**0.172**	0.071	0.000	**0.135**	0.021	

Supplementary Table 3.3. Values of pairwise genetic differentiation of Type β populations (below diagonal). Values higher than 0.1 are in red, between 0.5 and 0.1 are in orange font. Where P values were calculated, they are shown above diagonal. P values < 0.01 are in italic font, and statistically non-significant (p>0.01) values of genetic differentiation are grayed out. (a) pairwise D_{est} values, (b) pairwise uncorrected F_{st} values, (c) pairwise F_{st} values calculated with ENA correction in FreeNA, (d) pairwise standardised F'_{st} calculated in GenoDive with Meirmans' correction

(a)

		Northern GBR						Central GBR			
		LNW	LNNE	LE	LSE	LL	YON	FW	OSW	ONW	PW
Northern GBR	LNW		*0.002*	*0.001*	0.261	0.109	0.015	*0.000*	*0.000*	*0.000*	*0.000*
	LNNE	0.017		*0.002*	0.037	0.049	*0.004*	*0.000*	*0.000*	*0.000*	*0.000*
	LE	0.042	0.026		*0.008*	0.051	*0.007*	*0.000*	*0.000*	*0.003*	*0.009*
	LSE	0.000	0.009	0.026		0.049	0.014	*0.000*	*0.000*	*0.000*	*0.000*
	LL	0.004	0.010	0.013	0.011		0.011	*0.000*	*0.000*	*0.000*	*0.008*
	YON	0.009	0.020	0.023	0.013	0.026		*0.000*	*0.000*	*0.001*	*0.000*
Central GBR	FW	**0.111**	**0.138**	**0.145**	**0.126**	0.090	**0.129**		*0.000*	*0.000*	*0.000*
	OSW	0.060	0.095	0.090	0.064	0.098	0.098	0.055		*0.000*	*0.000*
	ONW	0.040	0.038	0.025	0.052	0.045	0.023	**0.100**	0.070		0.026
	PW	0.029	0.040	0.016	0.040	0.021	0.038	**0.120**	0.051	0.007	

(b)

		Northern GBR						Central GBR			
		LNW	LNNE	LE	LSE	LL	YON	FW	OSW	ONW	PW
Northern GBR	LNW		*0.000*	*0.002*	0.028	0.327	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
	LNNE	0.026		0.169	0.227	0.690	*0.002*	*0.000*	*0.000*	*0.000*	*0.000*
	LE	0.055	0.022		*0.007*	0.205	*0.006*	*0.000*	*0.000*	*0.001*	*0.008*
	LSE	0.002	0.009	0.057		0.335	0.368	*0.000*	*0.000*	*0.000*	*0.000*
	LL	0.019	0.010	0.016	0.013		0.046	*0.000*	*0.000*	*0.001*	*0.000*
	YON	0.029	0.042	0.058	0.022	0.061		*0.000*	*0.000*	*0.000*	*0.000*
Central GBR	FW	**0.104**	**0.139**	**0.166**	**0.128**	**0.144**	**0.151**		*0.000*	*0.000*	*0.000*
	OSW	0.087	**0.111**	**0.113**	**0.114**	**0.140**	**0.124**	0.082		*0.000*	*0.000*
	ONW	0.039	0.034	0.033	0.042	0.051	0.046	**0.118**	0.087		*0.001*
	PW	0.029	0.043	0.016	0.035	0.037	0.043	**0.101**	0.062	0.012	

Supplementary Table 3.3. continued

(c)

		Northern GBR						Central GBR			
		LNW	LNNE	LE	LSE	LL	YON	FW	OSW	ONW	PW
Northern GBR	LNW										
	LNNE	0.026									
	LE	0.058	0.037								
	LSE	0.005	0.019	0.062							
	LL	0.023	0.015	0.026	0.024						
	YON	0.032	0.051	0.064	0.033	0.066					
Central GBR	FW	**0.109**	**0.141**	**0.165**	**0.128**	**0.134**	**0.154**				
	OSW	0.076	0.097	0.095	0.099	**0.120**	**0.108**	0.083			
	ONW	0.038	0.033	0.037	0.042	0.047	0.055	**0.113**	0.070		
	PW	0.029	0.035	0.017	0.038	0.034	0.042	**0.103**	0.054	0.013	

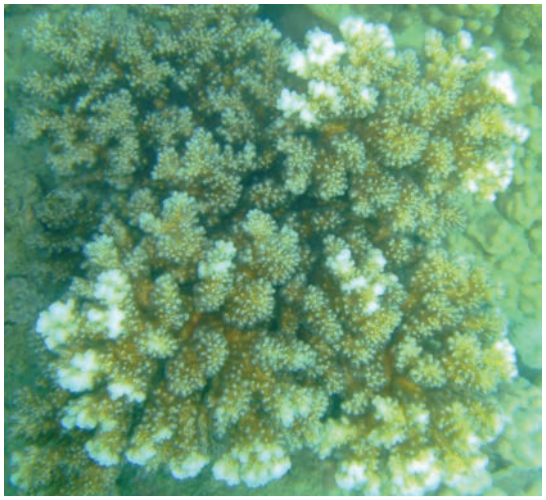
(d)

		Northern GBR						Central GBR			
		LNW	LNNE	LE	LSE	LL	YON	FW	OSW	ONW	PW
Northern GBR	LNW										
	LNNE	0.040									
	LE	0.091	0.008								
	LSE	0.000	0.000	0.060							
	LL	0.006	0.000	0.000	0.000						
	YON	0.047	0.073	0.089	0.010	**0.110**					
Central GBR	FW	**0.164**	**0.219**	**0.212**	**0.127**	**0.138**	**0.233**				
	OSW	**0.150**	**0.194**	**0.151**	**0.148**	**0.201**	**0.211**	0.000			
	ONW	0.049	0.015	0.000	0.001	0.000	0.036	**0.112**	0.070		
	PW	0.030	0.035	0.000	0.000	0.000	0.033	0.081	0.010	0.000	

(a)

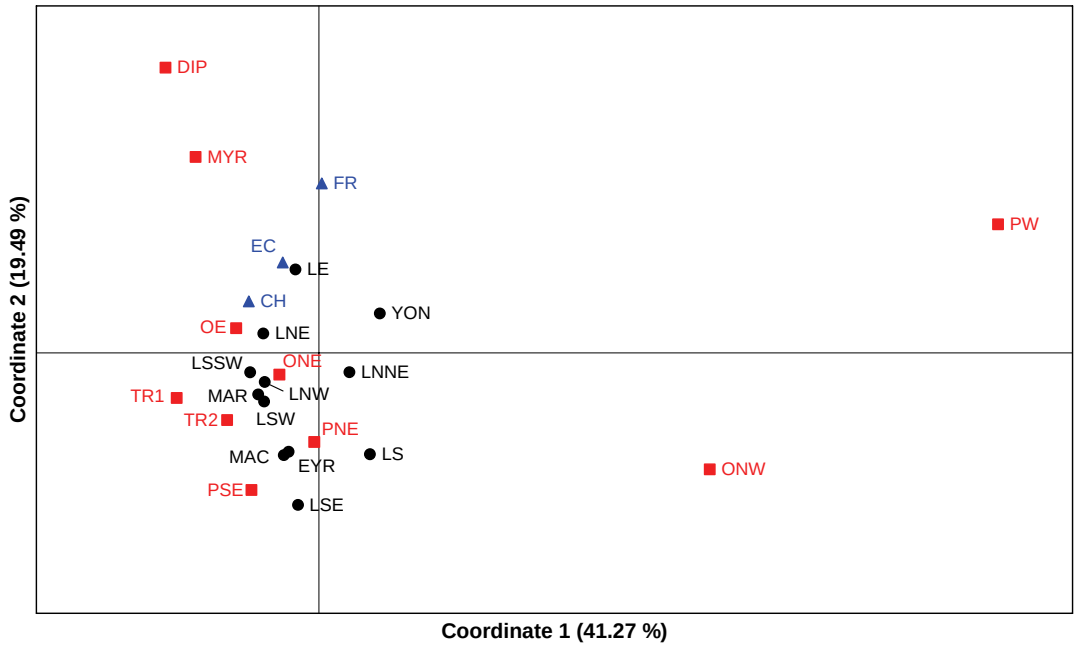
(b)

(c)

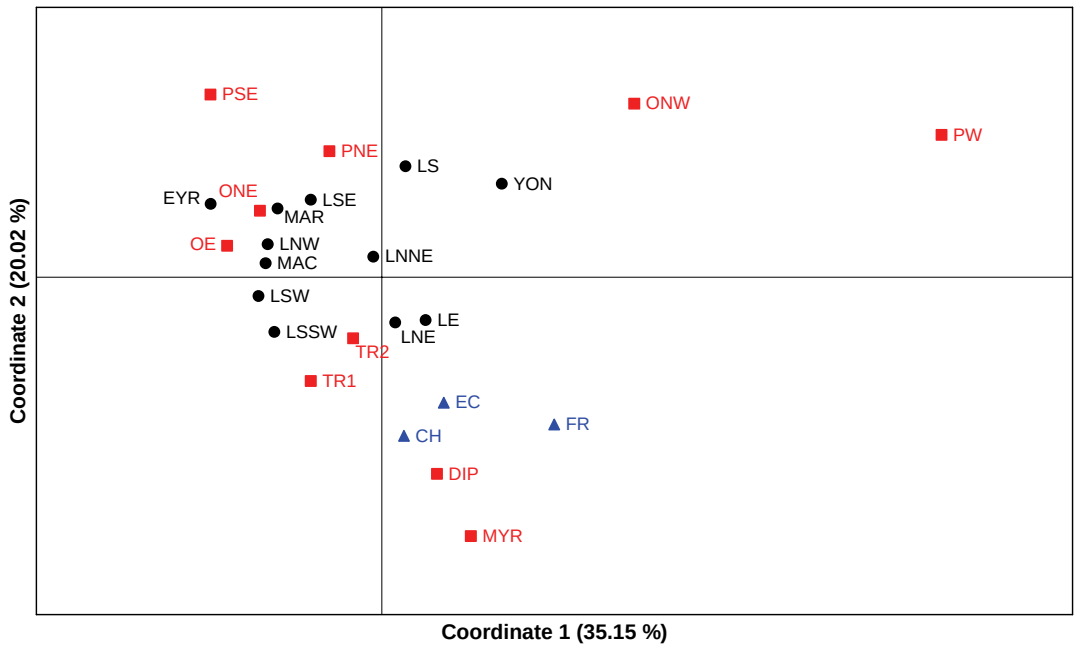


Supplementary Fig. 3.1. Photographs of "typical" *P. damicornis* Type α (A), β (B) and "other *Pocillopora*" (C). Morphology of the lineages often overlaps.

(a)

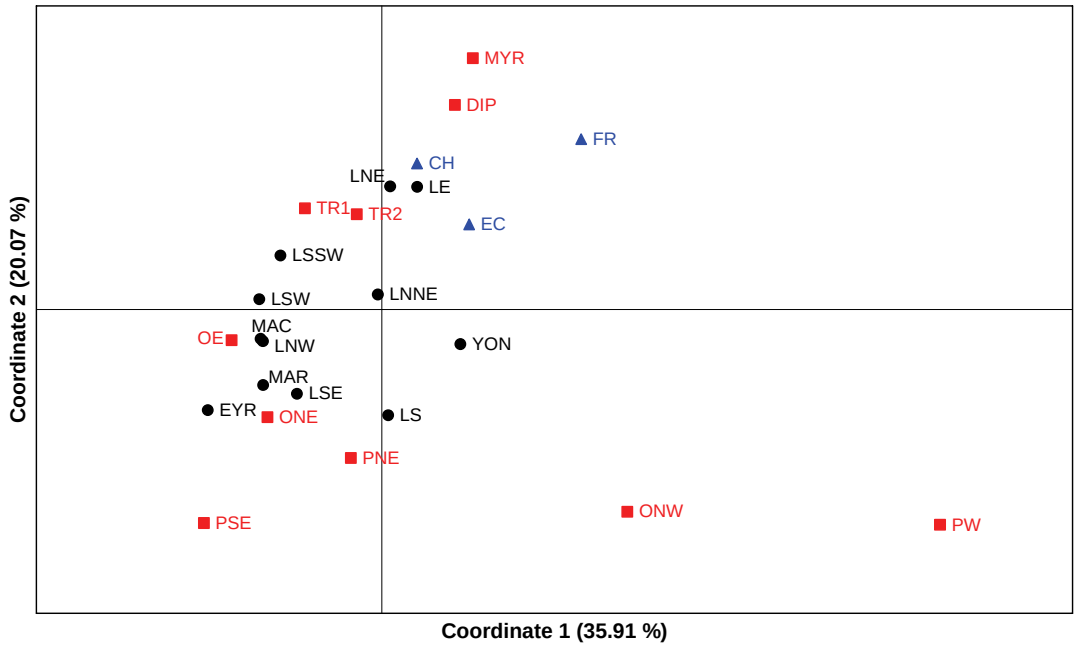


(b)



Supplementary Fig. 3.2. Principal coordinate analysis of pairwise D_{est} values (a), F_{st} values without correction (b), F_{st} values with ENA correction (c), and F_{st} values with Meirmans' correction (d) for Type α populations. The first two coordinates only represent 58.15 %, 56.77 %, 55.38 %, and 61.20 % of the variation of data for D_{est}, non-corrected F_{st}, ENA corrected F_{st}, and Meirmans-corrected F_{st}, values, respectively, and hence fail to reveal a large part of the relationships. Black dots = northern GBR, red squares = central GBR, blue triangles = Southern GBR

(c)



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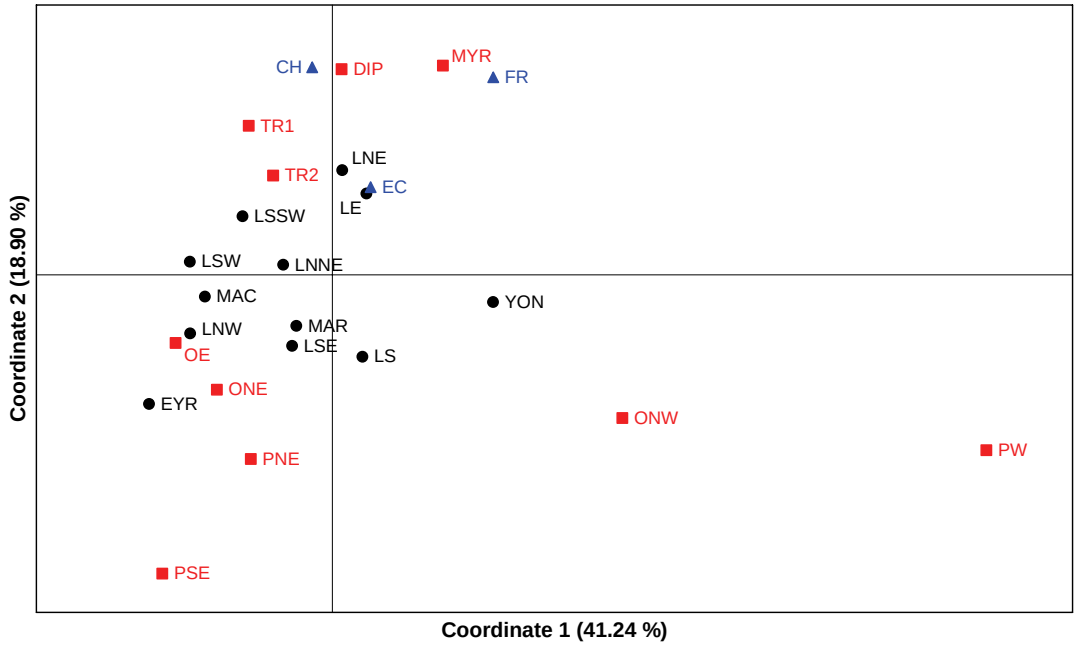
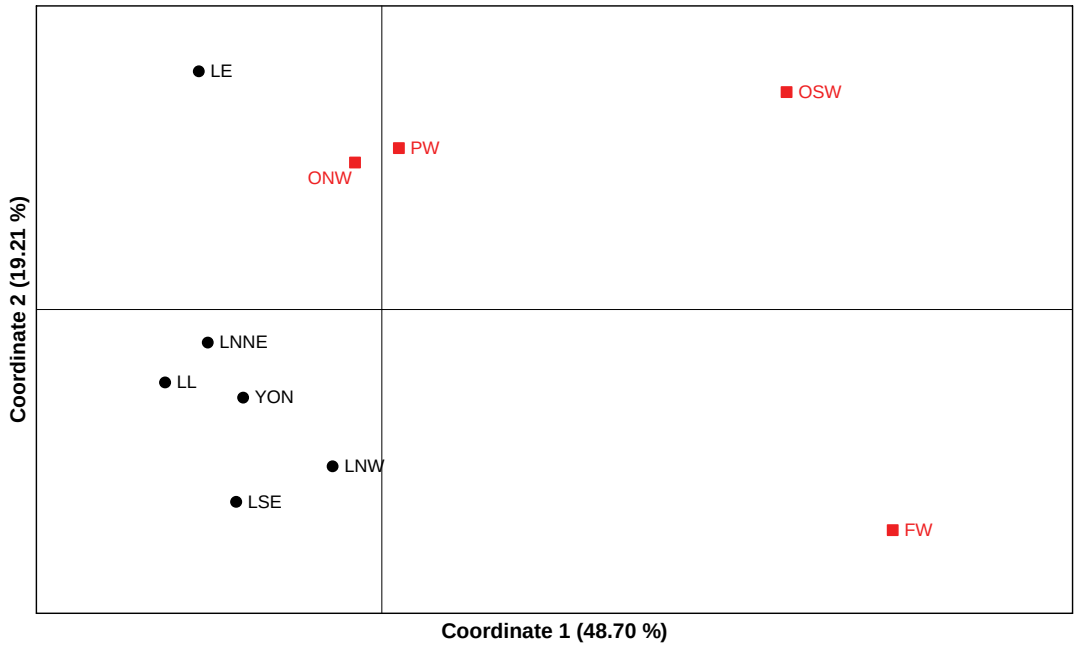


Supplementary Fig. 3.2. continued

(a)



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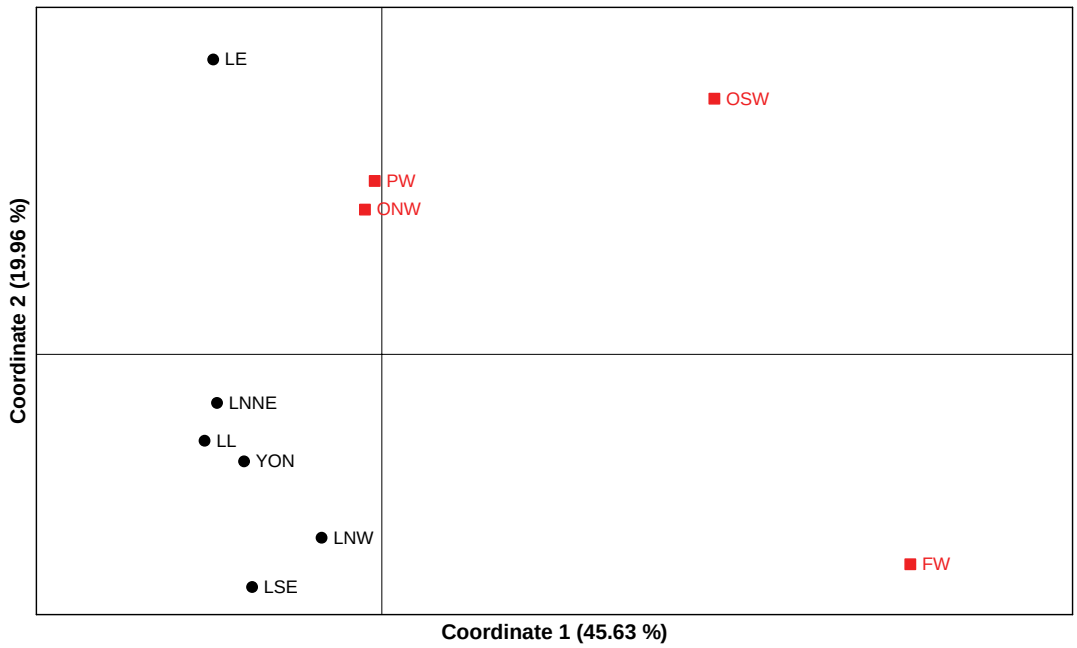
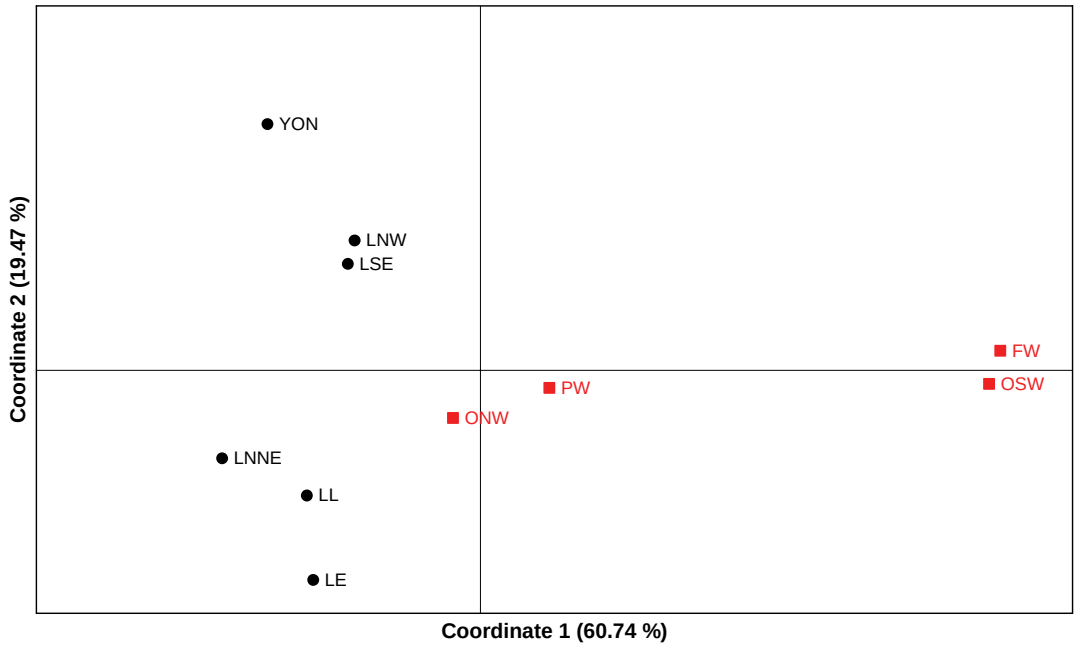
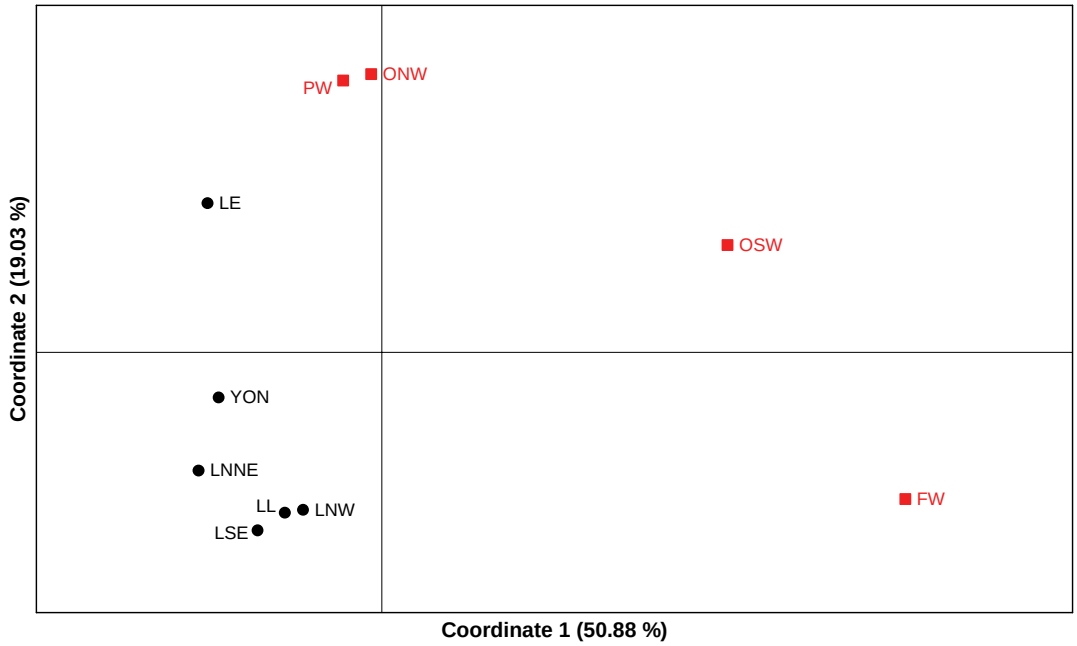


Supplementary Fig. 3.3. Principal coordinate analysis of pairwise D_{est} values (a), F_{st} values without correction (b), F_{st} values with ENA correction (c), and F_{st} values with Meirmans' correction (d) for Type β populations. The first two coordinates only represent 70.75 %, 70.38 %, 70.36 %, and 82.06 % of the variation of data for D_{est}, non-corrected F_{st}, ENA corrected F_{st}, and Meirmans-corrected F_{st}, values, respectively, and hence fail to reveal a large part of the relationships. Black dots = northern GBR, red squares = central GBR

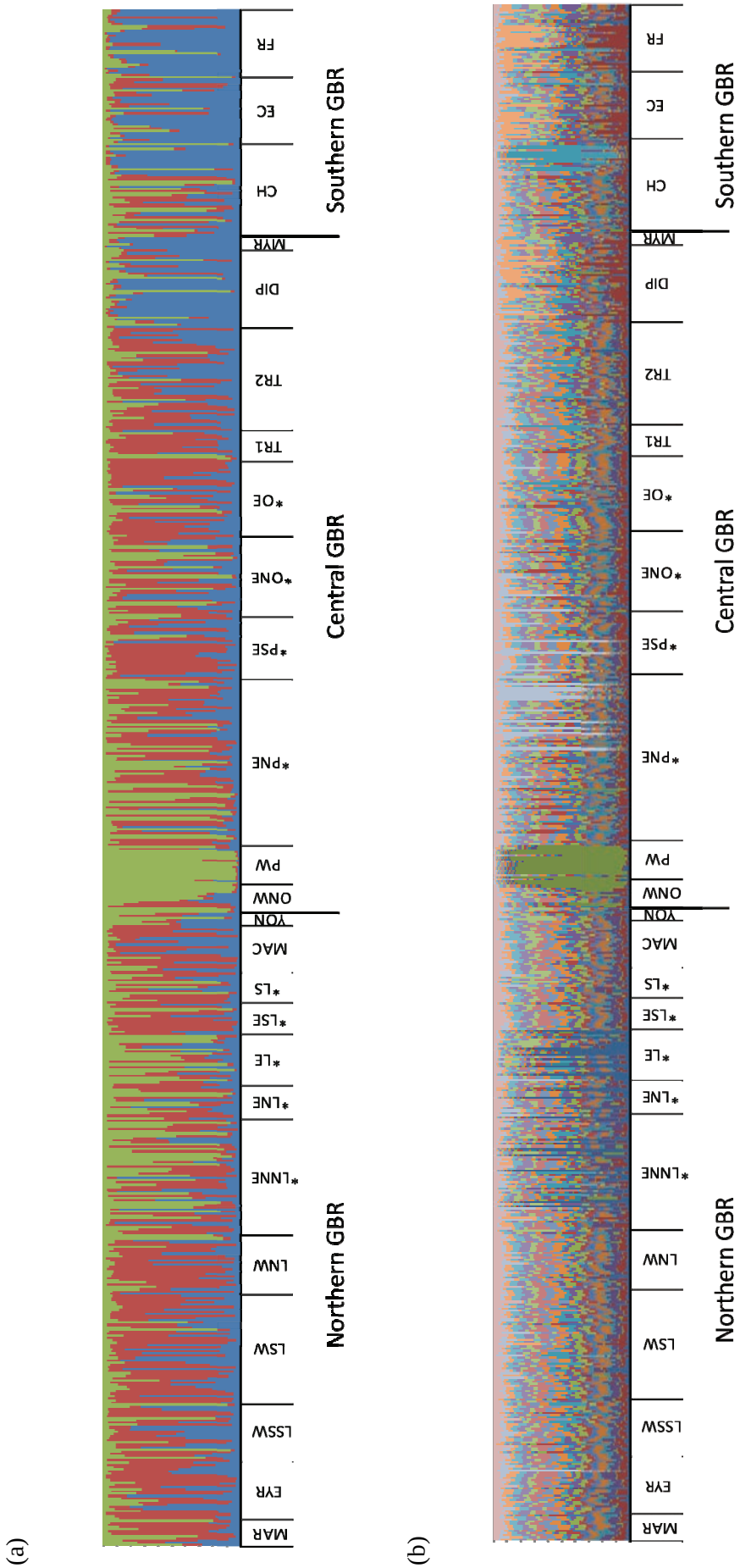
(c)



(d)

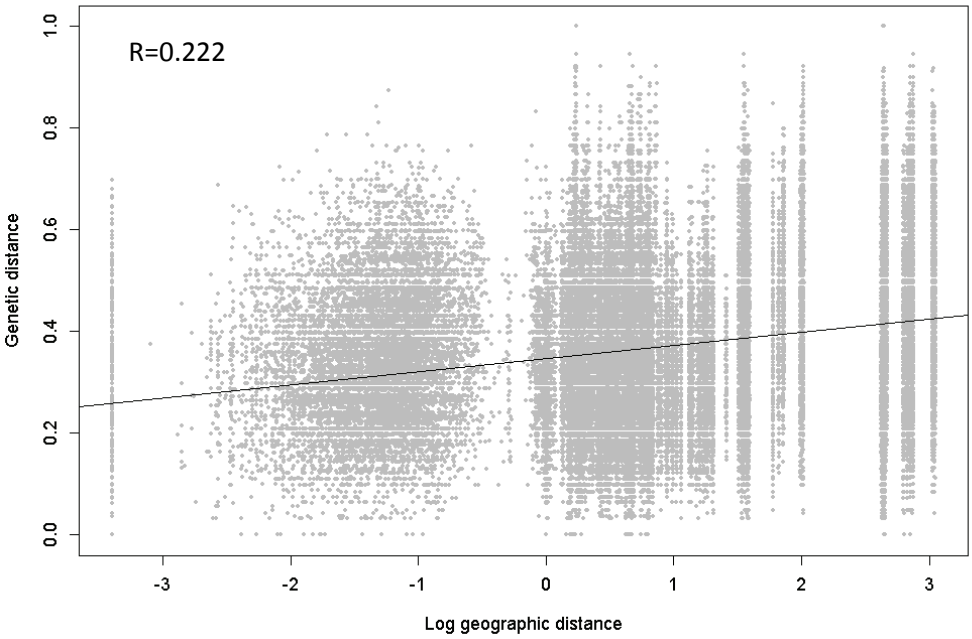


Supplementary Fig. 3.3. continued

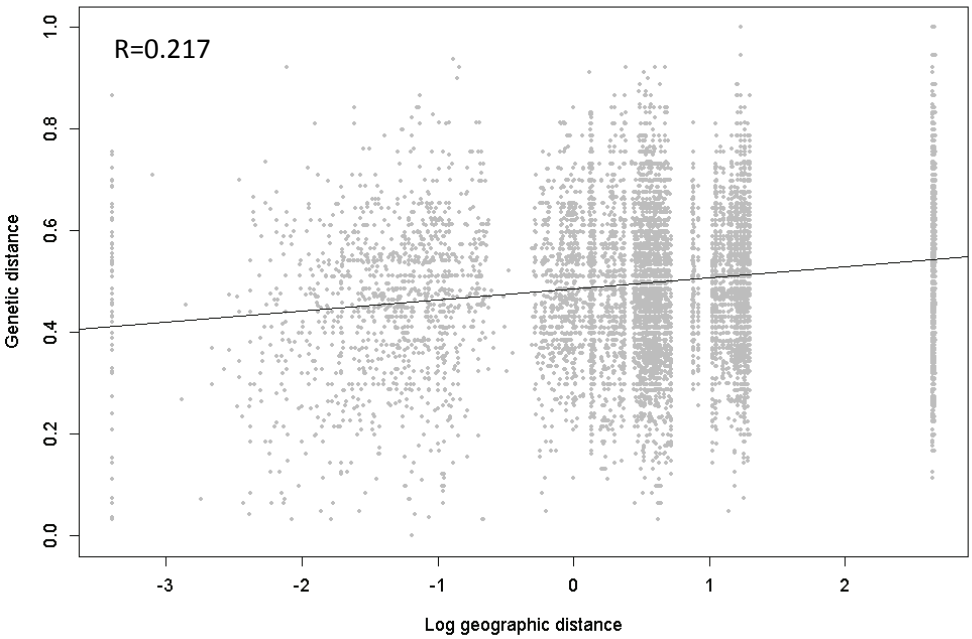


Supplementary Fig. 3.4. STRUCTURE plot of Type α individuals. Each column represents an individual, with the colours showing proportional cluster membership. a) K=3 identified by the Evanno method, b) K=20 identified by the NSp-C model in Geneland. Exposed populations are indicated with an asterisk (*).

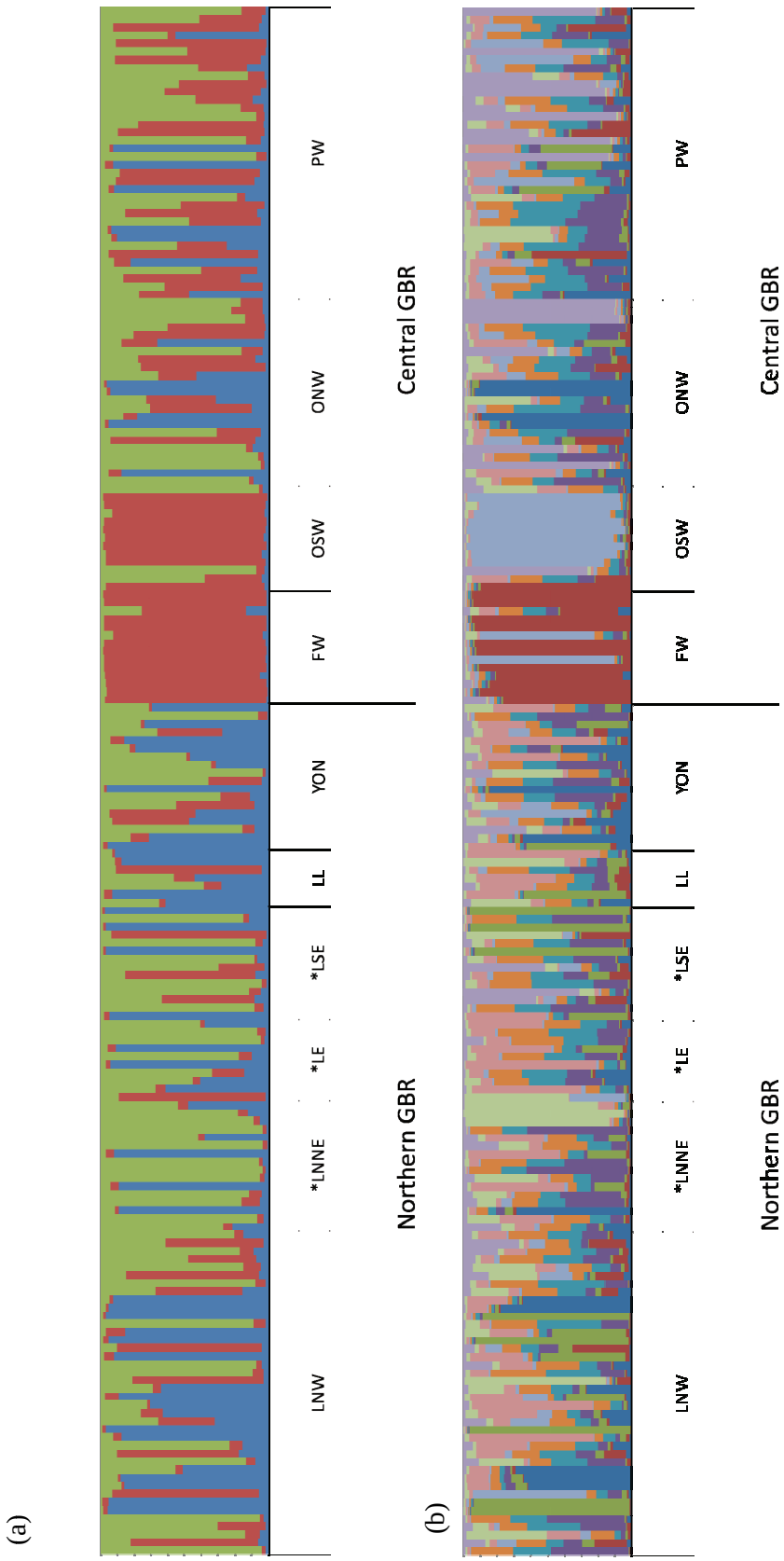
(a)



(b)



Supplementary Fig. 3.5. Significant but weak relationship between pairwise genetic distance and log transformed geographic distance in kms of Type α (a) and β (b) *P. damicornis* specimens. Large and small genetic distances can be observed at both large and small distances. The slope of the regression lines and their significance are driven by the relatively lower numbers of large within-site (i.e. approximately less than 100 m) genetic differences.



Supplementary Fig. 3.6. STRUCTURE plot of Type β individuals. Each column represents an individual, with the colours showing proportional cluster membership. a) K=3 identified by the Evanno method, b) K=10 identified by the NSp-C model in Geneland. Exposed populations are indicated with an asterisk (*).

Chapter 4

Should I stay or should I go? Long- and short-distance migration in two common lineages of *Pocillopora damicornis* on the Great Barrier Reef

Abstract

Understanding connectivity of populations of coral species among and within coral reefs over ecologically significant timescales is essential for developing evidence-based management strategies, including marine protected area design. In this chapter I present the first assessment of contemporary connectivity of populations of two lineages of the brooding coral *Pocillopora damicornis*. I used individual-based genetic assignment methods to identify philopatric and migrant specimens among newly settled recruits, sampled at regular time intervals over 12 months at sites around Lizard Island (northern GBR) and over 24 months at sites around the Palms Islands (central GBR). In both lineages I found evidence for spatially variable rates of self-recruitment as well as short- and long-distance migration, demonstrating the importance of local physical characteristics in driving dispersal processes. At the same time, differences between lineages in dispersal patterns indicate the effect of biological traits on the final outcome of larval dispersal, and, together with observed lineage-specific habitat preferences, support the recent delineation of lineages (putative cryptic species) within the *P. damicornis* morphospecies. The proportion of philopatric specimens among Type α recruits was 84% at sites around the Palm Islands, but only 48% around Lizard Island, while among Type β recruits it was 39% in both regions. Despite variation in dispersal patterns, both lineages combine self-recruitment and migration in a ratio that likely places them among the more resilient corals on the GBR. However, low survival rates of recruits during the first months following metamorphosis indicate that realised population replenishment in these coral species is likely much lower than suggested by recruit numbers.

While *P. damicornis* invests much energy into brooding clonal larvae, I found that only 15% and 7% of Type α and Type β recruits, respectively, were clones of sampled adult colonies or other recruits, further challenging the hypothesis that reproduction is predominantly asexual in this species. I explain high rates of self-recruitment and low rates of clonality in these lineages by speculating that locally retained larvae originate predominantly from spawned gametes, while brooded larvae are mainly vagabonds.

4.1. Introduction

Knowledge of population connectivity is fundamental to the design of marine protected area networks in tropical coral reef systems (Shanks et al., 2003; Salm et al., 2006; McLeod et al., 2008), but our understanding of ecological connectivity (i.e., connectivity over ecological, rather than evolutionary timescales) among coral populations is currently limited. Addressing gaps in current knowledge of coral population connectivity will increase our ability to evaluate the design and potential benefits of novel conservation and resource management strategies, and optimize resources to conserve viable metapopulations (Almany et al., 2009). The exchange of individuals among reefs contributes to maintaining high levels of genetic diversity and facilitates the spread of adaptive mutations among spatially dispersed populations of reef organisms, processes that are critical to the resilience of coral populations in the face of escalating anthropogenic threats. High levels of connectivity also play a key role in metapopulation dynamics that maintain healthy, viable populations, for example through re-stocking of depleted populations (Hanski, 1998).

The recent downfall of coral populations (e.g. De'ath et al., 2012), which constitute the cornerstone of the coral reef ecosystem, highlight the need to understand processes that drive population recovery (Hughes et al., 2011). Recovery of coral populations following major but localized mortality events may occur through re-growth of surviving tissue on remnants of local colonies (Diaz-Pulido et al., 2009), or through larval replenishment from external sources (Hughes and Tanner, 2000; Coles and Brown, 2007; Starger et al., 2010). As ocean warming and acidification escalates, and the frequency of acute disturbance events, including destructive tropical storms and crown-of-thorn starfish outbreaks increases (Webster et al., 2005; Hoegh-Guldberg et al., 2007; De'ath et al., 2012), the speed of recovery of coral populations will be the key to survival of coral metapopulations, and ultimately, of the coral reef ecosystem. It is therefore essential for science-based coral conservation schemes to understand the processes that drive recovery (Bellwood et al., 2004; Hughes et al., 2010), particularly patterns of population connectivity (Mumby and Hastings, 2008).

Patterns of connectivity among coral populations can only be assessed indirectly because the small size and body plan of coral larvae mean that it is not possible to apply

conventional tag and recapture or tracking methods (Van Oppen and Gates, 2006). Indirect techniques include dispersal modeling (Werner et al., 2007; Treml et al., 2007; Kool et al., 2011) and inferences made from population genetic and genetic parentage data (Hellberg et al., 2002; Harrison et al., 2012), or, ideally, a combination of both methods (e.g. Galindo et al., 2006; Foster et al., 2012). One major criticism of inferring connectivity from population genetic patterns of adult corals is that adult populations represent the culmination of connectivity processes over an undetermined period of time (Botsford et al., 2009). Also, sessile modular organisms like corals exhibit a number of life history traits that pose problems for traditional population genetic statistics, which are based on assumptions of random sexual reproduction and non-overlapping generations. For example, high potential for asexual reproduction in corals leading to long genotype lifespans and even "immortal" genotypes (Hughes and Jackson, 1980; Highsmith, 1982), which increase generation overlap, undermine conventional genetic statistics. Use of traditional equilibrium-based models, such as F-statistics, for descriptions of connectivity processes over ecological timescales have also been criticised because these are designed to characterize long-term genetic processes that reach a balance between opposing evolutionary forces (Manel et al., 2005). Advances in population genetic statistics now offer a wide range of tools to better assess contemporary connectivity from genetic data by focusing on individuals, rather than populations (Cornuet et al., 1999; Pritchard et al., 2000; Wilson and Rannala, 2003; Manel et al., 2005, 2007). These novel approaches have been applied in a small number of studies on reef corals (e.g. Baums et al., 2005; Underwood et al., 2007; van Oppen et al., 2008; Souter et al., 2010; Carlon and Lippé, 2011) and the results are promising for analysing genetic structures between and within populations, and identifying rates of recent admixture.

Corals have two primary reproductive modes that typically result in different dispersal capabilities of larvae and hence differ in their implications for inter-reefal population connectivity (e.g. Blanco-Martin, 2000; Underwood et al., 2009). Species with a broadcast spawning mode of reproduction release gametes into the water column for external fertilization and larval development, while brooding species have internal fertilization and develop planula larvae within coral polyps (Harrison and Wallace, 1990). Brooded larvae are capable of settling immediately after being released, but a

proportion of some broods has been shown to remain competent to settle for more than 100 days (Richmond, 1987; Harii et al., 2002), while spawned larvae have an obligatory pre-competency period of about three days (Babcock and Heyward, 1986; Harrison, 2006), followed by a competent period ranging from 23 days (Harrison et al., 1984) to 240 days (Graham et al., 2008). Accordingly, larval dispersal is typically thought to be much greater in spawning species than in brooding species, but realized dispersal does not always follow these predicted patterns (Ayre and Hughes, 2000).

Pocillopora damicornis (Linnaeus, 1758) is one of the few corals whose life history strategy encompasses both brooding and spawning reproductive modes, but generally, the implications of this mixed strategy for connectivity have not been considered. This species releases brooded larvae throughout the year, but seasonal peaks in larval release vary geographically (e.g. Harrigan, 1972; Harriott, 1983; Richmond and Jokiel, 1984; Villanueva et al., 2008). Despite indirect evidence of spawning from a number of studies (Stoddart and Black, 1985; Glynn et al., 1991; Ward, 1992), spawning has only been observed recently (Schmidt-Roach et al., 2012b). Interestingly, brooded larvae are asexually produced on the GBR (Stoddart, 1983; Ayre and Miller, 2004; Schmidt-Roach et al., 2012a) and vast numbers of clonal larvae are released in multiple planulation events each year. However, populations of *P. damicornis* are primarily sexually maintained on the GBR, a paradox that has taunted researchers for over a decade (Ayre et al., 1997; Ayre and Miller, 2004; Sherman et al., 2005).

In this study, I infer measures of larval dispersal from a genetic study of coral recruits of the common and widespread scleractinian coral *P. damicornis*. I compare individual-based population genetic statistics on genotypic data for recently settled coral recruits to those of adults in the same population to overcome the issue of cumulative and multi-generational patterns of connectivity when solely adult populations are considered. My study focuses on Great Barrier Reef (GBR) populations of two lineages of *P. damicornis*, Type α and β (Schmidt-Roach et al., 2012a). This coral recruit study complements my study of adult population genetics of *P. damicornis* Type α and β lineages on the GBR (Chapter 3), further enhancing understanding of dispersal processes in this model species. Results of this study provide new insights into the

paradox of prolific clonal larvae produced by adult populations that appear to be maintained predominately by sexual reproduction.

4.2. Materials and Methods

4.2.1. Sample collection

Coral recruits were sampled from 20 pre-conditioned, unglazed, terracotta tiles, deployed as described by Mundy (2000) at each of four sites around Lizard Island in the Northern Great Barrier Reef (GBR), and four sites around Orpheus and Pelorus Islands in the Palm Island group in the Central GBR (Fig. 4.1). Tiles were scattered haphazardly over an area approx. 100 m^2, at a depth of three to nine metres, and represented a total settlement surface area of 0.572 m^2. Tiles were recovered, sampled for coral recruits, cleaned and redeployed roughly every three months from May 2009 until May 2010 at Lizard Island sites, and every two months until May 2011 at Palm Island sites. In February 2011, a category five tropical cyclone, TC Yasi, hit the Palm Islands and destroyed the windward sites (NE Orpheus and NE Pelorus). All tiles at these exposed sites were lost and the last sampling was therefore carried out in early December 2010.

To assess survival rate of settled recruits, additional sets of 20 tiles were deployed at each of the Palm Island sites, and censused and sampled in May 2010 after roughly one year of exposure (henceforth referred to as “year-round tiles”). Each census and sampling of coral spat was done under a dissecting microscope by systematically scanning all sides and edges of each tile. Pocilloporid recruits were identified visually according to descriptions by Baird and Babcock (2000) and Babcock et al. (2003). Recruits sampled were photographed, snapped off tiles using a scalpel and stored in absolute ethanol in microcentrifuge tubes until genetic processing.

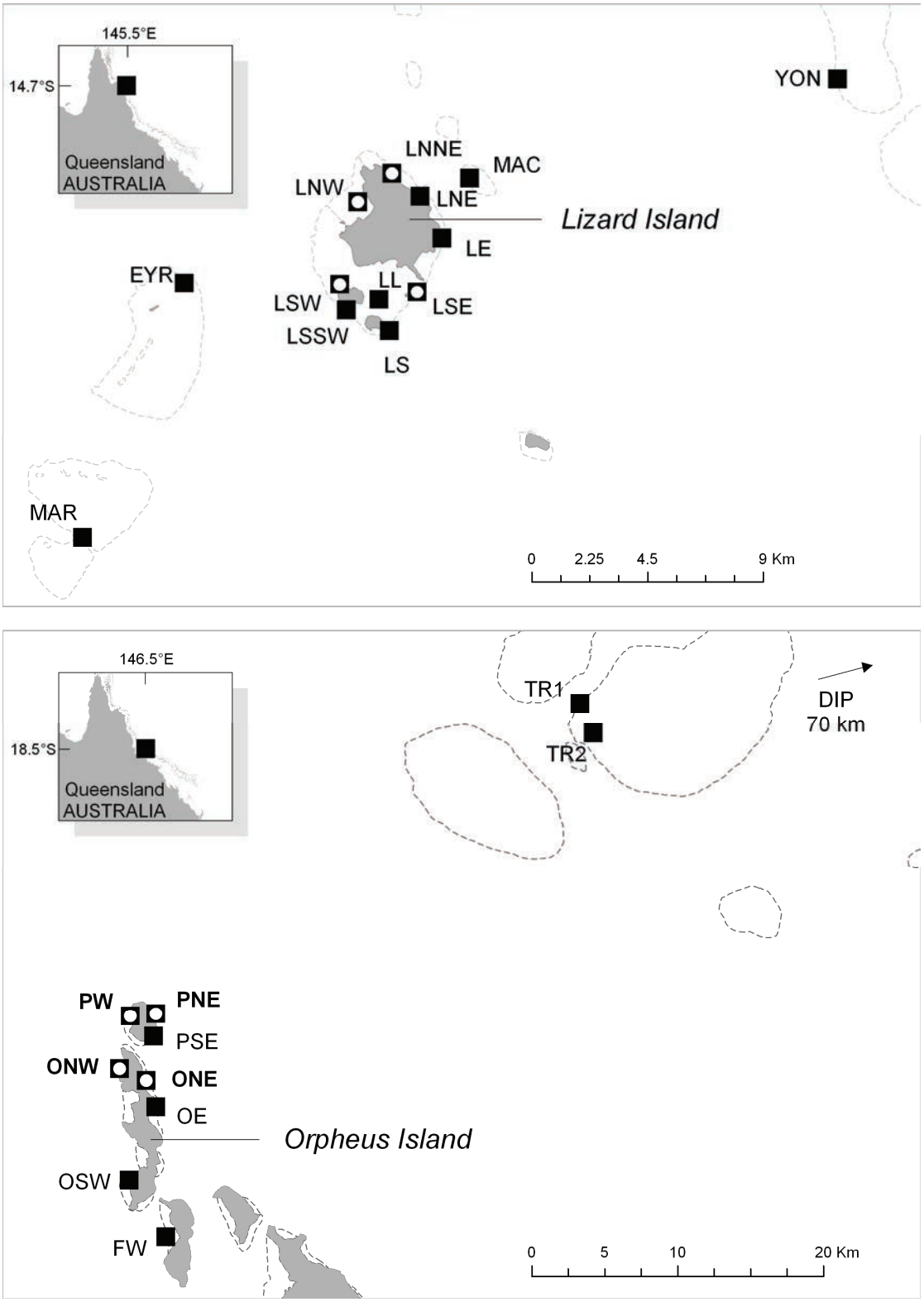


Fig. 4.1. Map showing sampling sites in the northern and central Great Barrier Reef (GBR) for the adult and juvenile population genetic studies. Black squares indicate collection sites of adult colony samples, white circles in black squares denote sites where settlement tiles have also been deployed. See Table 4.1 for site names.

I used 880 samples of adult colonies of *P. damicornis* (sensu Veron and Pichon, 1976) from 24 locations in the northern and central GBR that were used in the adult population genetic study described in Chapter 3 (Fig. 4.1; Table 4.1). The densities of adult coral colonies was estimated as described in Chapter 3. Additionally, colony density was estimated by sampling every colony in a 10 m x 10 m quadrat around the settlement tiles (henceforth "quadrat-method"), at all sites except NE Orpheus Island, which was destroyed by TC Yasi in February 2011.

4.2.2. DNA extraction and microsatellite genotyping

DNA of coral recruits was extracted following an adaptation of the protocol described by Gloor and Engels (1992). Samples were tested with the rapid genetic assay described in Chapter 1 to distinguish *P. damicornis* from other pocilloporid recruits, and to determine their respective lineage. Samples identified as *P. damicornis* Type α and β (Schmidt-Roach et al., 2012a) were genotyped at nine microsatellite markers using the multiplex groups, primers and protocols described in Chapter 1. Adult genotype data were from Chapter 3.

Table 4.1. Names and locations of sampling sites. Location of reefs indicated by I= inshore, M = midshelf, and O = outer reefs. Habitat type indicated by E = exposed windward, S = sheltered leeward, L = lagoon

		Site name	Code	WGS '84 coordinates Longitude	Latitude	Location	Habitat
Northern Region		Martin Reef	MAR	145.353	-14.777	I	S
		Eyrie Reef	EYR	145.388	-14.688	I	L
	Lizard Island sites	South-Southwest Lizard Island	LSSW	145.445	-14.697	M	S
		Southwest Lizard Island	LSW	145.443	-14.688	M	S
		Northwest Lizard Island	LNW	145.449	-14.659	M	S
		North-Northeast Lizard Island	LNNE	145.461	-14.649	M	E
		Northeast Lizard Island	LNE	145.471	-14.658	M	E
		East Lizard Island	LE	145.478	-14.672	M	E
		Southeast Lizard Island	LSE	145.470	-14.691	M	E
		Lizard Island Lagoon	LL	145.456	-14.694	M	L
		South Lizard Island	LS	145.460	-14.705	M	E
		MacGillivray Reef	MAC	145.488	-14.651	M	S
		Yonge Reef	YON	145.617	-14.616	O	S
Central Region	Palm Islands sites	West Fantome Island	FW	146.510	-18.684	I	S
		Southwest Orpheus Island	OSW	146.487	-18.649	I	S
		Northwest Orpheus Island	ONW	146.481	-18.580	I	S
		West Pelorus Island	PW	146.488	-18.547	I	S
		Northeast Pelorus Island	PNE	146.504	-18.546	I	E
		Southeast Pelorus Island	PSE	146.502	-18.560	I	E
		Northeast Orpheus Island	ONE	146.498	-18.587	I	E
		East Orpheus Island	OE	146.504	-18.603	I	E
		Trunk Reef 1	TR1	146.765	-18.354	M	S
		Trunk Reef 2	TR2	146.664	-18.125	M	L
		Dip Reef	DIP	147.457	-18.418	O	S

4.2.3. Data analyses

To analyse spatial and temporal patterns of recruitment, I normalised the number of recruits found at each site in each 2-3 month time-window to the exact number of exposure days of the settlement tiles. To assess survival rate of newly settled recruits, I compared the number and size (i.e., number of polyps) of recruits on the year-round tiles to the number and size of recruits found on regularly sampled tiles over the same time period. I compared adult population density to recruit numbers at each site by Pearson correlation.

I combined the genotype datasets of recruits and adults to assess the discriminative power of the microsatellite markers. Using the combined dataset, I calculated Genotype Probability (GP) for each locus and a combination of all loci in GENALEX 6.4 (Peakall and Smouse, 2006). Repeated multilocus genotypes (MLG) were considered to be clone mates at a GP < 0.001.

Assignment tests were carried out in GeneClass2 (Piry et al., 2004), using the adult genotypes from Chapter 3 as a reference dataset. The probability that each recruit belongs to each reference population was computed using the criteria and probability computation algorithm of Rannala and Mountain (1997), with 10,000 simulated genotypes, for Type α and β samples separately. Recruits were excluded from a reference population if the probability of assignment was < 0.1. When a recruit was not excluded from the local population, it was considered to be local, even if it was not excluded from other population(s).

To assign recruits into genetic clusters, the fully Bayesian model-based clustering method implemented in STRUCTURE v2.3.3 (Pritchard et al., 2000) was run without population information, under the admixture model and correlated allele frequencies, with a burn-in of 100,000 and another 100,000 MCMC replications after the burn-in. Five independent chains were run for each K from K = 1 to 26. To find the uppermost hierarchical level of structure, I used the Evanno-method (Evanno et al., 2005) implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). In addition to running this analysis on Type α and β recruit datasets separately, I also combined the corresponding recruit and adult datasets in order to obtain comparable cluster memberships between adult and recruit individuals.

4.3. Results

4.3.1. Recruit numbers and population densities

Type α

A total of 148 Type α recruits were identified out of the 1149 pocilloporid recruits analysed using the rapid genetic assay (Table 4.2). Thirty-one were collected at Lizard Island sites and 104 at Palm Island sites from the subset of regularly sampled tiles. The number of recruits at each site correlated strongly with the density of adult coral colonies in the immediate vicinity of the tiles, as determined using the quadrat method for assessing adult density (Fig. 4.2). In the Palm Islands, a similarly strong correlation was found between the number of recruits and the density of adult colonies in the wider neighborhood of the tiles, as determined using the tracklog-method, but only a moderate correlation was evident at Lizard Island (Figs. 4.2 and 4.3).

Type β

Ninety-five Type β recruits were identified out of the total sample of 1149 pocilloporid recruits with my assay, 51 of which were collected over 12 months at Lizard Island sites, and 41 collected over two years at Palm Island sites, from the set of regularly sampled tiles (Table 4.2). Correlations between recruit numbers and adult colony densities, as estimated from quadrats around the tiles, were strongest at Palm Island sites, and lower but still strong at Lizard Island sites. In contrast, correlations were poor (near zero) for both regions when adult densities were estimated using the tracklog-method (Figs.4.2 and 4.3).

Table 4.2. Number of recruits for two lineages (Type α and Type β) of *Pocillopora damicornis* at each of the sampling sites in the central (Palm Is.) and northern (Lizard Is.) GBR, in each sampling period.

Site	sampling period	Type α	Type β	Total
NW Pelorus	Apr-May 2009		2	2
	Jun-Jul 2009			
	Aug-Sep 2009			
	Oct-Nov 2009	2		2
	Dec-Jan 2010	1	1	2
	Feb-Mar 2010		1	1
	Apr-May 2010			
	Jun-Jul 2010	1		1
	Aug-Sep 2010			
	Oct-Nov 2010		1	1
	May-Jun 2011		1	1
	2009 - 2010			
	Total	4	6	10
NW Orpheus	Apr-May 2009	1	5	6
	Jun-Jul 2009		1	1
	Aug-Sep 2009			
	Oct-Nov 2009	2	2	4
	Dec-Jan 2010		1	1
	Feb-Mar 2010			
	Apr-May 2010	2	8	10
	Jun-Jul 2010		1	1
	Aug-Sep 2010			
	Oct-Nov 2010		3	3
	May-Jun 2011			
	2009 - 2010	1		1
	Total	6	21	27
NE Pelorus	Apr-May 2009			
	Jun-Jul 2009	38	1	39
	Aug-Sep 2009	4		4
	Oct-Nov 2009			
	Dec-Jan 2010			
	Feb-Mar 2010	2		2
	Apr-May 2010	9		9
	Jun-Jul 2010	16		16
	Aug-Sep 2010	3	2	5
	Oct-Nov 2010			
	May-Jun 2011	NA	NA	NA
	2009 - 2010	8		8
	Total	80	3	83

Table 4.2 continued

Site	sampling period	Type α	Type β	Total
NE Orpheus	Apr-May 2009		4	4
	Jun-Jul 2009	11		11
	Aug-Sep 2009	3		3
	Oct-Nov 2009		1	1
	Dec-Jan 2010	1	1	2
	Feb-Mar 2010	1	1	2
	Apr-May 2010	1	1	2
	Jun-Jul 2010	5	1	6
	Aug-Sep 2010		2	2
	Oct-Nov 2010	1		1
	May-Jun 2011	NA	NA	NA
	2009 - 2010	4	3	7
	Total	27	14	41
Palm Islands total		**117**	**44**	**161**
NW Lizard	Jun-Aug 2009			
	Sep-Nov 2009	1	1	2
	Dec-Feb 2010			
	Mar-May 2010	1	3	4
	Total	2	4	6
SW Lizard	Jun-Aug 2009	4		4
	Sep-Nov 2009		1	1
	Dec-Feb 2010			
	Mar-May 2010		2	2
	Total	4	3	7
NNE Lizard	Jun-Aug 2009	6	5	11
	Sep-Nov 2009	7	18	25
	Dec-Feb 2010	1	9	10
	Mar-May 2010	4	5	9
	Total	18	37	55
SE Lizard	Jun-Aug 2009	3	1	4
	Sep-Nov 2009			
	Dec-Feb 2010	1	6	7
	Mar-May 2010	3		3
	Total	7	7	14
Lizard Island total		**31**	**51**	**82**
Grand Total		**148**	**95**	**243**

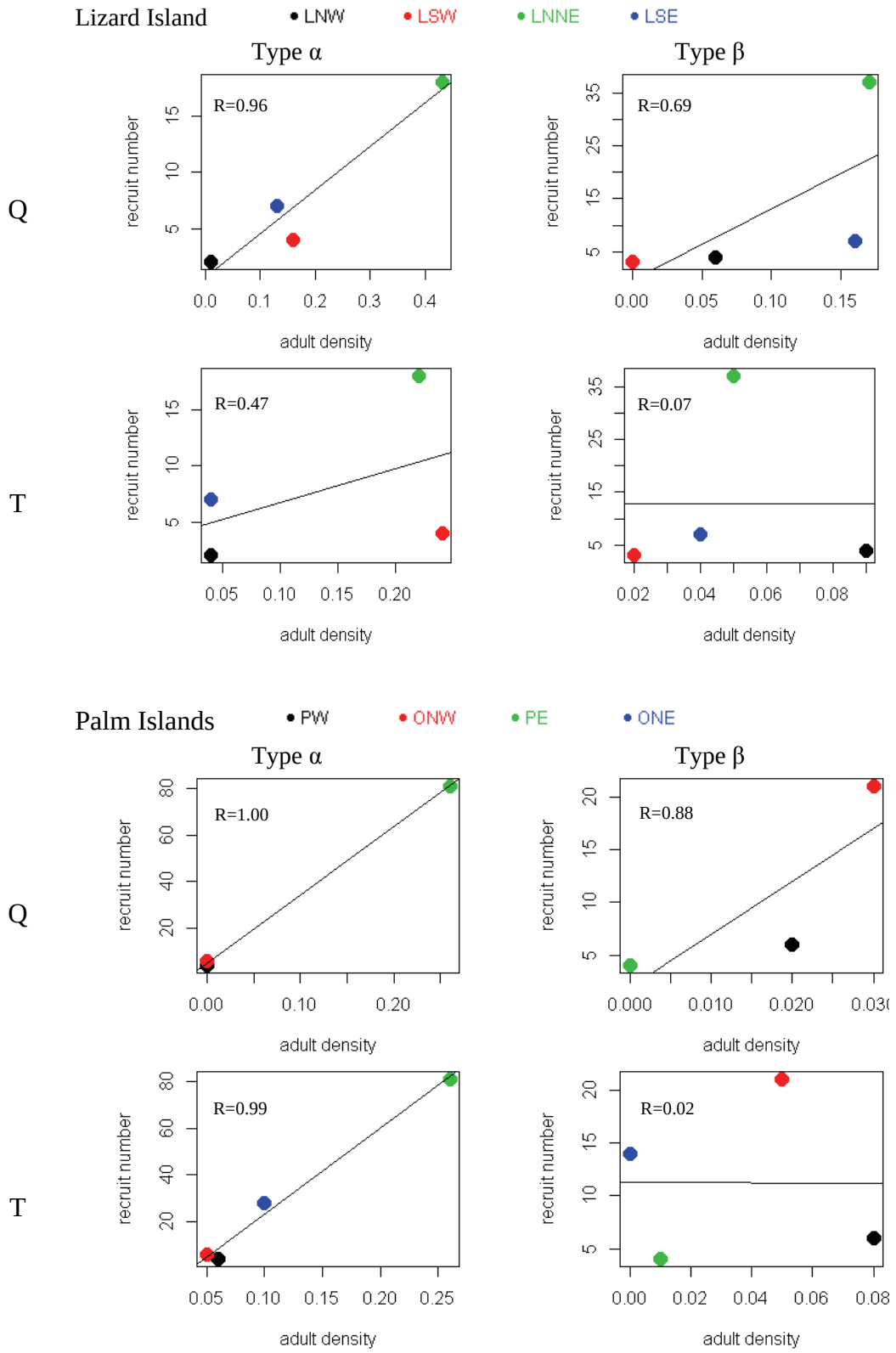


Fig. 4.2. Correlations between recruit numbers and density estimates of adult colonies of *Pocillopora damicornis* Type α and β at Lizard Island and the Palm Islands. The method used to estimate population densities is denoted as either Q for the quadrat-method or T for the tracklog-method (see Methods). Site names are abbreviated as per Table 4.1.

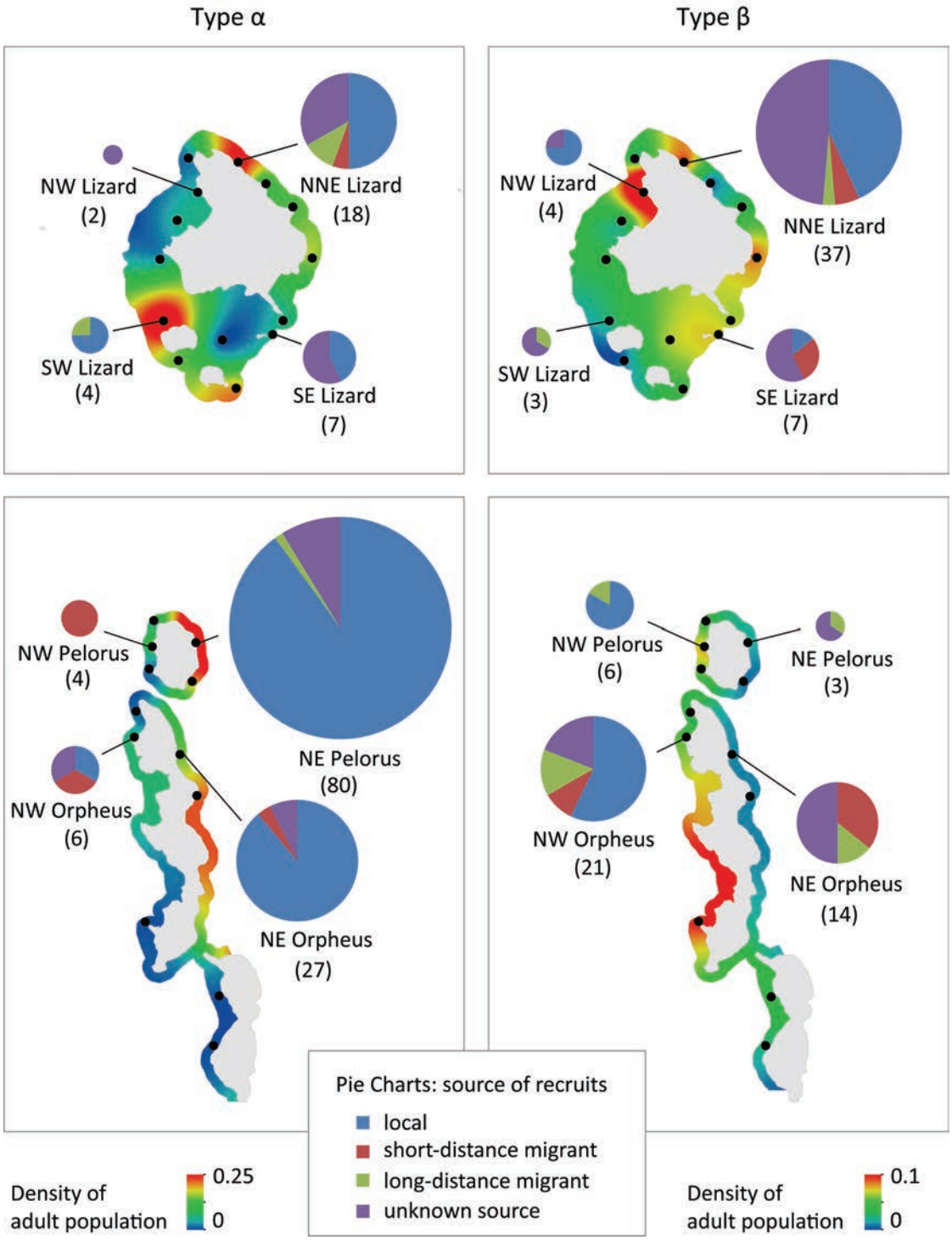


Fig. 4.3. Number and source of Type α and β recruits of *Pocillopora damicornis* (pie diagrams) in relation to population density maps around Lizard Island and the Palm Islands. Colony density was estimated by dividing the number of colonies recorded at each site (denoted by black dots) by the length of the collectors' tracklog (see Methods), and interpolated between sampling sites using the "spline with barrier" method in ArcGIS 10 (ESRI). Pie charts show the proportion of recruits from four source categories. Sizes of the pie charts are proportional to the numbers of recruits sampled at the corresponding site throughout the study period (also shown in brackets below the site names).

4.3.2. Survival of recruits

Thirteen Type α recruits were found on year-round tiles (four at NE Orpheus, one at NW Orpheus and eight at NE Pelorus) and three Type β recruits (all at NE Orpheus). The number of polyps per recruit ranged from one to 25 (mean 6.8 ± 7.2 SD) for Type α and one to six (mean 4.0±2.0 SD) for Type β specimens (Fig. 4.4). In comparison, the maximum number of polyps recorded for recruits from regularly sampled tiles in the Palm Islands was ten, therefore I assumed that recruits from year-round tiles with less than ten polyps had settled in the last two months of exposure. Only three Type α, and none of the Type β recruits from permanently sampled tiles had more than ten polyps (Fig. 4.4). Seventy-eight Type α and 30 Type β recruits were collected on regularly monitored tiles during the same 12 months (Table 4.2), suggesting ~ 4 % survival of Type α recruits in the first two months after settlement around the Palm Islands. The estimated survival of Type β recruits in the first two months is less than 3%.

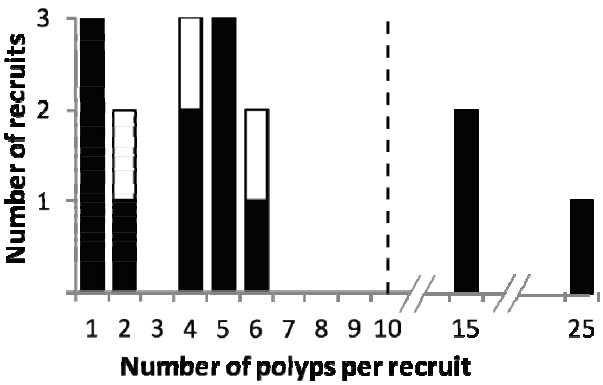


Fig. 4.4. Size distribution of Type α (black bars) and β (white bars) recruits on year-round tiles, measured by the number of polyps per recruit (on x axis). Dashed line indicates maximum number of recruits observed on regularly sampled tiles at Palm Islands (max 2 mo old recruits).

4.3.3. Clones

Type α

A total of 23 MLGs were repeated in the Type α recruit dataset (Table 4.3a), with Genotype Probability (GP) values ranging between 2.1E-06 – 1.5E-04. Overall, approximately 15% of recruits had at least one clone mate. Out of these, a clone pair and a clone sextet had no known adult counterparts, while 15 recruits were genetically identical to one of the adult colonies sampled. One recruit sampled at NW Pelorus was the clone of an adult from NE Orpheus, 13 recruits from NE Pelorus were clones of three adult colonies at the same site plus one colony at SE Pelorus, and one recruit from

NE Orpheus had two adult clone mates at the same site. These clonal larvae settled on the tiles at a variety of time-points throughout the study. The first specimen of a thirteen-clone cluster was sampled in June - July 2009 and the last one in August - September 2010, with clone mates showing up at almost all sampling events in between. Similarly, the clone sextet was sampled several times over the course of a year. Two of the 23 clones were found on year-round tiles (Table 4.3a) and were in an advanced stage of development (15 and 25 polyps, respectively).

Type β

Seven MLGs were repeated in the Type β recruit dataset, with GP values ranging from 2.1E-10 to 7.7E-06, representing roughly 7% of recruits (Table 4.3b). One clone pair with an unknown adult counterpart was sampled at each of the NE Orpheus, NNE Lizard Island and SE Lizard Island sites. Each of these recruits settled within the same sampling period as its clone mate (Table 4.3). One clonal offspring of a NW Pelorus colony settled at NW Orpheus (Table 4.3b).

Table 4.3. Settlement sites and times of clonal recruits of *Pocillopora damicornis* Type α (a) and β (b) lineages. Euclidean distances from their adult clone mates are shown in meters.

(a)

Site	Settlement time	No. of recruits	Location of adult clones	Distance (m)
NW Pelorus	2010 Jun-Jul	1	NE Orpheus	4433
NE Pelorus	2009 Jun-Jul	1	-	-
	2010 Apr-May	1	-	-
NE Pelorus	2009 Jun-Jul	1	-	-
	2010 Apr-May	2	-	-
	2010 Jun-Jul	3	-	-
NE Pelorus	2009 Jun-Jul	6		
	2009 Aug-Sep	1	NE Pelorus	16
	2010 Apr-May	3	NE Pelorus	56
	2010 Jun-Jul	1	NE Pelorus	98
	2010 Aug-Sep	1	SE Pelorus	1551
	2009 - 2010	1		
NE Orpheus	2009 - 2010	1	NE Orpheus	29
			NE Orpheus	148

(b)

Site	Settlement time	No. of recruits	Location of adult clones	Distance
NW Orpheus	2010 Apr-May	1	NW Pelorus	3720
NE Orpheus	2010 Aug-Sep	2	-	-
NNE Lizard	2009 Sep-Nov	2	-	-
SE Lizard	2010 Dec-Feb	2	-	-

4.3.4. Exclusions, assignments and clustering

Most recruits were assigned to several reference populations, with high likelihood of membership (Fig. 4.5). However, 113 Type α (76%) and 37 Type β (39%) recruits were not excluded from the population where the settlement tiles, to which they recruited, had been placed (Fig. 4.5, Table 4.4). The proportion of philopatric (i.e., self-recruiting) specimens among Type α recruits was 84% at sites around the Palm Islands, but only 48% around Lizard Island, while among Type β recruits it was 39% in both regions (Fig. 4.3, Table 4.4). Because I did not find sufficient Type β colonies around the tiles at NE Pelorus, NE Orpheus and SW Lizard Island to make reliable reference populations for assignment tests, self-recruiters were not determined at these sites. It cannot be ruled out, however, that the few colonies present at these sites were responsible for some of the recruitment. Potential migrants from neighboring sites (or short-distance migrants) were defined as recruits that were excluded from the immediately surrounding adult population, but were assigned to another adjacent reference population within 10 km. Eight such recruits were found for Type α and eleven for Type β and classified as short distance migrants (Table 4.4). Recruits that did not belong to any of the above categories, i.e. those that were excluded from all reference populations within 10 km of the collection site, but were assigned to distant populations with at least 0.1 probability, were categorized as long-distance migrants. Four Type α and nine Type β long-distance migrants were found (Table 4.4). Twenty-three Type α, and 38 Type β recruits were excluded from all known populations. These recruits are more likely to be long-distance migrants than either local recruits or short distance migrants, because of the extensive nested sampling design that was completed centered around the tiles. No-assignment cases predominantly occurred at Lizard Island sites, for both lineages.

Recruits or groups of recruits that had one or more known adult clone mates were never excluded from reference populations containing the corresponding adult clones based on GeneClass analyses. Reference populations that were characterized as genetically highly divergent (Chapter 3), such as Type α at the PW site, and Type β at the FW and OSW sites, had none or very few recruits assigned to them above the 0.1 probability threshold.

(a)

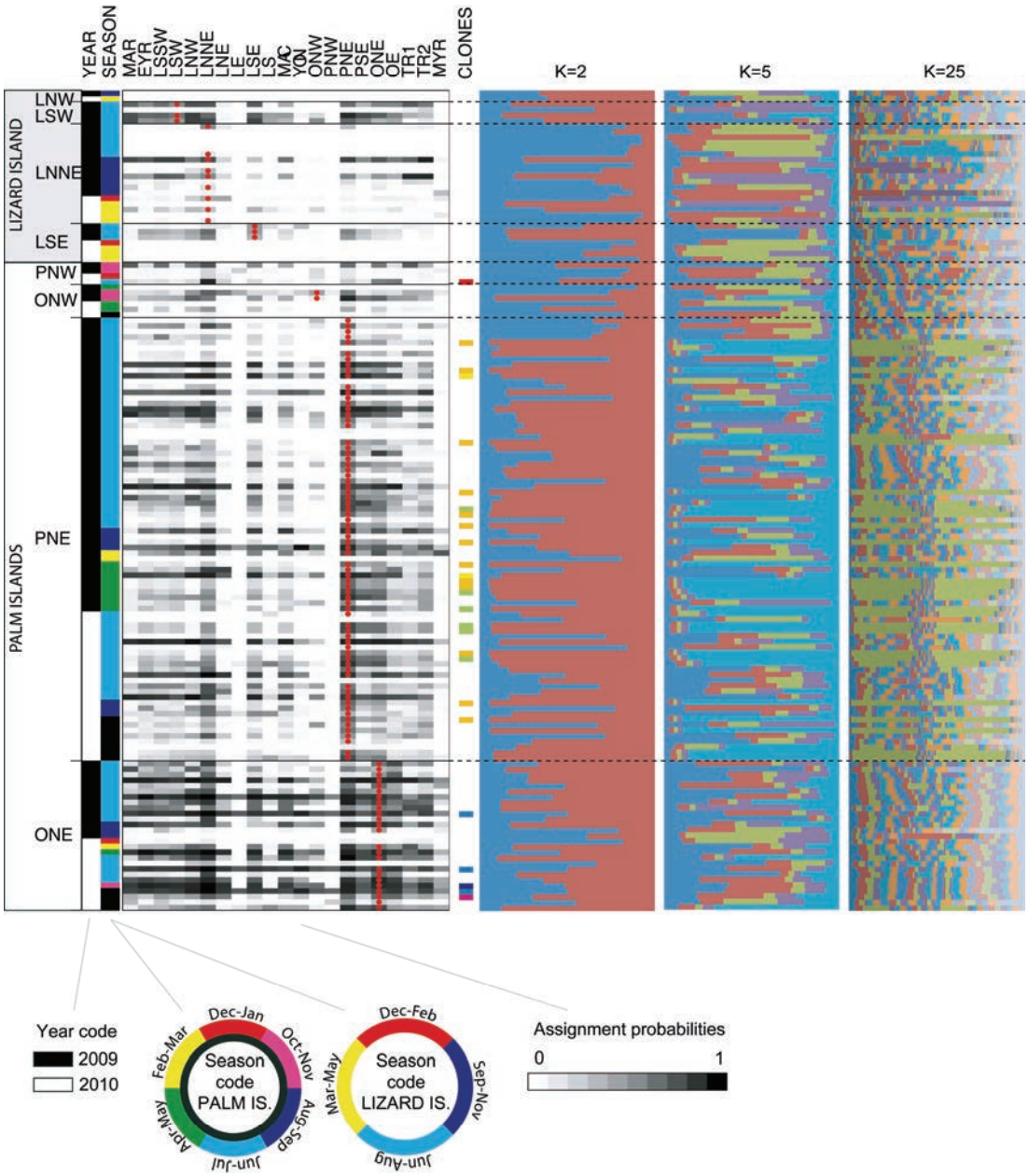


Fig. 4.5. Probability of assignment and proportional membership in *a posteriori* genetic clusters of recruits of *Pocillopora damicornis* Type α (a) and Type β (b) lineages. The gray scale matrix shows the assignment probabilities of each recruit (in rows) to each adult reference population (in columns, names as per Table 4.1). Non-exclusion from the surrounding reference population is indicated with a red dot. The annotation and color codes to the left-hand side show the site, the year and the period of collection. Identical MLGs are color coded in the "Clones" column. To the right-hand side, proportional cluster membership in *a posteriori* genetic clusters of recruits is shown on a barplot (STRUCTURE plot), at K=2, 5 and 25 for Type α, and K=2, 10 and 23 for Type β recruits.

(b)

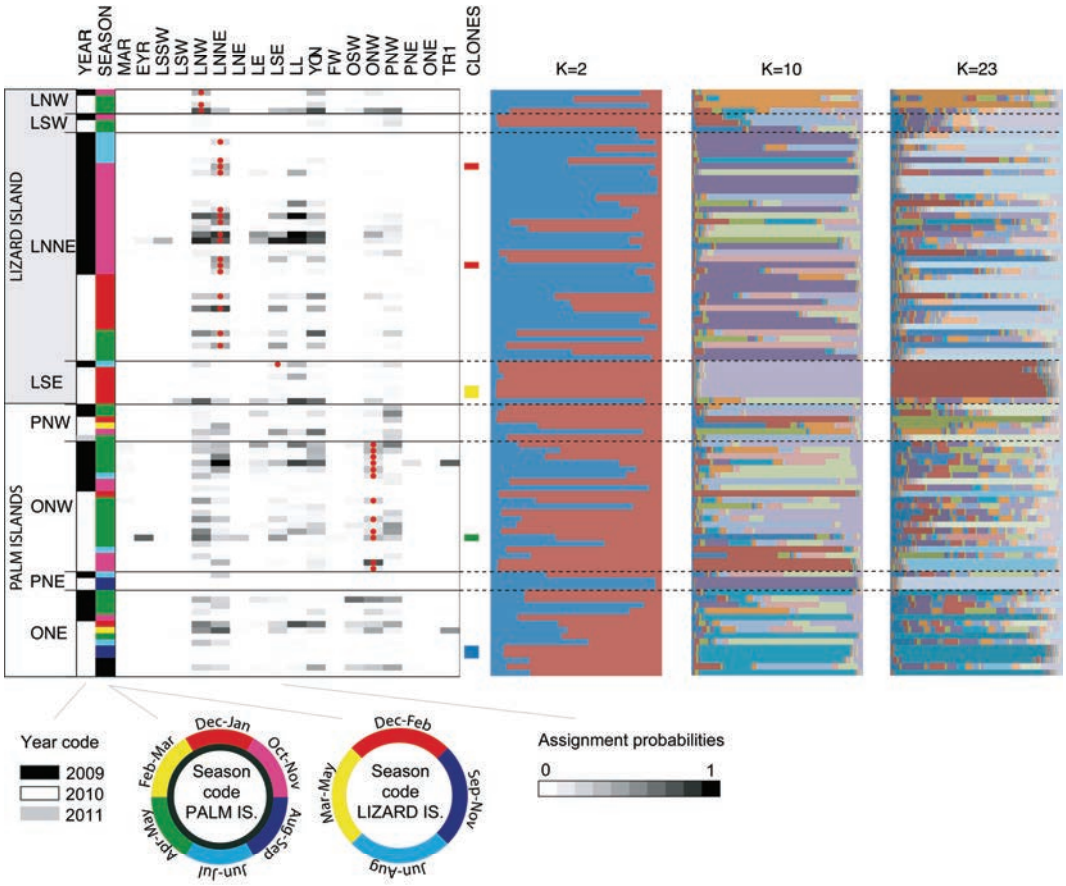


Fig. 4.5. continued

The Evanno-method gave two as the uppermost hierarchical level of structure for recruits of both lineages. However, I also explored genetic patterns at higher numbers of clusters, corresponding to the plateau of the L(K) curve (K=5 for Type α and K=10 for Type β) and at the second highest peak of the ΔK plot (K = 25 for Type α and K = 23 for Type β) (Fig. 4.5).

Clustering at K=2 did not delineate geographically or temporally homogeneous groups of Type α recruits (Fig. 4.5a). With five genetic clusters, some recruits at NE Pelorus were clearly distinct from others (shown by predominant turquoise blue cluster membership on Fig. 4.5). At K=25, the majority of Type α recruits were made up of a balanced proportion of all genetic clusters (Fig. 4.5a). There were, however, outliers that predominantly belonged to one or two genetic clusters, most commonly recruits at the NE Pelorus site. Most of these outliers were not excluded genetically from the

surrounding adult population, and were either clone mates of known adult colonies, or were genetically linked to a handful of adult colonies in the proximity (Figs. 4.5 and 4.6). Genetic outliers at Lizard Island, on the other hand, were mostly excluded from all known reference populations.

Genetic diversity was proportionally higher among Type β than Type α recruits. Similarly to Type α, analysis at K=2 did not yield a spatially or temporally meaningful grouping. At K=10, most recruits belonged predominantly to one genetic cluster and the genetic composition of recruit cohorts at most sites was very complex. Exceptions were at the LSE site, where all seven recruits belonged predominantly to the same genetic cluster, and the PNE site, where low sample number contributed to the low genetic diversity of recruits (Fig. 4.5). Some of the recruits that were excluded from all known reference populations showed high similarity in their proportional genetic cluster membership to one or a few adult individuals in the nearest adult population (Figs. 4.5 and 4.6; e.g. in NNE Lizard the "light blue" or in NW Orpheus the "turquoise" cluster dominated recruits). Self-recruiters could not be determined at SW Lizard, NE Orpheus and NE Pelorus because of low adult sample sizes and hence absence of reference populations around the settlement tiles.

Table 4.4. Potential origin of recruits based on assignment / exclusion test results. Exclusion threshold from a population was set to 0.1 assignment probability. Recruits not excluded from the local populations are termed "local". "Short-distance migrants" are recruits that were excluded from the local adult population, but were assigned to another adjacent reference population within 10 km. Recruits that were excluded from all reference populations within 10 km of the collection site, but were assigned to distant populations are called "long-distance migrants". Recruits excluded from all known populations are termed "unknown source".

	Local		**Short-distance migrant**		**Long-distance migrant**		**Unknown source**	
	Type A	**Type B**	**Type A**	**Type B**	**Type A**	**Type B**	**Type A**	**Type B**
NW Pelorus		5	4			1		
NW Orpheus	2	12	2	2		3	2	4
NE Pelorus	72				1	1	7	2
NE Orpheus	24		1	5		2	2	7
NW Lizard		3					2	1
SW Lizard	3				1	1		2
NNE Lizard	9	16	1	2	2	1	6	18
SE Lizard	3	1		2			4	4
Total	**113**	**37**	**8**	**11**	**4**	**9**	**23**	**38**

Type α K = 25

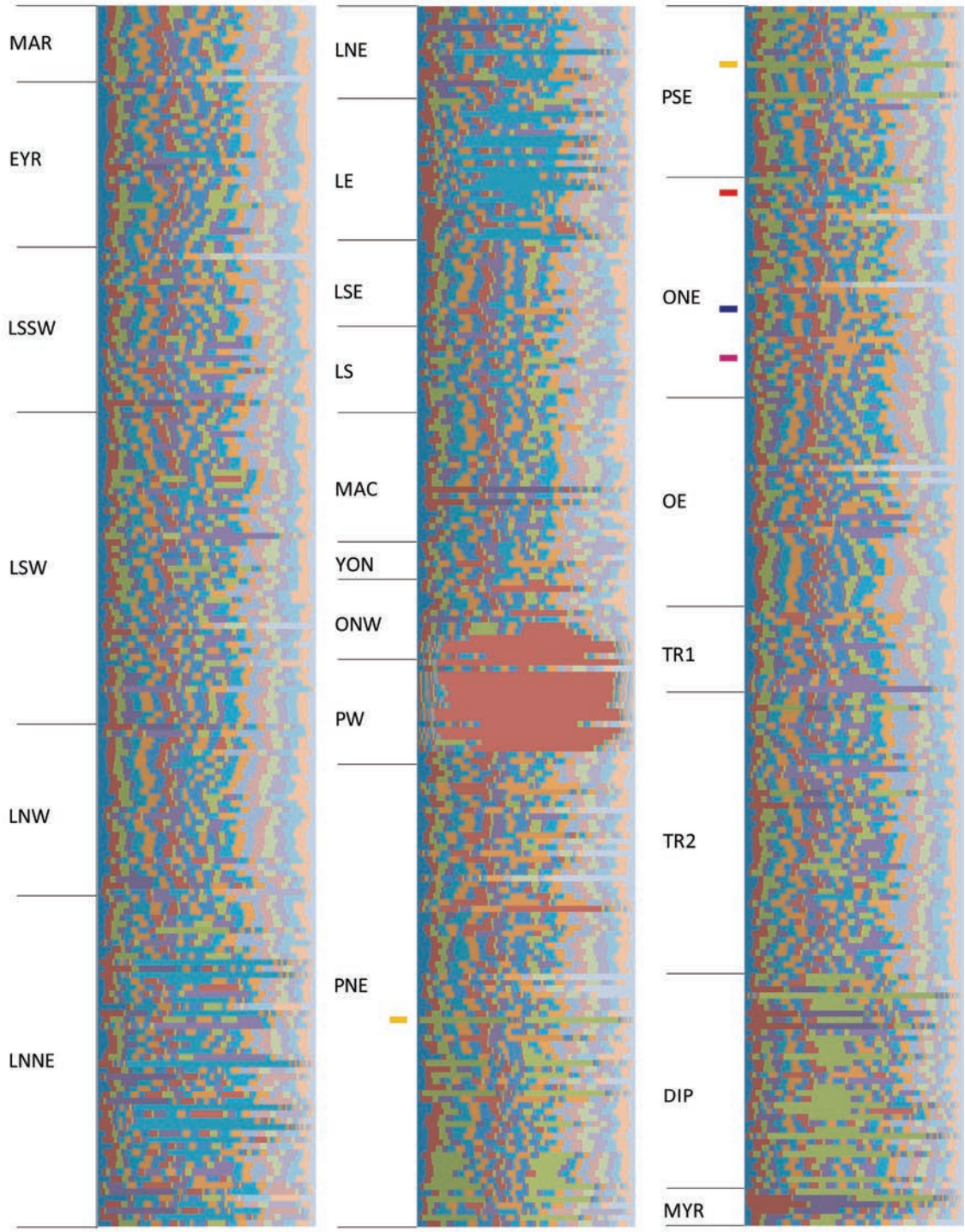


Fig. 4.6. Proportional membership in *a posteriori* genetic clusters of adult (reference) samples. Genetic clusters correspond to those in Fig. 4.5. Adult clone mates of recruits are indicated by colored rectangles to the left of each column and color coded as per Fig. 4.5. Population names as per Table 4.1.

Type β K = 23

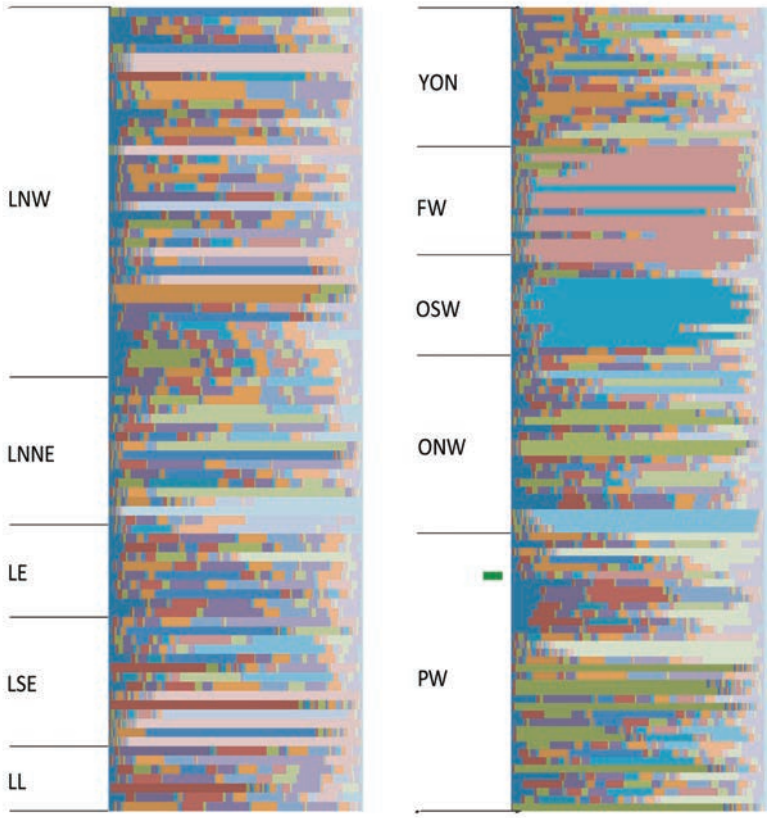


Fig. 4.6. continued

4.3.5. Spatio-temporal patterns of recruitment

Type α

The distribution of Type α recruits was unbalanced in space and time (Fig. 4.7). Average recruitment rate, calculated from sample numbers on regularly censused tiles before TC Yasi, was four and eight recruits per day per 100 m^2 at Lizard Island and the Palm Islands, respectively. With the exception of SE Lizard Island, windward sites in general received an order of magnitude more recruits than leeward sites (Fig. 4.3, Table 4.2). At the windward sites of the Palm Islands, recruitment clearly peaked in June – July in both 2009 and 2010 (Fig. 4.7). These peaks were mainly caused by locally produced larvae, but a few short- and long-distance migrants were also detected. Much smaller and less regular peaks were observed at the leeward sites; in 2009, the peak was in October – November at both Pelorus and Orpheus sites, but in 2010, recruitment was highest in June – July and April – May at NW Pelorus and Orpheus sites, respectively.

None of the recruits at NW Pelorus were assigned to the local adult population, but are thought to have originated from neighboring populations. There were no recruits on the tiles after TC Yasi (Fig. 4.7).

At Lizard Island, Type α recruitment was much lower overall (Fig. 4.3, Table 4.2), and timing of the reproductive peak was not consistent among sites (Fig. 4.7). On average, minima in the number of recruits occurred during the December – February period (Fig. 4.7). Local larvae were found throughout the year at NNE Lizard Island, but only in June-August at two other sites. In contrast, no locally produced larvae recruited to the NW Lizard Island site. Recruits from unknown source were detected in all seasons, except December – February (Fig. 4.7).

Type β

On average, Lizard Island received more than two times as many Type β recruits as the Palm Islands (seven and three recruits per day per 100 m^2, respectively). There were no clear windward-leeward patterns in the abundance of Type β recruits, either at Lizard Island or at Palm Island sites, despite the higher density of the adult population on the sheltered side of the Palm Islands (Figs. 4.3 and 4.7). However, recruitment was not evenly distributed among sites; NW Orpheus received more than 45% of recruits in the Palm Island region, and NNE Lizard Island more than 70% of recruits in the Lizard Island region.

Consistent seasonal peaks in recruit numbers were not observed. Locally produced larvae were detected throughout the year at least at one of the Lizard Island sites, and in all sampling periods except August-September and February-March at least at one of the Palm Islands sites (Fig. 4.7). The low population density (Fig. 4.3) and hence small sample sizes for adult populations at the SW Lizard Island site, as well as at the NE Pelorus and NE Orpheus sites, precluded genetic assignments to these reference populations, therefore self-recruiting could not be determined at these sites. Consequently, at these sites, some recruits that were excluded from all known reference populations could have been locally produced. Recruitment did not cease completely at NW Pelorus following TC Yasi.

(a)

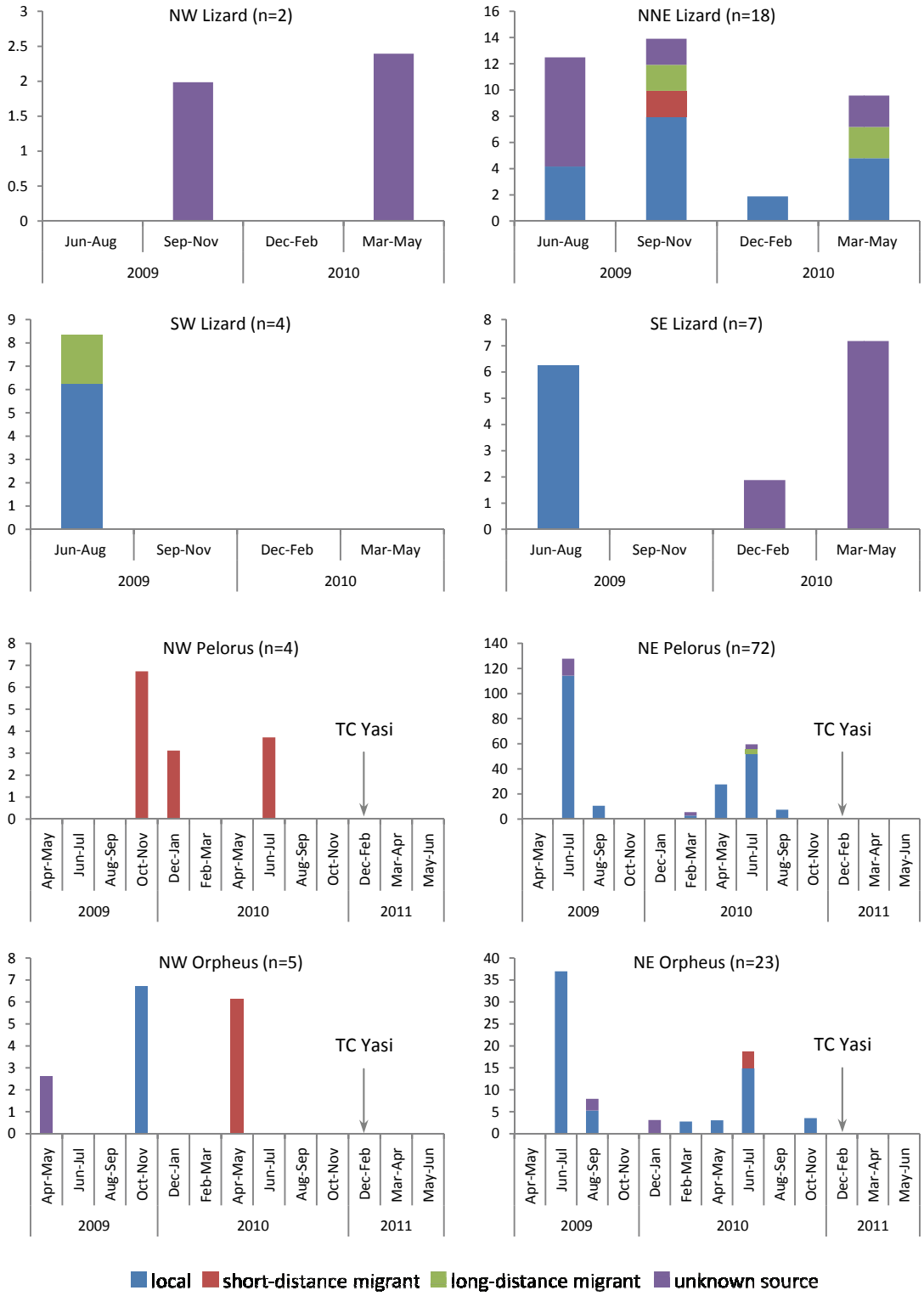


Fig. 4.7. Stacked bar charts comparing recruitment rates of *Pocillopora damicornis* Type α (a) and β (b) lineages at leeward (left charts) and windward (right charts) sites through time. For each site, the y axis represents the normalized number of recruits per day per 100 $m^{2,}$ while n in brackets behind each site name shows the actual number of recruits collected during the sampling period. Dispersal classes were identified by genetic assignment tests (see Methods). Self-recruiters could not be identified among Type β recruits at NE Pelorus, NE Orpheus and SW Lizard Island because of insufficient numbers of adult colonies for assignment tests.

(b)

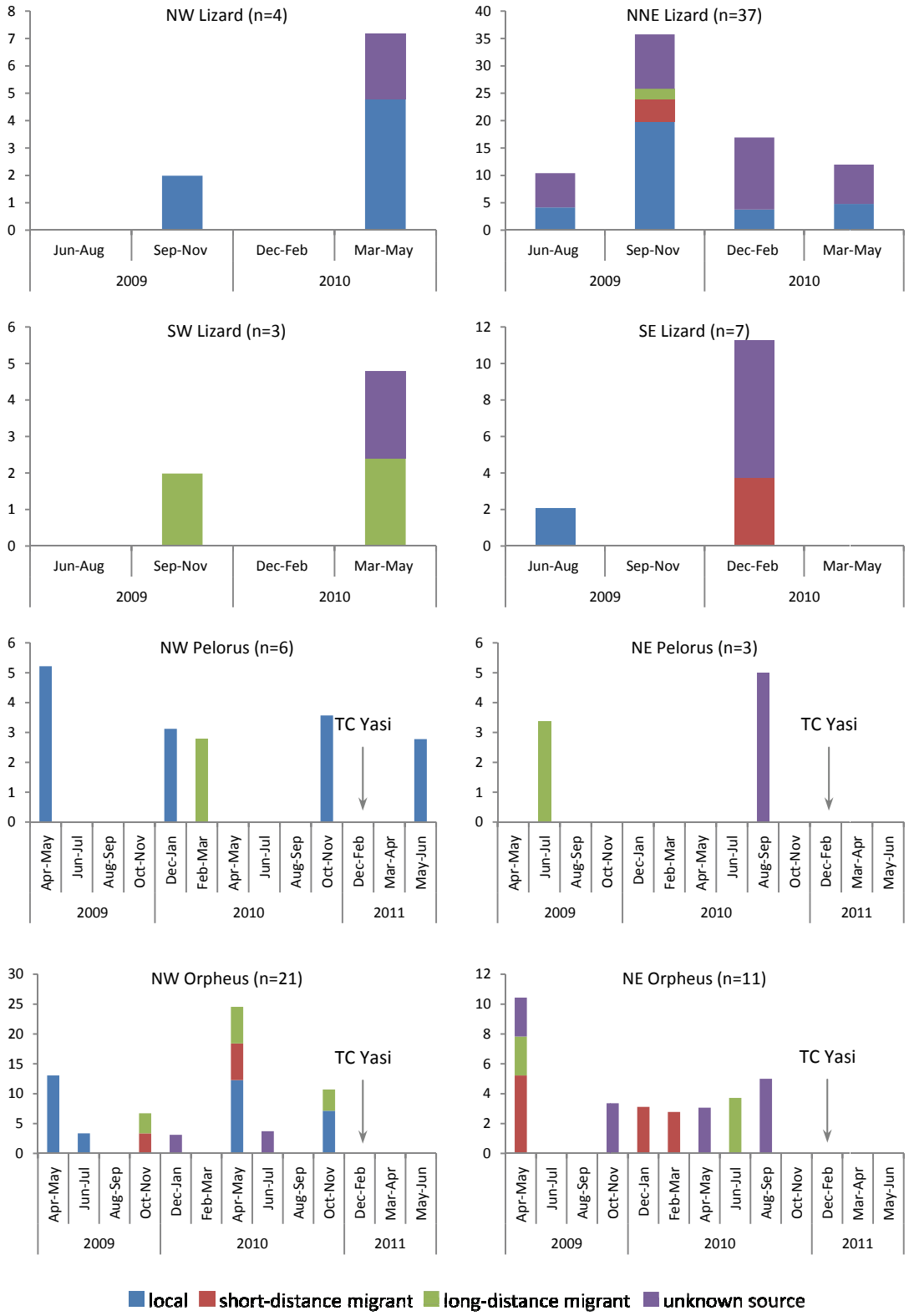


Fig. 4.7. continued

4.4. Discussion

4.4.1. Variability in recruitment patterns

I found clear differences in recruitment intensity and patterns of self-seeding versus short- and long-distance larval dispersal between two common lineages of *Pocillopora damicornis* on the Great Barrier Reef (GBR) (see Schmidt-Roach et al., 2012a for descriptions of lineages). Type α recruits were predominantly philopatric (locally produced), while 61% of Type β recruits were migrants with a known putative source population or genetically excluded from all known populations (assumed migrants). This pattern is in agreement with findings of Richmond and Jokiel (1984), that the brooded planulae of the Hawaiian *P. damicornis* Type B, which is assumed to correspond to Type β on the GBR (Schmidt-Roach et al., 2012a), usually do not settle immediately upon release. The observed high number of migrants also explains the two-fold higher recruitment rate of the Type β lineage compared to that of the Type α lineage at Lizard Island, despite lower density of Type β colonies in this region. Conversely, the recruitment rate of the Type α lineage was two-fold higher than that of Type β at the Palm Islands (central GBR), and this was mainly driven by strong self-seeding of Type α populations on the exposed sides of the islands. This demonstrates that recruitment patterns do not only differ between lineages, but also show regional and habitat-specific variation within lineages.

Large proportions of long-distance migrants and of recruits from unknown source populations at Lizard Island are in contrast with patterns of strong larval retention at Palm Island sites, and correspond to differences in prevailing water circulation patterns between these two regions. A branch of the South-Equatorial Current is likely to deliver recruits to Lizard Island sites from regions beyond the GBR, particularly from Papua New Guinea (PNG; Chapter 3), while the Palm Islands are known to have complex tidal circulation features (King and Wolanski, 1996; Luick et al., 2007; Steinberg, 2007) that may be responsible for high larval retention and/or recruitment stochasticity (Van Oppen et al., 2011b). My conclusion that rates of larval dispersal are higher around Lizard Island than the Palm Islands is also in accordance with the observed timing of planula release in previous studies; at Lizard Island, larvae are released on the spring tides (Harriott, 1983), while in the central GBR planulation occurs at either quarter or

three-quarter moon (Muir, 1984), which corresponds to neap tides. However, it must be noted that since my estimates of self-recruitment were based on genetic exclusion tests, it is possible that some long-distance migrants remained undetected due to high genetic similarity of adult populations along the GBR. Such undetected long-distance migrants are likely to be more frequent in the central GBR, where the dominant East Australia Current arrives after sweeping southward along the length of the northern GBR, whereas at Lizard Island, particle dispersal modeling suggests that the dominant source of long-distance migrants is likely from sites beyond the GBR (Chapter 3). At the same time, in Chapter 3 I have also shown that strong genetic breaks may exist over small spatial scales in these *P. damicornis* lineages, therefore I cannot rule out the possibility that some recruits that were excluded from all known source populations originated from unsampled near-by shoals and reef patches. Furthermore, some rare adult genotypes may be under-represented in the reference populations, as suggested by some level of mismatch between results of assignment tests and Bayesian clustering analysis (Figs. 4.5 and 4.6). Therefore it is possible that recruits excluded from a population (or all known populations) in fact originate from a handful of local genetic outlier colonies.

Patterns of dispersal in recruits of Type α and β *P. damicornis* correlate well to the proportion of putative first generation migrants identified in their adult populations (12% for Type α and 27% for Type β, Chapter 3). While the trend is similar between adults and recruits, the magnitudes differ by a factor of ~1.5, suggesting higher post-settlement mortality among migrants than local recruits. This may be due to decreased energy reserves and overall fitness of migrants at the point of settlement as they approach senescence (Graham et al., 2008), which may have a critical impact on the first and most vulnerable phase of colony development of the metamorphosed recruit (Hunt and Scheibling, 1997). In addition, migrants may be maladapted to the environmental conditions at the site of recruitment and therefore suffer higher mortality.

4.4.2. Temporal stochasticity in recruitment

Inconsistent patterns of temporal variability in recruitment between sites and years may reflect seasonal patterns in reproduction (e.g. Harriott, 1983; Tanner, 1996) that are modified or masked by stochasticity in recruitment success (Wallace, 1985; Connell et

al., 1997; Hughes et al., 2000; Smith et al., 2005; Adjeroud et al., 2007), and variability in PLD before settlement. Seasonal patterns of recruitment of both Type α and β lineages were found to be irregular at Lizard Island sites, in contrast to previously reported strong reproductive seasonality of *P. damicornis* from the same location (Harriott, 1983). The resolution of this apparent paradox may be that despite higher planulation rates in winter months, slower larval metabolism and development rates in winter, driven by colder water temperatures (Hoegh-Guldberg and Pearse, 1995; Nozawa and Harrison, 2002), and/or stronger current activity during the Trade wind season in winter (March – September) (Frith et al., 1986) that may sweep larvae away from local sites, potentially result in decreased local settlement success. While it is difficult to draw parallels between recruitment rates observed in this study and planulation rates described in the study of Harriott (1983) at Lizard Island, partly due to the above mentioned sources of bias, and partly because of the poor temporal resolution of my data for this region, recruitment rate at the Palm Islands clearly shows a temporal pattern not yet described for *P. damicornis* on the GBR. Here two distinct peaks of recruitment can be observed, one in approximately April-June and one in October-November. The complete annual pattern did not occur within any one site throughout the whole study period, which may explain why the only other modern study that described reproductive seasonality of the species based on histological surveys of gonad and planula development combined with observations of planula release in aquaria (Harriott, 1983) may have missed the second peak. Alternatively, it is plausible that reproductive seasonality is different in the northern GBR from that in the central GBR, or that reproduction is synchronized among some individuals but not among populations (Marshall and Stephenson, 1933), thus locally produced recruits in my sample-set originated from only a handful of larger colonies (e.g. Hall and Hughes, 1996) that reproduce synchronously to optimize their reproductive output to ideal local environmental conditions (e.g. Guest et al., 2005).

4.4.3. Low proportions of clones, but high levels of self-recruitment

A central paradox in the reproductive biology and population genetics of *P. damicornis* on the GBR is that brooded larvae are produced asexually but local adult populations reflect predominately sexual reproduction (Ayre et al., 1997; Ayre and Miller, 2004;

Sherman et al., 2005). Based on the growing body of evidence from mating and other studies that all brooded larvae are clones of the parent colony (Stoddart, 1983; Ward, 1992; Ayre and Miller, 2004; Schmidt-Roach et al., 2012a; for counter example see Yeoh and Dai, 2010) and are capable of settling immediately upon release (Atoda, 1947; Harrigan, 1972; Harii et al., 2002), substantial proportions of populations are expected to be clones of a limited number of genotypes. Such clonal populations were found in south-western Australia (Stoddart, 1984), Kenya (Souter et al., 2009), and Southern Taiwan (Yeoh and Dai, 2010) but not on the GBR (Benzie et al., 1995; Ayre et al., 1997; Ayre and Hughes, 2000; Ayre and Miller, 2004; Sherman et al., 2005). My study helps to explain this discrepancy between reef regions by providing an estimate of the rate of recruitment of asexually produced larvae into newly settled juvenile populations. My finding that clones were represented by 15% and 7% of Type α and Type β recruits, respectively, is similar to the proportion of clones found in adult populations (14% for Type α and 6% for Type β) (Chapter 3). The remarkable similarity in the estimated clone frequencies of adults and newly settled recruits suggests that most clonal larvae disappear from the local population soon after release and prior to settlement.

My estimates of the frequency of occurrence of clones are considerably lower than expected from our current understanding of the life history strategy of this coral. It is important to realize, however, that my estimates are not error-free. Firstly, the proportion of clones in the adult population that originate from broods is likely underestimated in Chapter 3, because sampling of colonies adjacent to those that had already been sampled was avoided to minimise including clones produced by fragmentation. This sampling strategy potentially missed some clones produced by asexual brooding. Secondly, clonal recruits could only be detected if they originated from one of the adults that had been sampled, or if several of the settled larvae had identical MLGs, both of which would contribute to underestimating rates of occurrence of clones among recruits. To minimise this latter error, all adult colonies were sampled within a 10 x 10 m quadrat around each settlement tile site.

My finding that clonal larvae of *P. damicornis* do not recruit back to their natal reefs could reflect either prevailing current patterns at the time of their release (Ayre and

Miller, 2004; Chapter 3) or exceptionally high mortality rates in early life history stages. Graham et al. (2008) have shown that coral larvae follow a combination of Type I and Type III mortality curves, i.e. they have high initial rates of mortality, followed by a lower and approximately constant mortality rate, which ultimately increases sharply when they reach a senescence threshold. Although their study included only azooxanthellate larvae from broadcast spawning species, it showed substantial inter-specific variation in the shape of these curves. While it is possible that initial mortality rates of *P. damicornis* broods are high and exceed previous assumptions on the GBR, it does not provide an evolutionarily satisfactory explanation, since brooding is energetically more expensive mode of reproduction than spawning (Ward, 1995). The relatively larger sized broods that already contain zooxanthallae are well suited for long-distance migration (Richmond, 1987; Harii et al., 2002, 2007), therefore it is more reasonable to assume that clonal larvae are dispersed away from their natal population.

In tandem with low numbers of local clonal recruits, results of exclusion tests, as well as strong correlation between colony abundance and recruit numbers at each site suggest that a substantial proportion of settlers are philopatric, and are therefore mainly sexually produced. One possible explanation is that brooded larvae are not exclusively clonal. Simultaneous occurrence of both clonal and non-clonal larvae in *Pocillopora* has been shown by Yeoh and Dai (2010). Although, there are no reports of sexually produced brooded planulae in GBR populations of *P. damicornis*, the studies that addressed this question used allozyme electrophoresis and only a few markers, therefore it is a question that warrants further testing with higher resolution molecular markers. Another and more likely explanation is that sexually produced larvae are products of reproduction through broadcast spawning and external fertilisation. Indirect evidence for spawning in *P. damicornis* has been reported from earlier histological studies (Stoddart and Black, 1985; Ward, 1992, 1995), and recently, Schmidt-Roach et al. (2012b) observed spawning in *P. damicornis* at One Tree Island in the southern GBR. Spawned eggs were negatively buoyant, which supports my hypothesis that locally retained larvae came predominantly from spawned gametes. In agreement with this hypothesis, strong correlations between the number of Type β recruits and the density of surrounding adults (based on the quadrat-method), but near-zero correlations with the densities of neighboring adult populations (tracklog-method) in both study regions

suggest that there are two classes of Type β larvae. One class of larvae settles immediately around the parent colony, and another class drifts substantial distances away from their natal reef. In combination, given evidence that spawned eggs are negatively buoyant (Schmidt-Roach et al., 2012b), and considering the lower proportion of clones in Type β populations, I further hypothesize that larvae retained locally originate from spawning, while clonal larvae disperse. In combination with observations of spawning at various times during the summer season (Schmidt-Roach et al., 2012b), the high year-round proportion of non-clonal recruits found in my study suggests that such spawning events happen several times a year. This hypothesis explains why previous studies found predominantly sexually produced adult populations on the GBR (Ayre and Miller, 2004; Sherman et al., 2005), but contradicts classical models that seek to explain the role of mixed reproductive strategies by asserting that asexual reproduction is selected for under conditions favouring local dispersal, whereas sexual reproduction is selected for under conditions favouring long distance dispersal (Williams, 1975; Maynard-Smith, 1978).

4.4.4. Potential for metapopulation resilience and implications for conservation

Both Type α and β lineages combine self-recruitment with short and long-distance migration, indicating that metapopulations of both lineages have good potential for resilience. According to theoretical considerations, self-recruitment is an important factor in stabilizing dynamics of metapopulations because it enhances local population viability (Hastings and Botsford, 2006), and ensures that once a few individuals settle at a new location, their retained offspring will efficiently colonise the site. At the same time, migration provides the first settlers at new sites and fortifies depleted populations following major disturbance events, or, in extreme cases, contributes to recolonising habitats following local extinctions (Hanski and Gilpin, 1997). Indeed, Starger et al. (2010) have demonstrated that populations of pocilloporid corals regained high abundance and intra-population genetic diversity just decades following their complete extirpation from Krakatau island, and concluded that good larval connectivity with distant reefs provided the first colonizers, but self-recruitment contributed to the bulk of the population recovery. My data supports the hypothesis that as a consequence of their

complex reproductive strategies (Highsmith, 1982; Stoddart, 1983; Stoddart and Black, 1985; Ward, 1992; Yeoh and Dai, 2010; Schmidt-Roach et al., 2012b) and related broad dispersal capacities (Richmond, 1987; Harii et al., 2002; Schmidt-Roach et al., 2012b), populations of Type α and β *P. damicornis* are likely to be among the more resilient corals to inhabit the GBR.

Very low rates of recruit survival during the first months following metamorphosis, however, warns that even though dispersal suggest good potential for metapopulation resilience, high early lifestage mortality may substantially slow down natural recruitment rates. Determining the age of recruits/juvenile corals is difficult, due to variable growth rates under different conditions (Raymundo and Maypa, 2004). Baird and Babcock (2000) describe the correlation of the age of pocilloporid recruits and the diameter of their basal discs, however, how their observations can be generalized to environmentally different sites remains unknown. Early fusion of recruits can further increase uncertainty in recruit age estimates; ten polyp-colonies of *P. damicornis* were approximately 16 weeks old in the study of Raymundo and Maypa (2004) when one recruit built the colony, but were only approximately eight weeks old when two recruits fused. To account for the variability originating from environmental differences, I estimated the age of recruits in this study from observations of polyp numbers of maximum two-months-old recruits from the same sites where the year-round tiles were deployed. The size distribution of recruits on the year-round tiles show that even including uncertainty, survival rate during the first two months after settlement on the artificial substrate was not more than 4% and 3% for Type α and β recruits, respectively (Fig. 4.3). However, because both recruitment rate (Penin et al., 2010) and post-settlement mortality (Wilson and Harrison, 2005) is higher on artificial substrates than natural substrates, how these estimates reflect true success of natural recruitment remains unknown.

Dispersal modeling (Chapter 3) and genetic assignment of recruits suggest that the "GBR metapopulations" of *P. damicornis* lineages may encompass populations of SE Papua New Guinea, which emphasizes the importance of coordinated conservation efforts across national borders. *Pocillopora damicornis* is one of the most widespread coral species (Veron, 2000), with a capacity for long-distance dispersal (Richmond,

1987; Harii et al., 2002). My results that long-distance migration occurs over ecological timescales indicate that the species has good potential to form resilient metapopulations, but also suggest that important metapopulation nodes are likely to be distributed across two or more countries. Preservation of such large-scale metapopulation dynamics requires international cooperation (Chester, 2006) which is potentially the key to the conservation of many coral reef organisms with large larval dispersal capacities.

High variability of dispersal patterns among lineages, geographic regions and habitat types highlight the significance of assessing ecological connectivity separately for each site and species of conservation interest when designating networks of protection areas. The interplay of species-specific life-histories and dispersal behavior with local environmental conditions, such as water circulation (Black, 1993; Andutta et al., 2012) or temperature (O'Connor et al., 2007) may create unique connectivity patterns for different species at the same site, or for the same species at different sites. Temporal variability adds a further layer of complexity to dispersal patterns, which is very difficult to include in static management plans (Siegel et al., 2008), however since marine protected areas (MPAs) are designated for many decades, seasonal and annual fluctuations even out over time, and have less importance than in fisheries management, where, for example, annual harvest planning assumes temporally stable recruitment, or incorporates in recruitment stochasticity.

While my results cannot be automatically extrapolated to other reefs or coral taxa due to the above sources of variation, the coupling of my genetic connectivity data plus results from similar studies with high resolution realistic water circulation models (e.g. Galindo et al., 2006; Foster et al., 2012) may help develop management strategies on large spatial domains.

4.5. Conclusions

Populations of *P. damicornis* Type α and β lineages showed spatially variable rates of self-recruitment, short- and long-distance migration, demonstrating the importance of local physical characteristics in driving dispersal processes. I explain higher rates of self-seeding in the inshore Palm Island group in the central GBR by complex circulation patterns, mainly driven by tidal forces (King and Wolanski, 1996; Luick et

al., 2007) and larval release at neap tides (Muir, 1984). Conversely, lower rate of self-recruitment at Lizard Island is explained by larval release on spring tides (Harriott, 1983) and prevailing currents that favour the dispersal of propagules (Frith et al., 1986). The high frequency by which recruits were excluded from all sampled populations further support the hypothesis that recruits may arrive at Lizard Island from populations beyond the GBR (Chapter 3), and emphasize the importance of coordinated conservation efforts across national borders.

Differences in dispersal patterns between lineages indicate the effect of biological traits on the final outcome of larval dispersal, and, together with the lineage-specific habitat preferences observed, support the recent delineation of lineages (putative cryptic species) within the *P. damicornis* morphospecies. Among the two lineages, Type α showed higher rates of self-recruitment, especially at exposed sites, in contrast to Type β's preference for sheltered habitats, especially in the Palm Island group. However, both lineages combine self-recruitment and migration in a ratio that likely places them among the more resilient corals to inhabit the GBR.

The low proportion of clones among recruits of both lineages challenges the hypothesis that reproduction is predominantly asexual in *P. damicornis*. Because brooded larvae are thought to be exclusively clonal (Stoddart, 1983; Ayre and Miller, 2004), and spawning of sperm and negatively buoyant eggs has been recently reported (Schmidt-Roach et al., 2012b), I explain high rates of self-recruitment and low rates of clonality by speculating that locally retained larvae originate predominantly from spawned gametes, while brooded larvae are mainly vagabonds.

Chapter 5

Spatial and temporal recruitment patterns of *Seriatopora hystrix* inferred from genotypic analysis of adults and recruits

This chapter is a result of a collaboration between G. Torda and P. Warner. Coral colony samples were collected, DNA extracted and genotyped by P. Warner, for a population genetic study as part of her Ph.D. research. G. Torda collected additional samples, extracted DNA, identified and genotyped coral recruits, and carried out all analyses that are presented in this chapter. The chapter was written by G. Torda with intellectual contribution of P. Warner.

Abstract

The importance of knowledge on contemporary connectivity among populations of hard corals in developing effective spatial management strategies for tropical coral reefs is increasingly recognised, yet there are few studies providing empirical data on this matter. In this chapter I used assignment methods on microsatellite genotype data from newly settled recruits of the common brooding coral *Seriatopora hystrix*, to estimate rates of self-recruitment and larval immigration in populations around Lizard Island in the northern GBR and the Palm Islands in the central GBR. I found that patterns of recruitment intensity and larval dispersal are highly variable among sheltered and exposed sites in the two regions, with primarily philopatric recruitment around the Palm Islands, and mainly exogenous recruitment around Lizard Island. A substantial proportion of genotyped recruits (16% at the Palm Islands and 86% at Lizard Island) were excluded from all sampled adult populations, and were genetically highly dissimilar from any adult specimen. I hypothesize that such recruits originate from deep populations of *S. hystrix* that are maladapted to the shallow reef environment and hence selectively removed post settlement. New recruits were found year-round, with peaks in September-November and April-June in the central GBR. This is in agreement with previous studies from this region, and other reef systems. However, biannual peaks of recruitment were not observed at sites in the Northern GBR. In tandem with seasonality, strong inter-annual variation in recruit numbers was observed, especially on sites where the source of recruitment was primarily exogenous. This indicates that snapshot or short-term recruitment data should be interpreted carefully. I found that the proportion of asexually produced recruits in the sexual brooder *S. hystrix* are on par with those of the closely related asexual brooder/sexual spawner *Pocillopora damicornis*, suggesting that the importance of asexual reproduction may have been underestimated in the former.

5.1. Introduction

The decline of tropical coral reef ecosystems in the past decades has been associated with several direct and indirect anthropogenic stressors (e.g. Connell et al., 1997; Hoegh-Guldberg et al., 2007; De'ath et al., 2012), and has called for improved conservation management tools. One proposed improvement is to (re-)design networks of marine protected areas (MPAs) in a manner that maximizes benefits of population connectivity to increase the resilience of the metapopulations of reef species (Botsford et al., 2009; McCook et al., 2009; Steneck et al., 2009; Almany et al., 2009). These improvements require better understanding of metapopulation dynamics, including source-sink dynamics, and demographically significant dispersal distances of key marine organisms (e.g. Crowder et al., 2000; Bode et al., 2006; Cowen and Sponaugle, 2009). However, knowledge gaps in connectivity over ecological, rather than evolutionary timescales, among coral populations have hindered practical applications of this information in coral reef conservation.

Obtaining direct estimates of larval retention and dispersal in corals is exceedingly difficult due to the size and body plan of their larvae, which inhibits traditional tagging (Van Oppen and Gates, 2006). Slicks of coral spawn have been tracked by drogues over tens of km (Willis and Oliver, 1988, 1990), but not all coral larvae aggregate into slicks during spawning (Willis and Oliver, 1990), therefore this method does not give exhaustive information about actual dispersal in spawning corals. Furthermore, these remote sensing techniques are not suitable for tracking brooded coral larvae, because these are released in smaller numbers, usually more than once a year (e.g. Harriott, 1983; Tanner, 1996), and do not form conspicuous slicks. Instead, the analysis of genetic differentiation between populations has been extensively used to estimate larval retention and exchange between reefs in various scleractinian taxa (e.g. Ayre et al., 1997; Yu et al., 1999; Ayre and Hughes, 2000, 2004; Nishikawa and Sakai, 2005; Underwood et al., 2007; van Oppen et al., 2008, 2011b; Souter and Grahn, 2008; Sammarco et al., 2012). The population genetic approach is powerful, as long as the statistical methods applied match the questions of the study (Manel et al., 2005). For example, the widely used F-statistics assume random sexual reproduction in non-overlapping generations, while in reality, larger coral colonies may contribute more to next generations than their smaller peers (Hall and Hughes, 1996), and fragmentation or

other forms of asexual reproduction may lead to long genotype lifespans, or even "immortal" genotypes (Hughes and Jackson, 1980; Highsmith, 1982), which potentially result in largely overlapping generations. A further limitation of F-statistics is that they are based on equilibrium models and are therefore not sensitive to changes in gene flow over contemporary timescales (Manel et al., 2005), especially in long-lived organisms, and have unrealistic assumptions that are rarely met (Whitlock and McCauley, 1999).

In addition to population genetics, stock-recruitment correlations (Hughes et al., 1999, 2000; Hughes and Tanner, 2000) have also been used to assess population self-sustainability. The major limitation of these studies is that coral recruits can rarely be identified to the species level by their morphology, therefore several species and genera with potentially differing biological characteristics (including reproductive and dispersal characteristics) are often pooled in the analyses. To overcome these difficulties, I developed a genetic assay that identifies two lineages of the scleractinian coral *Pocillopora damicornis* (Chapter 2). In this chapter I extend this assay to include the closely related species *Seriatopora hystrix*. The needle coral, *S. hystrix*, provides a useful comparative model to *P. damicornis* (Chapters 2 and 3) for connectivity studies, because it exhibits a sexual brooding reproductive strategy (Ayre and Resing, 1986) in which planulae are released several times a year (reviewed in Fadlallah, 1983). These broods may originate from outcrossed gametes, but the species has a tendency for self-fertilisation (Sherman, 2008). Brooded coral larvae are thought to have a large variance in dispersal distances, because they attain settlement competency shortly after release (Harrison and Wallace, 1990), but remain competent to settle for extended periods of time enabling long-distance dispersal (Richmond, 1987; Harii et al., 2002). Accordingly, existing estimates of connectivity, dispersal and larval retainment in *S. hystrix* are complex and often contradicting. Primarily self-seeding populations were found on the GBR (Ayre and Dufty, 1994; Ayre and Hughes, 2000) and the Red Sea (Maier et al., 2005) with limited larval dispersal on scales of a few hundred meters to a few tens of kilometers, but panmixia was later described from the same region in the Red Sea over ~ 8 km (Maier et al., 2009); while some evidence for occasional long-distance dispersal has been shown for reefs of NW Australia (Underwood et al., 2007) and by a GBR-wide population genetic study (Van Oppen et al., 2008). Additional to the horizontal structuring of population, *S. hystrix* populations seem to form two

genetically isolated ecotypes that are segregated by depth (Bongaerts et al., 2010b, 2011; van Oppen et al., 2011a).

A further peculiarity of *S. hystrix* is that its polyps can detach themselves from the colony if stressed, and float away to re-settle and form a new colony - a phenomenon termed “polyp bail-out” (Sammarco, 1982). While this phenomenon has been described as a stress escape mechanism, its potential as a means of reproduction has yet to be assessed.

With the present study I aim at resolving some of these controversies by analyzing single-species recruitment patterns and by describing contemporary connectivity in *S. hystrix*. To avoid the potential confounding effect of overlapping generations, as well those presented by equilibrium model-based statistics, I examine genetic characteristics of newly settled recruits of *S. hystrix*, and identify potential sources of new recruitment by genetic assignment methods. This chapter complements a similar study on *P. damicornis* (Chapter 4), and provides opportunities for comparison between dispersal characteristics of the closely related sexual brooder *S. hystrix*, and the asexual brooder/sexual spawner *P. damicornis*, hence contributes to understanding dispersal mechanisms in brooding corals.

5.2. Materials and Methods

5.2.1. Sample collection

Twenty pre-conditioned, unglazed, terracotta tiles, with a total surface area of 0.572 m^2, were deployed haphazardly over approximately 100 m^2 of reef between three to nine metres of depth following Mundy (2000) at each of eight sites on the Great Barrier Reef. Four of the sites were located around Lizard Island in the Northern GBR, and four around the Palm Islands (Pelorus and Orpheus Islands) in the Central GBR (Fig. 5.1). The tiles were recovered and scanned systematically under a dissecting microscope for pocilloporid recruits roughly every three months from May 2009 to May 2010 at Lizard Island, and every two months from May 2009 to May 2011 at the Palm Islands (see Chapter 4 for details). An additional set of 20 tiles was deployed at each of the same Palm Island sites and left exposed for roughly one year, from May 2009 to May 2010,

and from May 2010 to May 2011 (henceforth referred to as “year-round tiles”) to assess mortality of newly settled coral recruits. All tiles were lost from the NE Pelorus and NE Orpheus sites due to a category five tropical cyclone (TC Yasi) in February 2011.

One branch fragment was collected from each of 875 colonies of *S. hystrix* at 18 sites located in the vicinity of the settlement tiles (Fig. 5.1). The track log of divers was recorded as per Chapter 4 to obtain the coordinates of each colony. Between 23 and 58 specimens were sampled at each site (Table 5.1) in a depth range of 2 to 10 meters on the upper reef slope (therefore only “shallow” ecotypes *sensu* Bongaerts et al. (2011) were sampled), along a haphazard zigzag transect. Colonies within 1 m of a colony already sampled were avoided to minimise collection of clone mates by fragmentation. Coral branches were fixed in absolute ethanol.

5.2.2. DNA extraction, genetic identification and microsatellite genotyping

DNA from coral branch samples was extracted following a modified protocol of Wilson et al. (2002) optimized for coral tissues. Coral recruit DNA was extracted by an adaptation of the method from Gloor and Engels (1992). The species and lineage identity of pocilloporid recruits was determined by a modified genetic assay from Chapter 2. Originally, this assay was designed to identify lineages of the closely related genus *Pocillopora*, however in this study I expanded the assay with an additional primer pair, ShMT662F YTT SAG AGA GTG TGT MST WAG G and ShMT960R CAA CTG CCT CAT CAA KGT T that amplifies a variable region in the mitochondrial putative control region of the *Seriatopora* genus, but not in any other coral taxa (NCBI BLAST, 1st October 2012). The amplicon of this primer pair is 272 bp in *S. caliendrum*, and is dimorphic in *S. hystrix*, giving 248 (Type “S”) or 299 (Type “L”) bp products. Following Alu 1 enzyme digestion, the expanded assay now results in banding patterns on an agarose gel that enable the identification of six major pocilloporid groups, including *P. damicornis* Type α and β lineages (Schmidt-Roach et al., 2012a), a pooled group of all other *Pocillopora* species and lineages, *S. hystrix* types “S” and “L”, and *S. caliendrum* (Fig. 5.2). Following identification, all *S. hystrix* samples were genotyped by ten microsatellite markers described in Underwood et al.

(2006). PCR amplifications were carried out in 10 µL reactions using Qiagen Multiplex PCR Kits (Qiagen®) and following the manufacturer's protocol. Multiplex groups, primer concentrations and annealing temperatures are given in Table 5.2. PCR products were separated on a MegaBACE 1000 DNA Analysis System. Electropherograms were analyzed using MegaBACE Fragment Profiler v1.2 (Amersham Biosciences). All automatic scoring was checked manually.

Table 5.1. Names and locations of sampling sites of *S. hystrix* adult colonies. Habitat type indicated by E = exposed windward, S = sheltered leeward, L = lagoon

				WGS '84 coordinates		
	Site name	**Code**	**Sample size**	**Longitude**	**Latitude**	**Habitat**
Lizard Island	NW Lizard Is 1	LNW1	50	145.4506	-14.6568	S
	NW Lizard Is 2	LNW2	50	145.4534	-14.6608	S
	SW Lizard Is 1	LSW1	23	145.4409	-14.6834	S
	SW Lizard Is 2	LSW2	50	145.4434	-14.6875	S
	Lizard Is Lagoon	LL	50	145.4543	-14.6860	L
	NE Lizard Is 1	LNE1	50	145.4607	-14.6492	E
	NE Lizard Is 2	LNE2	44	145.4632	-14.6514	E
	SE Lizard Is 1	LSE1	50	145.4696	-14.6917	E
	SE Lizard Is 2	LSE2	50	145.4661	-14.6941	E
Palm Islands	W Pelorus Is 1	PW1	50	146.4879	-18.5477	S
	W Pelorus Is 2	PW2	50	146.4884	-18.5506	S
	NNW Orpheus Is	ONNW	50	146.4831	-18.5717	S
	NW Orpheus Is	ONW	50	146.4818	-18.5800	S
	W Orpheus Is 1	OW1	50	146.4866	-18.6105	S
	W Orpheus Is 2	OW2	50	146.4872	-18.6091	S
	SE Pelorus Is 1	PSE1	50	146.5002	-18.5635	E
	SE Pelorus Is 2	PSE2	50	146.5018	-18.5602	E
	NE Orpheus Is	ONE	58	146.4981	-18.5910	E
	Total		**875**			

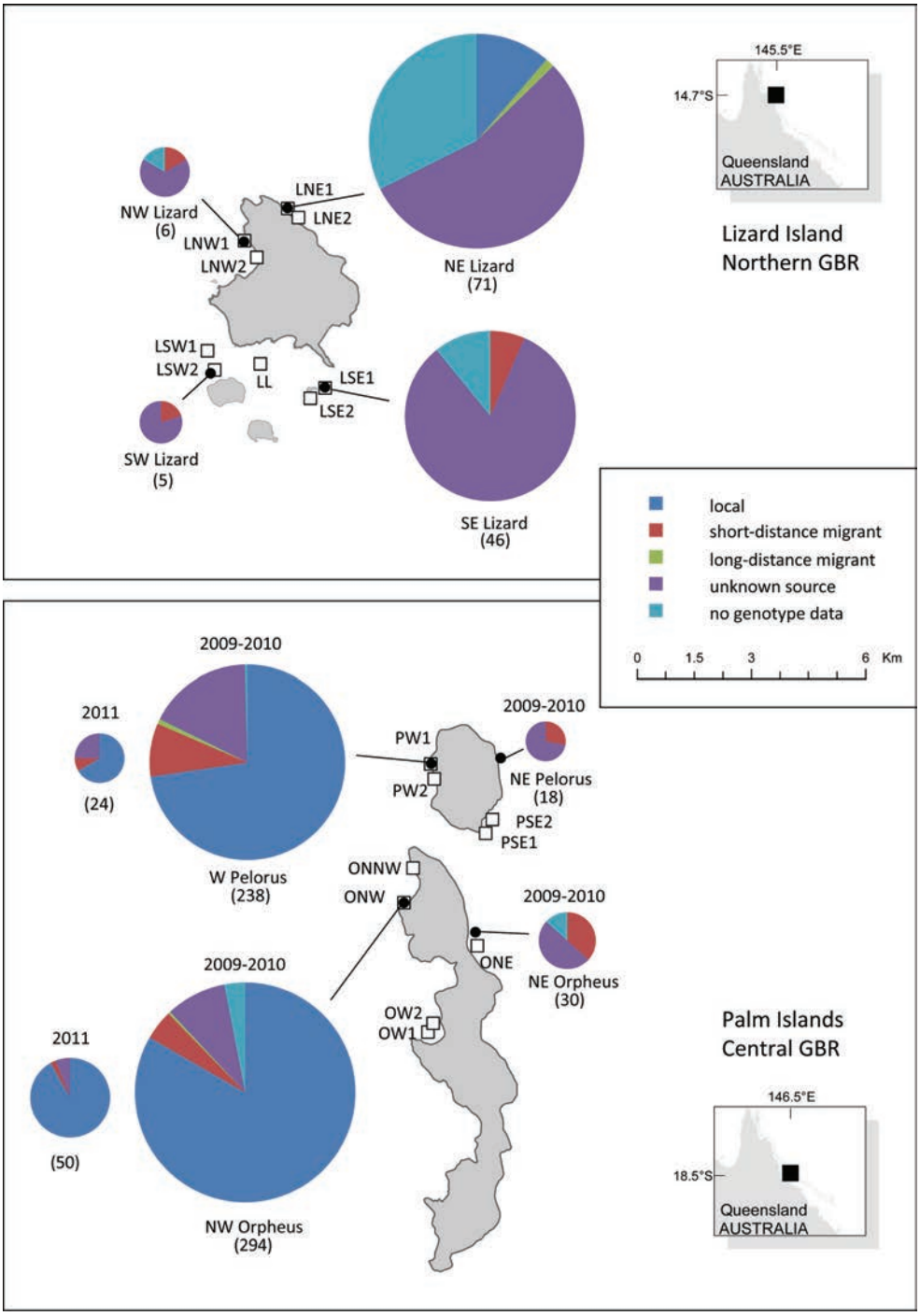


Fig. 5.1. Map of the study area. White squares indicate collection sites of colony samples, black dots indicate recruit sampling sites. The pie charts show the proportion of recruits from four source categories plus those without genotype data. The sizes of pie charts are proportional within a region to the numbers of recruits (shown in brackets) sampled at the corresponding site throughout the study period (May 2009 – May 2010 around Lizard Island; May 2009 – May 2011 around the Palm Islands). Recruits were only sampled at NW Pelorus and NW Orpheus in 2011, therefore data from this year is shown on separate pie charts left of the main pies to make inter-habitat comparisons easier. Note that local recruits were not identified at NE Pelorus, due to the lack of local reference population.

5.2.3. Data analyses

Spatial and temporal patterns of recruitment were analysed after normalising the number of recruits found at each site in each time-window by the exact number of exposure days of the settlement tiles. The proportions and size distributions of recruits on the year-round tiles and recruits found on regularly sampled tiles over the same time period were used to estimate a survival rate of recently settled recruits (see Chapter 4).

To assess annual variation of recruitment rate, and the impact of TC Yasi thereon, the numbers of recruits per each tile in corresponding sampling time-windows of consecutive years were compared by two-tailed paired t-tests, following normalization to a standard 60-day period, to account for differences in the length of sampling time-windows. At W Pelorus and NW Orpheus, this comparison was continued after the cyclone impact, but since the sampling time-windows were similar but not identical pre- and post-Yasi, comparisons were made between the most similar pairs of sampling periods (i.e., Dec-Jan 2010 and Dec-Feb 2011; Feb-Mar 2010 and Mar-Apr 2011; Apr-May 2010 and May-Jun 2011).

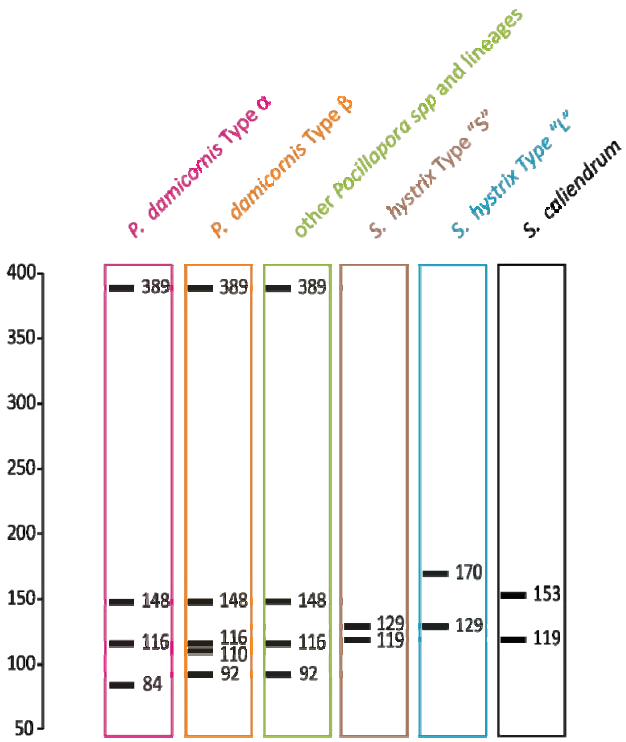


Fig. 5.2. Schematic figure of banding patterns of products of the expanded genetic assay from Chapter 2 after electrophoresis on an agarose gel. Numbers show fragment sizes in bp.

Table 5.2. Multiplex groups of primers from Underwood et al. (2006) used for microsatellite genotyping of *S. hystrix* recruits (a) and adults (b).

(a)

Group	Running Tm (°C)	Locus	5' MOD	concentration (µM)
M1	54	Sh3_003	HEX	0.2
		Sh2_006	TET	0.2
		Sh3_004	FAM	0.2
		Sh4_010	HEX	0.2
M2	54	Sh3_007	HEX	0.2
		Sh4_001	TET	0.2
		Sh3_008	FAM	0.2
M3	54	Sh2_005	FAM	0.2
		Sh2_002	HEX	0.2
		Sh3_009	TET	2

(b)

Group	Running Tm (°C)	Locus	5' MOD	concentration (µM)
M1	52	Sh4_001	TET	0.2
		Sh3_003	HEX	0.2
		Sh3_004	FAM	0.2
		Sh3_009	HEX	0.2
M2	54	Sh2_002	TET	0.2
		Sh2_005	FAM	0.2
		Sh4_010	HEX	0.2
M3	54	Sh2_006	HEX	2
		Sh2_007	TET	0.2
		Sh3_008	FAM	0.2

The density of reproductively mature *S. hystrix* colonies was estimated by counting every specimen that was larger than 8 cm in diameter (minimum size of adult colonies sensu Harrison and Wallace, 1990) along three replicates of 20 x 2 m belt transects crossing each of the recruitment sampling sites. Adult colony density was correlated by Pearson correlation with the number of recruits at each site. Only recruits collected between May 2009 and May 2010 were considered, to ensure identical sampling effort between Lizard Island and the Palm Islands.

The discriminative power of the microsatellite marker set was assessed by combining the recruit and adult genotype datasets. Genotype Probability (GP) of each sample for each locus and a combination of all loci was calculated in GENALEX 6.4 (Peakall and Smouse, 2006). Repeated multilocus genotypes (MLG) were considered to be clone

mates at a GP < 0.001. Genetic assignment of recruits to potential source populations was carried out in GeneClass2 (Piry et al., 2004), using the adult genotypes as reference dataset. The probability that each recruit belongs to each reference population was computed using the criteria and probability computation algorithm of Rannala and Mountain (1997), with 10,000 simulated genotypes. Recruits were excluded from a reference population if the probability of assignment was < 0.1.

The Bayesian clustering algorithm implemented in STRUCTURE v2.3.3 (Pritchard et al., 2000) was run without population information, under the admixture model and correlated allele frequencies, with 100,000 MCMC of burn-in, followed by 100,000 MCMC replications to assign recruits into genetic clusters. In order to obtain comparable cluster memberships between adult and recruit specimens, I combined the recruit and adult datasets, and ran five independent chains for each of K = 1 to 10. To find the most informative number of clusters, I plotted the mean of the estimated log probability of the data (Evanno et al., 2005) over the number of clusters using STRUCTURE HARVESTER (Earl and vonHoldt, 2012). The lowest K corresponding to the plateau of the plot was considered the most meaningful number of clusters.

5.3. Results

5.3.1. Recruit numbers and survivorship

A total of 784 *S. hystrix* specimens were identified among 1149 pocilloporid recruits (Table 5.3). One hundred and twenty-eight were collected from sites around Lizard Island and 656 from sites around the Palm Islands. The number of recruits varied greatly among different habitat types. The sheltered sites of Pelorus and Orpheus received an order of magnitude more recruits (153 recruits per day per 100 m^2 on average) than the exposed sites (12 recruits per day per 100 m^2 on average). In contrast, at Lizard Island recruits at exposed sites outnumbered those at sheltered sites by a factor of ten (61 and 6 recruits per day per 100 m^2 on average, respectively). The number of recruits between May 2009 and May 2010 correlated strongly with the density of adult colonies around the settlement tiles in the Palm Islands, but only moderately in Lizard Island (Fig. 5.3). This latter correlation is weakened by the SE Lizard Island site, where high numbers of recruits were collected despite the low density of local colonies (Fig.

5.3). Highest colony density was found on the exposed NE site at Lizard Island, and on the sheltered sites of the Pelorus and Orpheus Islands.

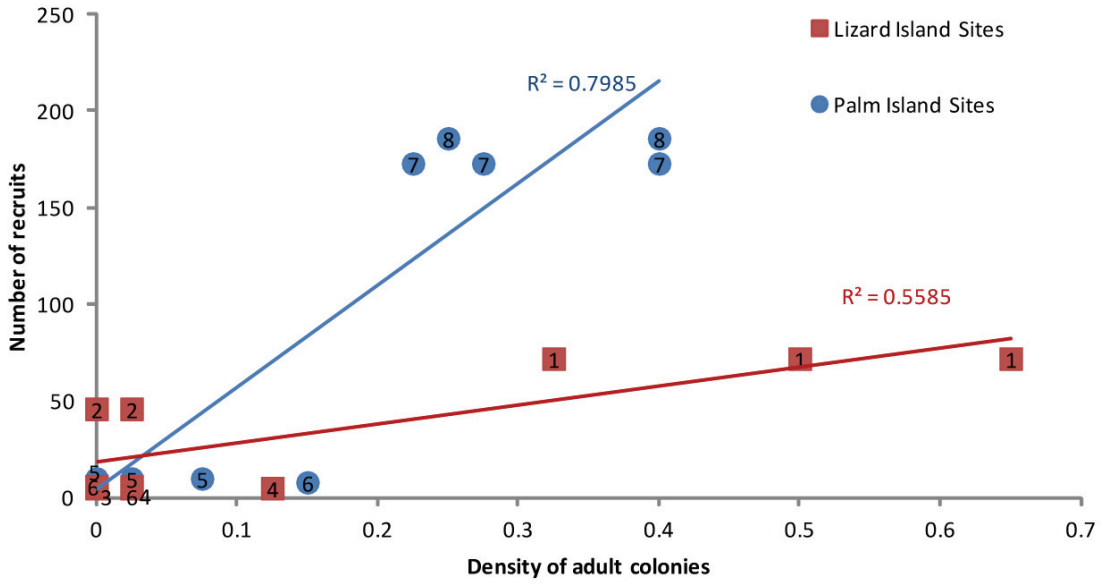


Fig. 5.3. Correlation between the density of adult *S. hystrix* colonies and recruits at sites around Lizard Island (red squares) and the Palm Islands (blue dots). Site codes are 1 – NE Lizard, 2 – SE Lizard, 3 – NW Lizard, 4 – SW Lizard, 5 – NE Pelorus, 6 – NE Orpheus, 7 – W Pelorus, 8 – NW Orpheus.

Forty-six recruits were found on year-round tiles at sites around the Palm Islands; seven were sampled from tiles at the exposed sites, 25 from tiles at sheltered sites in 2010 and fourteen from tiles at sheltered sites in 2011 (Table 5.3). The maximum number of polyps per recruit was 25 on year-round tiles (mean 4.9 ± 5.0 SD), and 14 (mean 4.0 ± 2.0 SD) on regularly sampled tiles, therefore the estimated maximum size of recruits two months after settlement was 14 polyps. Only three of the recruits on year-round tiles had more than 14 polyps (Fig. 5.4), all collected in 2010 at W Pelorus. In the period May 2009-2010 a total of 418 recruits settled on regularly sampled tiles around the Palm Islands, 182 of which were found at W Pelorus (Table 5.3). The survival of recruits during the first two months following settlement in 2009 – 2010 is estimated to be 1.6% for W Pelorus, or 0.7% for all four sites around the Palm Islands. In the May 2010 – May 2011 period, early life stage survival was zero, within the limits of detection.

Table 5.3. Number and source of recruits at each of the sampling sites, in each sampling period. SDM = short-distance migrant, LDM = long-distance migrant, Unknown = source unknown, N/A = no genotype data

Site	Year	Period	Local	SDM	LDM	Unknown	N/A	Total
NE Lizard (71)	**2009**	Jun-Aug	2	0	1	14	11	**28**
		Sep-Nov	6	0	0	19	12	**37**
	2010	Dec-Feb	0	0	0	6	0	**6**
		Mar-May	0	0	0	0	0	**0**
SE Lizard (46)	**2009**	Jun-Aug	0	1	0	8	4	**13**
		Sep-Nov	0	2	0	19	1	**22**
	2010	Dec-Feb	0	0	0	11	0	**11**
		Mar-May	0	0	0	0	0	**0**
NW Lizard (6)	**2009**	Jun-Aug	0	1	0	0	1	**2**
		Sep-Nov	0	0	0	1	0	**1**
	2010	Dec-Feb	0	0	0	3	0	**3**
		Mar-May	0	0	0	0	0	**0**
SW Lizard (5)	**2009**	Jun-Aug	0	0	0	3	0	**3**
		Sep-Nov	0	1	0	0	0	**1**
	2010	Dec-Feb	0	0	0	1	0	**1**
		Mar-May	0	0	0	0	0	**0**
NE Pelorus (18)	**2009**	Apr-May	0	0	0	0	0	**0**
		Jun-Jul	0	1	0	7	0	**8**
		Aug-Sep	0	0	0	0	0	**0**
		Oct-Nov	0	0	0	1	0	**1**
	2010	Dec-Jan	0	0	0	0	0	**0**
		Feb-Mar	0	0	0	0	0	**0**
		Apr-May	0	1	0	0	0	**1**
		Jun-Jul	0	0	0	0	0	**0**
		Aug-Sep	0	1	0	3	0	**4**
		Oct-Nov	0	0	0	1	0	**1**
	year-round 2009-2010		0	2	0	1	0	**3**
NE Orpheus (32)	**2009**	Apr-May	0	0	0	0	0	**1**
		Jun-Jul	0	0	0	0	2	**3**
		Aug-Sep	0	0	0	1	1	**2**
		Oct-Nov	0	0	0	1	0	**1**
	2010	Dec-Jan	0	0	0	1	0	**1**
		Feb-Mar	0	0	0	1	0	**1**
		Apr-May	0	0	0	0	0	**0**
		Jun-Jul	0	0	0	0	0	**0**
		Aug-Sep	0	1	0	0	0	**1**
		Oct-Nov	0	9	0	8	1	**18**
	year-round 2009-2010		0	1	0	3	0	**4**

Table 5.3. continued

Site	Year	Period	Local	SDM	LDM	Unknown	N/A	Total
W Pelorus (262)	**2009**	Apr-May	3	0	0	5	1	**9**
		Jun-Jul	13	0	0	1	0	**14**
		Aug-Sep	3	0	0	3	0	**6**
		Oct-Nov	49	9	0	11	0	**69**
	2010	Dec-Jan	25	0	0	4	0	**29**
		Feb-Mar	14	1	2	0	0	**17**
		Apr-May	30	4	0	4	**0**	**38**
		Jun-Jul	2	2	0	1	0	**5**
		Aug-Sep	4	1	0	3	0	**8**
		Oct-Nov	21	3	0	6	0	**30**
	2011	Dec-Feb	1	0	0	2	0	**3**
		Mar-Apr	3	0	0	1	0	**4**
		May-Jun	10	2	0	1	0	**13**
	year-round 2009-2010		9	1	0	3	0	**13**
	year-round 2010-2011		2	0	0	2	0	**4**
NW Orpheus (344)	**2009**	Apr-May	16	5	0	9	3	**33**
		Jun-Jul	8	1	0	0	0	**9**
		Aug-Sep	0	0	0	0	0	**0**
		Oct-Nov	82	4	0	6	1	**93**
	2010	Dec-Jan	39	1	0	2	2	**44**
		Feb-Mar	21	0	0	3	0	**24**
		Apr-May	15	0	0	0	1	**16**
		Jun-Jul	16	0	0	1	0	**17**
		Aug-Sep	0	0	0	1	1	**2**
		Oct-Nov	39	2	1	1	1	**44**
	2011	Dec-Feb	9	0	0	1	0	**10**
		Mar-Apr	14	0	0	0	0	**14**
		May-Jun	14	0	0	2	0	**16**
	year-round 2009-2010		9	0	0	3	0	**12**
	year-round 2010-2011		9	1	0	0	0	**10**
Total			**488**	**58**	**4**	**189**	**43**	**782**

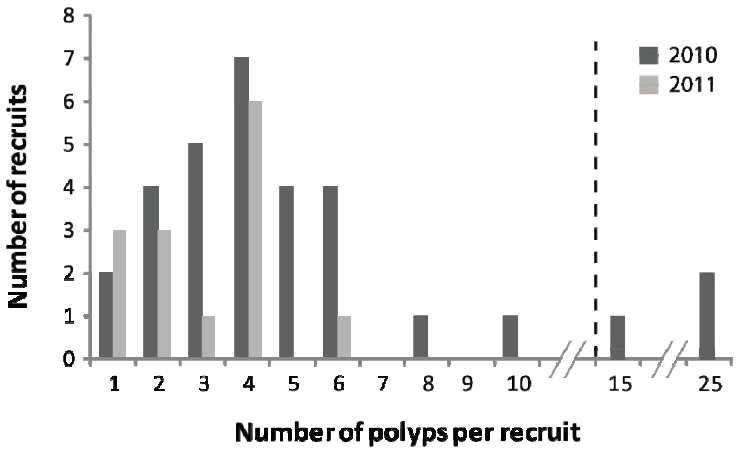


Fig. 5.4. Size distribution of *Seriatopora hystrix* recruits on year-round tiles in the May 2009 – May 2010 period (dark grey bars) and the May 2010 – May 2011 period (light grey bars). Recruit size is shown on the x axis by the number of polyps per recruit, and the number of recruits within each size-class is shown on the y axis. The dashed line indicates maximum number of recruits observed on regularly sampled tiles at Palm Islands, and therefore represents the maximum estimated size of two-months-old recruits.

5.3.2. Spatial – genetic patterns of recruitment

Local recruitment was the dominant process identified in the sampled coral recruits; however, patterns were also strongly influenced by geography and habitat. In total, 66% (n=488) of the 739 successfully genotyped recruits were not excluded from the local adult populations and hence determined to be "locally produced" (henceforth "local" or "philopatric" recruits; Table 5.3). Fifty-eight recruits (8%) were excluded from the local adult population, but not from neighboring populations within 10 km ("short-distance migrants"). Four recruits (1%) were considered "long-distance migrants" and excluded from all neighboring populations, but not from populations sampled in the other region. And, the remaining 27% of recruits (n=189) could not be "assigned" membership to any of the sampled adult populations ("recruits with unknown source" or "unassigned recruits"; Table 5.3). However, patterns in recruitment source and the number of recruits varied greatly among sites (Fig. 5.1). Recruits of unknown source were dominant at all Lizard Island sites, as well as exposed sites in the Palm Islands. Of these six locations, local recruits were only detected at the exposed NNE Lizard Island site (11%). In contrast, the two sheltered sites of the Palm Island group, NW Pelorus and NW Orpheus, were dominated by local recruitment (72% and 85%, respectively) as well as producing the greatest numbers of recruits across all sites in both regions (Fig.

5.1). Because the adult population was not sampled at NE Pelorus, I was unable to discern local recruits for that site.

Fourteen recruits (1.9%) were detected to be clones of a sampled adult colony, two of which were identical to the same adult. All clonal recruits were found on sheltered western sites of the Palm Islands; nine at NW Orpheus and five at NW Pelorus. Five clonal recruits (35.7%) matched adult genotypes within the same site. One recruit arrived to W Pelorus from W Orpheus (6.8 km); one assumed migrant clone came to NW Orpheus from W Pelorus (3.6 km), one from E Orpheus (3.4 km), and six from NNW Orpheus (0.9 km). The maximum assumed dispersal that I detected for clones in this study is therefore 6.8 km, and the Euclidean distance between clone mates follows a logarithmic distribution (R^2= 0.9) (Fig.5.5). Identical MLGs were shared by an additional ten pairs and two triplets of recruits (3.5%). Three clone pairs and two members of a clone triplet settled in the same sampling period, while other clone mates were sampled with as much as 18 months difference. All clone mates settled at the same site except for one instance in which one member of a clone pair settled at NW Orpheus and the other at W Pelorus, 3.6 km from each other, and 10 months difference apart. In spite of assumed migration, genetic assignment tests did not exclude clonal recruits from the local adult populations where they settled, even when the corresponding adult clone mate was located at a different site. This contradiction is due to the low genetic differentiation of populations on the sheltered sites of Pelorus and Orpheus Islands (P. Warner unpublished thesis), and demonstrate that genetic exclusion tests may fail to detect migrants among genetically similar populations (Berry et al., 2004).

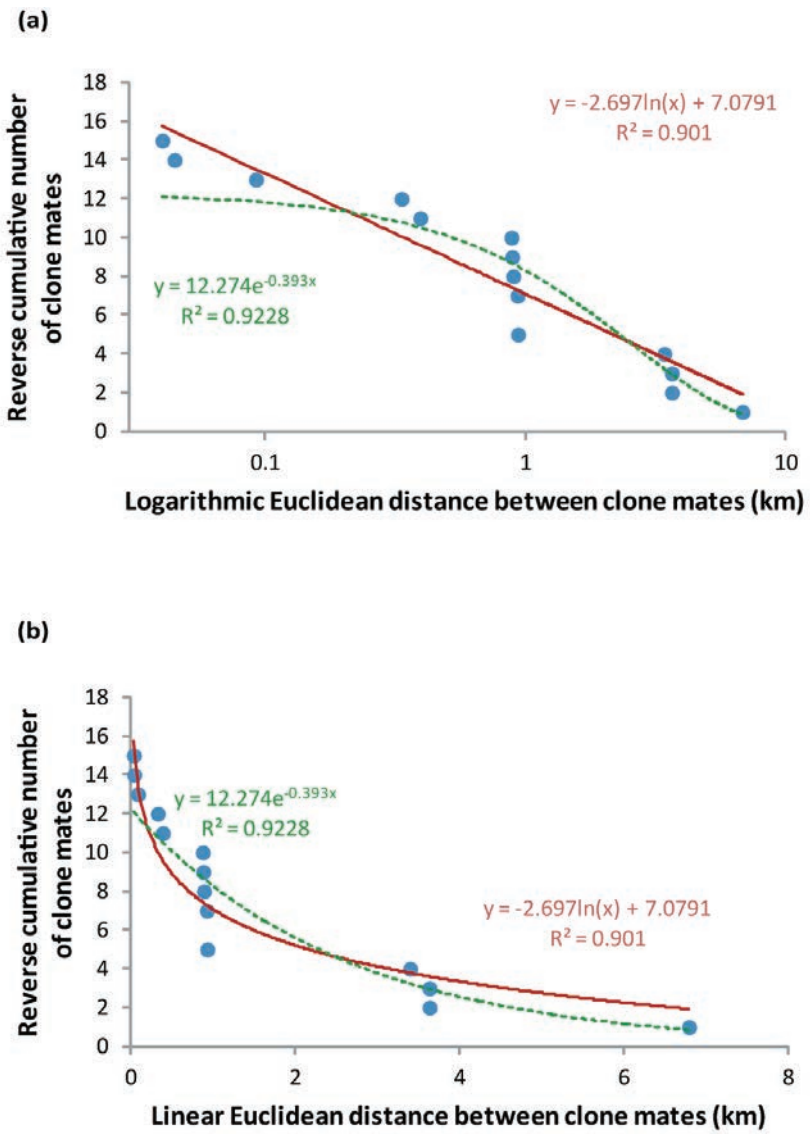


Fig.5.5. Reverse cumulative number of clones as a function of Euclidean distance between clone mates on a logarithmic scale (a) and on a linear scale (b). Red lines show logarithmic and green lines exponential fitted curves, with respective functions and R^2 values similarly color-coded.

5.3.3. Temporal patterns of recruitment

The distribution of recruits was also unbalanced in time (Fig. 5.6). At exposed sites of Lizard Island, most recruitment happened in the September – November period, while recruitment on sheltered sites was irregular. The few local recruits at NE Lizard were detected in the June – August and September – November time windows. Some recruits were collected in December – February as well, but no recruits settled on the tiles during the March – May period at Lizard Island (Fig. 5.6).

At sheltered sites of the Palm Islands recruit settlement was most intense in the October-November period (Fig. 5.6). Recruitment at exposed sites of the Palm Islands was irregular compared to sheltered sites. Altogether, apart from the October - November recruitment peak, a second peak was also observed in the austral late autumn, early winter months (April – June) at the Palm Islands, and recruitment did not stop throughout the year.

Recruitment did not stop after cyclone Yasi at the two sheltered sites where sampling could be continued (W Pelorus and NW Orpheus; Fig. 5.6), but was significantly lower in the sampling period when the cyclone impacted the sites (December – February) than in the same period in the previous year (Fig. 5.7). In the two subsequent sampling periods, recruitment was significantly lower than in the previous year at W Pelorus, as well as the two sheltered sites combined, but not at NW Orpheus (Fig. 5.7; note that the sampling time-windows were similar but not identical pre- and post-Yasi). However, the number of recruits in corresponding time-windows showed substantial and often statistically significant annual variation at each site even before the cyclone impact (Figs. 5.6 and 5.7). At W Pelorus, for example, recruitment rate was significantly different from the previous year's corresponding time-window in all instances, except for the period August-September 2010 compared to 2009 (Fig. 5.7). Furthermore, a significant drop in recruitment was observed before the cyclone, in October-November 2010 compared to 2009 at both sheltered sites of the Palm Islands. Therefore conclusions regarding the impact of cyclone Yasi on recruitment rate must be drawn carefully from this dataset. Nevertheless, the magnitude of recruitment decline in the time-window of the cyclone (77% decline at NW Orpheus and 90% decline at W Pelorus) was the greatest throughout the study period at both sites, and showed gradual recovery in subsequent months (Fig. 5.7).

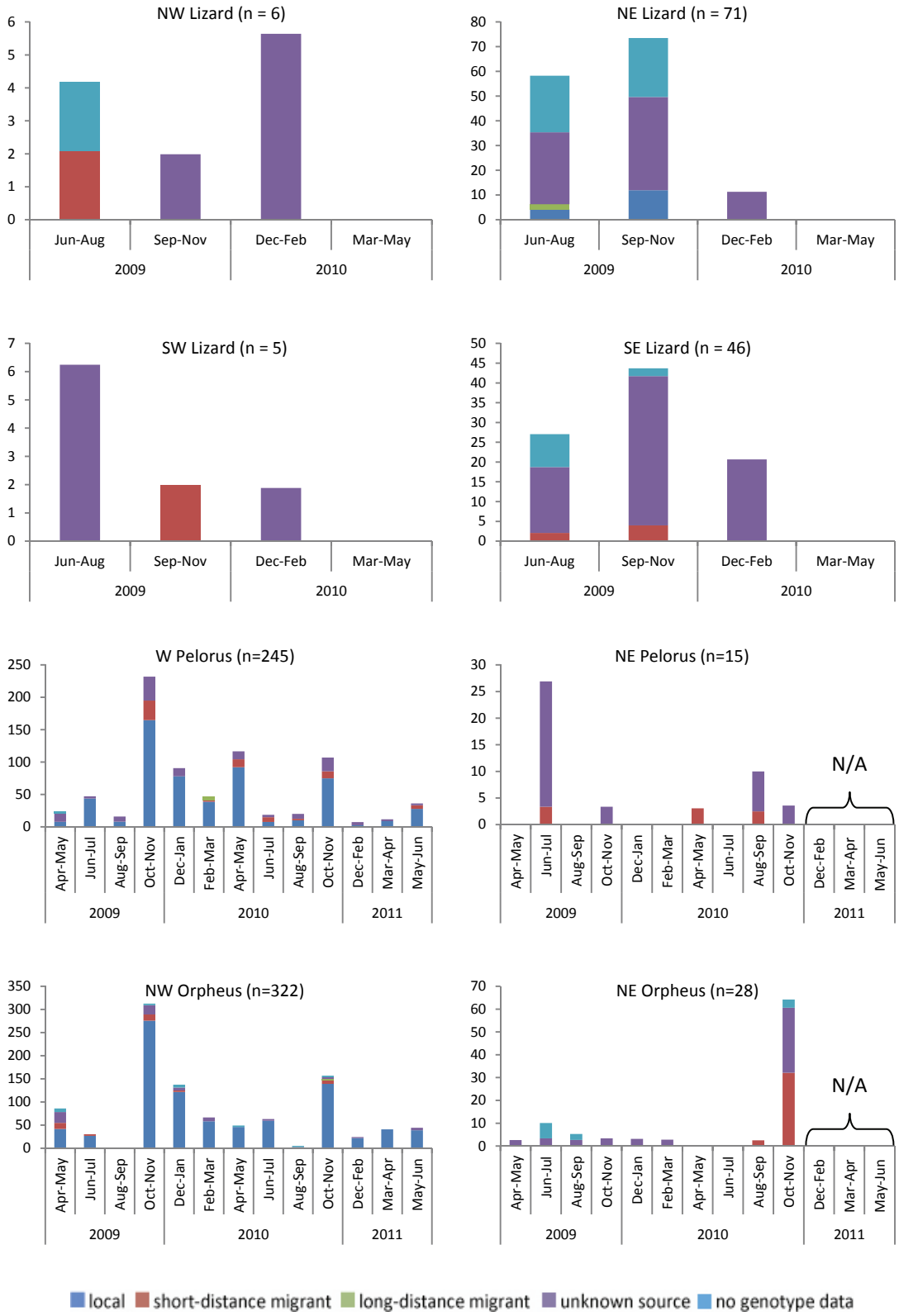


Fig. 5.6. Stacked bar chart of recruitment rate at each sampling site, dispersal distance category, and time-window. For each site, Y axis shows normalized mean number of recruits per day per 100 m^2. Potential dispersal distance classes were identified by genetic assignment tests. The actual numbers of recruits collected during the sampling period (n) are shown in brackets.

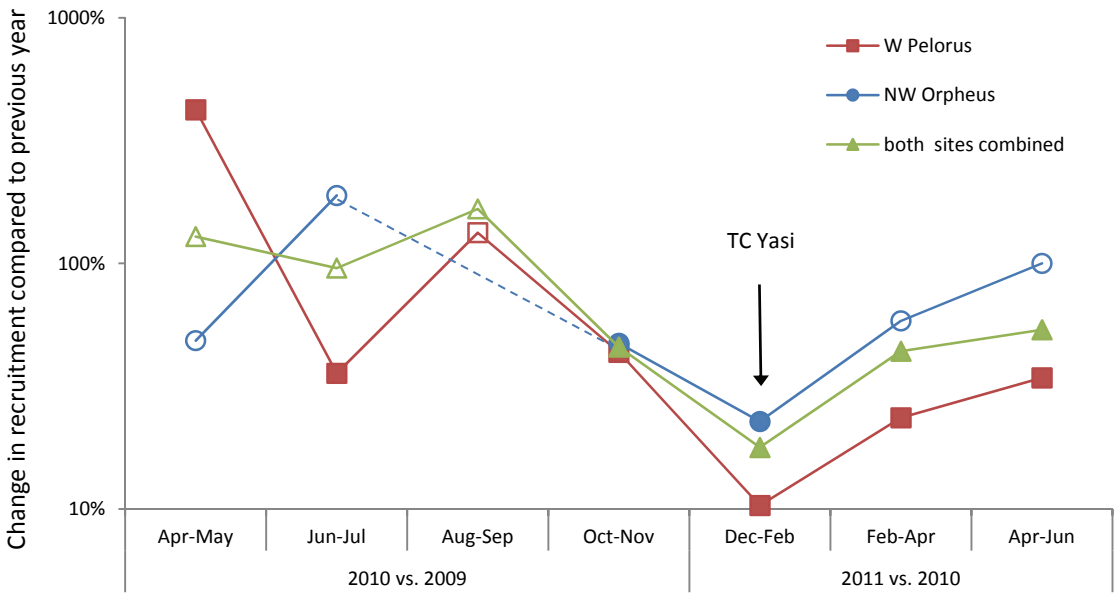


Fig. 5.7. Variation in recruitment rate between corresponding time-windows in consecutive years at sheltered sites of the Palm Islands. Y axis shows changes between the number of recruits in each time-window in a given year, compared to the previous year. Significance of the change in recruitment rates was tested by paired t-tests. Significant values are indicated by filled markers, non-significant values by hollow markers. Change could not be calculated for NW Orpheus in Aug-Sep 2010 vs. 2009 because the denominator was zero.

5.3.4. Genetic structure of adults and recruits

The curve of the mean estimated log probability of the Bayesian clustering results showed a plateau beginning at K=7 (Fig. 5.8), therefore I describe genetic structures at this number of clusters. To better interpret the results of the STRUCTURE analyses, I grouped adult specimens within each site based on delineation of putative cryptic species by P. Warner (unpublished thesis; Fig 5.9).

The recruits from Lizard Island that were genetically excluded from all known populations mainly clustered together, but were distinct from any of the adult populations (greyish-blue cluster; Fig. 5.9). Those recruits that were identified as philopatric by assignment tests, were similar to “exposed” type adults from both northern and central GBR populations. In the Palm Islands, recruits at W Pelorus and NW Orpheus mainly resembled the respective local adult populations that predominantly consisted of “sheltered” type specimens, with signs of admixture between the all sheltered sites of the region (Fig. 5.9). Approximately half of the recruits at NE Pelorus, and a few recruits at NE Orpheus belonged to the greyish –blue

cluster (Fig. 5.9) of the recruits of Lizard Island, that did not have members from the adult populations. The rest of the recruits at exposed sites of the Palm Islands resembled genetically adult populations in the sheltered sites. In general, the results of assignment tests and Bayesian clustering analysis were in agreement.

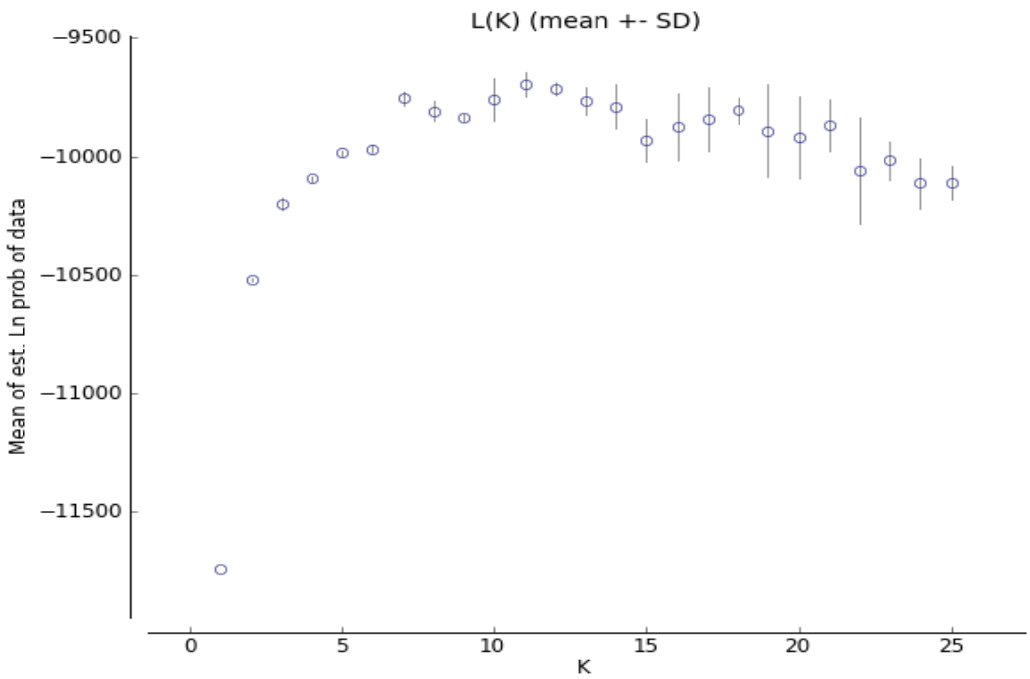


Fig. 5.8. Mean estimated log probability of the Bayesian clustering results of *Seriatopora hystrix* recruits. The curve reaches a plateau at K=7.

5.4. Discussion

5.4.1. Spatially variable recruitment patterns

This study shows that patterns of recruitment intensity and larval dispersal of *S. hystrix* are highly variable among sheltered and exposed sites in the northern and central GBR. Recruitment was most intense at sheltered sites of the Palm Islands, and at exposed sites of Lizard Island, and the origin of larvae settling at these well recruited sites showed remarkable similarities to those of two *P. damicornis* lineages (Chapter 4). Primarily philopatric recruitment around the Palm Islands, and mainly exogenous (or of unknown source) recruitment around Lizard Island are in agreement with expectations of an opportunistic settlement time in brooded coral larvae (Richmond, 1987; Isomura and Nishihira, 2001; Harii et al., 2002) combined with variable water circulation conditions

at these sites (Frith et al., 1986; King and Wolanski, 1996; Luick et al., 2007; Andutta et al., 2012) during multiple planulation events.

The fact that sites with relatively low recruitment rates always had low densities of mature colonies, supports previous conclusions of Underwood et al. (2009) that philopatric larvae are the primary source of recruitment in this species. Indeed, genetic assignments showed that at two out of three sites with high densities of reproductively mature colonies (W Pelorus and NW Orpheus), self-seeding was the dominant source of recruitment. In contrast, at the third high density site (NE Lizard) most recruits were excluded from the local adult population, and despite having the highest density of mature colonies among all sites surveyed, it did not have the highest number of recruits, or the highest rate of self-seeding (but note that 32% of recruits could not be genotyped at this site). This suggests that locally produced larvae are largely washed away from NE Lizard, but in turn currents bring substantial numbers of migrant larvae, underpinning the importance of inter-site variability of water movements on larval dispersal. The importance of larval import is further demonstrated at SE Lizard Island, where despite the low density of mature colonies, high numbers of recruits had settled, almost all of which were genetically excluded from all known adult populations. Stochasticity of larval dispersal (e.g. Siegel et al., 2008) could cause an unusually strong recruitment pulse at otherwise weakly seeded sites. However, the almost continuous year-round inflow of exogenous larvae to this site suggests that the cause of low population density is likely not due to the lack of larval supply, but rather to high post-settlement mortality. It is possible that recruits that were genetically excluded from all populations have travelled vast distances before settlement and approach senescence (Graham et al., 2008), and/or originate from very different habitats and are maladapted to the local environmental conditions, hence have higher than usual mortality rates. In summary, while self-seeding may be the general form of recruitment in *S. hystrix* where strong reproductive stocks are present and water movements favour larval retention (Andutta et al., 2012), other sites with lower larval output and/or stronger current activity may highly depend on exogenous larval replenishment.

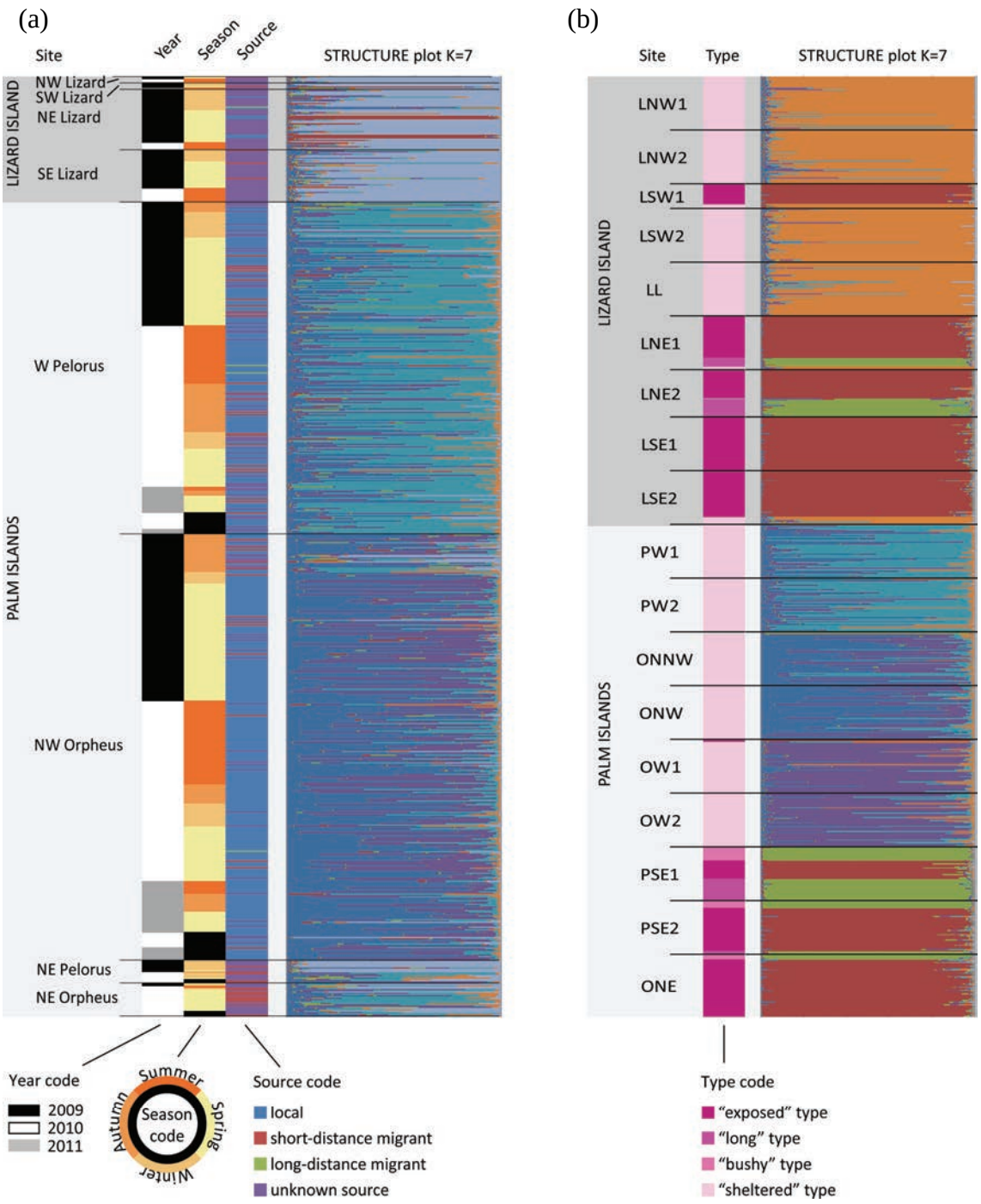


Fig. 5.9. Structure plots of *S. hystrix* recruits (a) and adults (b) at K=7. Bayesian clustering was run on the merged genotype database of adults and recruits, therefore clusters (colours) of the two plots are identical. Recruits are grouped hierarchically by site, year, and season of settlement. Estimated natal origin of each recruit is shown by a colour-code consistent with Figs. 5.1 and 5.6. Adult specimens are grouped by site and putative cryptic species identity, as identified by P. Warner (unpublished thesis).

5.4.2. Recruitment from unknown sources

While recruits excluded from all sampled populations made up a substantial proportion of genotyped recruits (16% at the Palm Islands and 86% at Lizard Island), the STRUCTURE analysis did not detect genetically similar adult specimens in any of the populations. This makes it unlikely that excluded recruits originate from a few under-represented genotypes in my reference dataset, and suggests that exclusion from all sampled populations is not a statistical artefact caused by the masking effect of other dominant genotypes among adults. However, it also raises questions as to where these recruits originate from and show no genetic affiliation to the sampled adult populations.

Given the low genetic differentiation between *S. hystrix* populations from similar habitats of Lizard Island and the Palm Islands (P. Warner unpublished thesis), it is reasonable to assume that the recruits that were genetically excluded from all sampled populations, originate from geographically distant areas. Populations of *S. hystrix* on the GBR are genetically highly differentiated (Ayre and Dufty, 1994; Ayre and Hughes, 2000; van Oppen et al., 2008). Given the small number of populations used as reference in genetic assignment tests in this study, it is a valid hypothesis that the unassigned recruits originate from other reefs on the GBR. Furthermore, a passive particle dispersal simulation showed that in a period half as long as the maximum documented pelagic larval duration (PLD) of brooded larvae of the closely related coral species *P. damicornis* (Richmond, 1987; Harii et al., 2002), particles may reach Lizard Island from Papua New-Guinea (Chapter 3). Since brooded larvae of *S. hytrix* are comparably well suited for long planktonic journeys (Isomura and Nishihira, 2001), unassigned recruits may also originate from populations beyond the GBR.

As an alternative hypothesis, I speculate that unassigned recruits originate from deep populations of *S.hystrix* that are maladapted to the shallow reef environment. This species is known to occur from the intertidal zone to mesophotic depths (Bridge et al., 2012), and strong genetic structuring has been observed along the depth gradient (Bongaerts et al., 2010b; van Oppen et al., 2011a). Although the fringing reefs of Lizard Island and the Palm Islands rarely extend below 20 meters, some deeper shoals and, especially in the case of Lizard Island, the continental shelf in their proximity may host genetically very distinct populations that have adapted to light and temperature

conditions that govern greater depths. Evidence for recruitment of larvae of deep water origin into shallower habitats was found in Scott Reef (Western Australia), but not on the GBR (Van Oppen et al., 2011a). Because these authors inferred migration from genotypic data of adult colonies, and I hypothesize that recruits of deep water origin have close to 100% mortality rate due to maladaptation to higher light and temperature levels on shallow reefs, their results do not contradict my hypothesis of vertically migrant larvae. Survival of such vertical vagabonds at Scott Reef but not on the GBR may reflect differences in the environmental conditions of shallow reefs of the two regions, or less stenoecious deep water lineages of *S. hystrix* in Western Australia. Assuming that unassigned recruits are of deep water origin, the larger number of deep habitats around Lizard Island also explains why such recruits were more common in this region.

5.4.3. Clonality in S. hystrix

Seriatopora hystrix is known as an exclusive sexual brooder (Ayre and Resing, 1986), therefore it is unexpected that 5.4% of all recruits were found to be clones of adult colonies or of other recruits. Clonal recruits were only found at W Pelorus and NW Orpheus, a result not surprising, considering that roughly 95% of all self-seeding and short-distant migrant recruits were collected at these sites, and the chance of sampling the clone mate of a recruit rapidly drops with increasing dispersal distance. Narrowing down the comparison to these two sites, the proportion of clones among recruits increases to 6.7%, a figure very similar to that found among recruits and adults of the closely related asexual brooder *P. damicornis* Type β (7% and 6%, respectively; Chapter 4). While it is an unexpectedly high ratio for the sexual brooder *S. hystrix*, this rate of clonality is surprisingly low for an asexual brooder species, and is thought to be the result of a combination of wide dispersal of clonal broods and retention of larvae produced via spawning of gametes (Chapter 4). Clonality in *S. hystrix* may originate from several sources. Polyps of *S. hystrix* can detach themselves from the colony and potentially re-settle, a mechanism termed polyp bail-out by Sammarco (1982) and popularly assumed to be a stress response. This phenomenon may be more common than initially assumed by Sammarco (P. Warner unpublished thesis), and may explain a moderate frequency of clonal propagules. A further potential source of clones in *S.*

hystrix, as it is for a wide variety of unrelated taxa, is polyembryony, i.e., the splitting of a sexually produced embryo into two or more (Craig et al., 1997). Yeoh and Dai (2010) argue that this mechanism created genotypically identical larvae in broods of sexually derived larva arrays in *P. damicornis*. Polyembryony alone, however, can only explain the presence of genetically identical recruits within one cohort, and fails to explain recruit-adult clone mates or recruit clone mates in several cohorts. Finally, *S. hystrix* may be able to brood asexually, through parthenogenesis or pseudogamy, similarly to the closely related *P. damicornis* (Stoddart, 1983; Ayre and Miller, 2004; Schmidt-Roach et al., 2012a), but this has not yet been documented for *S. hystrix*.

Alternatively, the detection of clones may be biased if outcrossing is not the only way of sexual reproduction, because traditional Genotype Probability estimates – that determine whether identical multilocus genotypes shall be considered true clones – are based on the assumption of random mating (Waits et al., 2001). *Seriatopora hystrix* is capable of self-fertilization, especially in the absence of mates or under sperm-limited conditions (Sherman, 2008), therefore, genetically similar multilocus genotypes of sexual origin may slip through the 0.001 Genotype Probability threshold and create false positives. This is especially true when a large number of adult colonies are heterozygous for only a low number of loci. In this study, 0.9 % of samples were heterozygous at only one out of ten microsatellite loci, 4.8 % at two, and 19.5% at three. Therefore it cannot be ruled out that the observed MLGs are results of sexually produced, but self-fertilized gametes.

5.4.4. Temporal variability of recruitment

The presence of newly settled recruits at all sampling time-points indicates year-round reproduction in *S. hystrix*, in agreement with observations from Palau (Atoda, 1951) and previous studies from the GBR (Sammarco, 1982; Tanner, 1996; P. Warner unpublished thesis), but annual patterns in recruitment intensities are emerging. According to Sammarco (1982), in the central GBR the “normal period for peak planulation” of the species is November, when hundreds of larvae are released per colony, as opposed to other months when only a few are released. November is also the peak of reproduction based on my results of larval settlement intensities on tiles, however, I have also detected a second peak of settlement at the Palm Islands between

April-June. Tanner (1996) reports planulation of *S. hystrix* during the austral summer months from the southern GBR, but that planulae were also present in polyps during February-March. Similar biannual reproductive peaks were reported from Eniwetok Atoll (Stimson, 1978). In contrast, the second peak of recruit settlement was not obvious around Lizard Island, but here recruits were collected at trimonthly intervals, providing less temporal resolution. Additionally, since most recruits at these sites are assumed immigrants, variable time lags between the planula release event and settlement of larvae may mask seasonal patterns of planulation; and with increasing distance between the natal reef and the site of settlement, recruitment stochasticity (Wallace, 1985; Connell et al., 1997; Smith et al., 2005) is expected to increase. And finally, planulation time in the assumed source populations of the recruits that were genetically excluded from all sampled populations (i.e., deep habitats; see above 5.4.2. Recruitment from other sources) may be dissimilar to that of the widely studied shallow reef *S. hystrix*, confounding seasonal patterns.

Despite annual patterns of reproduction, actual recruit numbers were highly variable between the years in corresponding seasons and sites, which hinders the evaluation of the impact of category five cyclone Yasi on recruitment. Tropical cyclones affect reefs in a variety of ways, from mechanical destruction, through changes in sedimentation, to decreased salinity (Harmelin-Vivien, 1994). Coral stocks on the sheltered side of the Palm Islands were largely spared from the devastation by waves, but were affected by the consequences of heavy precipitation in the aftermath of the cyclone. Accordingly, a major drop in recruitment rate was observed in the affected sampling period, and total mortality was observed for recruits beyond the first two months of settlement in this year. However, recruitment rate seemed to be on a recovery track two months after the cyclone, and the proportion of self-seeders vs. migrants did not change compared to pre-cyclone proportions, i.e. these sites remained predominantly self-seeding.

5.4.5. Implications for conservation

Differences in the intensity and origin of recruitment of *S. hystrix* between geographic regions, as well as between habitats of the same reef system within the same region suggest that different management strategies are required for the effective conservation

of these populations. While at first sight high recruit numbers at W Pelorus and NW Orpheus indicate that these sites are well seeded compared to others, their vulnerability becomes evident when we assess their connectivity to other reefs by looking at recruitment rate from non-local sources. All non-local categories of recruitment source combined, these sites received on average 33 recruits per day per 100 m^2, placing them behind the exposed Lizard Island sites in this respect, where average number of non-local recruits was 43 per day per 100 m^2. Given the low survival of *S. hystrix* recruits, these results suggest that if the local population is extirpated on the sheltered side of Orpheus and Pelorus, the population would not recover faster from exogenous larval import than the populations around Lizard Island. However, despite mass mortality of *S. hystrix* at the Palm Islands during the 1998 bleaching event (Marshall and Baird, 2000), the quick recovery of their populations underpin the hypothesis that small amounts of surviving coral tissue may provide good basis for stock regeneration following stress events (Underwood et al., 2007; Diaz-Pulido et al., 2009); and that high rates of self-recruitment enhances local population viability (Hastings and Botsford, 2006). Therefore, because the sheltered populations of the Palm Islands seem to be less connected to other reefs, and are highly self-recruiting, their persistence is highly dependent on local broodstock. In contrast, populations on the exposed sites of the Palm Islands, as well as sites around Lizard Island, proportionally rely more on exogenous larval replenishment, therefore their long-term conservation requires safeguarding upstream populations. A remaining grey area is the true origin and post-settlement faith of the genetically unassigned recruits, the resolution of which is required before drawing more detailed conclusions for conservation management.

5.5. Conclusions

I found that the source and number of *S. hystrix* larvae settling at exposed and sheltered sites of the northern and central GBR are highly variable, and I suggest that management strategies should follow these natural patterns. Heavily self-recruiting populations on the sheltered side of the Palm Islands likely require local protection, while sites around Lizard Island, which strongly rely on external larval supplement, would benefit more from an ecologically scaled network of MPAs (i.e. a network of

MPAs where protected areas are spaced in a way that the gap between them is bridgeable by organisms of conservation interest).

My results that the brooder *S. hystrix* are on par with those of the closely related asexual brooder *P. damicornis* (Chapter 3 and 4), suggest that the importance of asexual reproduction may have been underestimated in this species. Potential mechanisms behind the production of clonal propagules may be polyp bail-out (Sammarco, 1982), parthenogenesis, and polyembryony. Distance between clone mates provided direct evidence for routine dispersal up to 6.8 km in *S. hystrix*.

The annual patterns of recruitment reflected results of previous studies on the seasonality of planulation (Sammarco, 1982; Tanner, 1996), and confirmed the existence of a suspected second peak of reproduction in the austral late autumn (Tanner, 1996), additional to the austral late spring peak. However, in tandem with seasonality, strong inter-annual variation in recruit numbers was observed, especially on sites where the source of recruitment was primarily exogenous. This calls for caution in interpreting snapshot or short-term recruitment data. Accordingly, the impact of TC Yasi was partly masked by the “normal” variation in recruitment; nevertheless my results suggest that following this extreme disturbance event, recruitment levels returned to pre-cyclone levels a few months after the event.

Unveiling the true origin of the large numbers of recruits that have been excluded from all sampled populations calls for more research. Because these recruits belong to genetic clusters that are not represented by adult colonies, I suggest that their natal environment may be substantially different from the site of settlement, e.g. deep habitats, and their disappearance from the adult populations is the consequence of extreme mortality due to maladaptation.

Chapter 6

General discussion: major results and future research

6.1. Major results and conclusions of the thesis

1. A rapid genetic assay has been developed that identifies pocilloporid species and lineages, suitable for the identification of both coral adults and recruits (Chapters 2 and 5)
2. Populations of *Pocillopora damicornis* on the GBR are well connected over evolutionary timescales (Chapter 3)
3. The realized dispersal of *P. damicornis* and *Seriatopora hystrix* larvae varies from meters to potentially hundreds of kilometres (Chapters 3, 4 and 5)
4. Dispersal patterns are highly site- and species-specific; in general, larval retention is characteristic of Palm Island populations, and high larval dispersal is more common for Lizard Island populations (Chapters 4 and 5)
5. Recruitment in pocilloporid corals is year-round with seasonal peaks, but there are strong inter-annual variations in the intensity of recruitment (Chapters 4 and 5)
6. Both *P. damicornis* and *S. hystrix* are likely to be relatively resilient coral species on the GBR because substantial long-distance larval dispersal provides good population connectivity that is complemented by highly self-recruiting populations, which enables quick population recovery from local resources.

6.2. General discussion

6.2.1. Species identification of coral recruits – a new era for recruitment studies

Recruitment studies have been widely used to infer sustainability of coral populations (Hughes et al., 2002 and references therein), but the inability to determine the species identity of coral recruits has confounded species-specific patterns. The development of a genetic assay that groups pocilloporid recruits into seven taxonomic categories (1. *P. damicornis* Type α; 2. *P. damicornis* Type β; 3. other *Pocillopora* species and lineages; 4. *S. hystrix* Type S; 5. *S. hystrix* Type L; 6. *S. caliendrum*; and 7. non-*Pocillopora* and non-*Seriatopora* pocilloporids; Chapters 2 and 5) opens up new horizons for recruitment studies of pocilloporid corals. The importance of species identification in the analysis of recruitment patterns is well demonstrated by the species-specific patterns

found in the spatial and temporal variability of recruit numbers in Chapters 4 and 5. For example, by pooling all pocilloporids in an analysis of the impact of cyclone Yasi on recruitment around the Palm Islands, one would conclude that recruitment of *Pocillopora* species did not cease on the sheltered side of the islands following the cyclone. However, species-level analyses show that post-cyclone pocilloporid recruitment was dominated by *S. hystrix*, and very few *P. damicornis* larvae recruited into sheltered populations. Such species-specific differences in recruitment patterns reflect impacts of the cyclone on populations on the exposed side of the island, the main source of recruitment of Type α *P. damicornis*, where all mature coral colonies were removed by the extreme wave action of the cyclone (*pers. obs.*). Similarly, stock-recruitment relationships (e.g. Hughes et al., 2000) may be masked by pooling coral recruits on supra-specific levels. My results highlight that, as molecular resources advance to include a wider range of taxa, species-identification genetic assays should be developed for other scleractinian families and applied to coral ecology studies.

6.2.2. Anything is possible – scale-free patterns of genetic differentiation and connectivity in pocilloporid corals

Due to their maturity at the time of release, larvae of brooding corals are competent for immediate metamorphosis (Harrison and Wallace, 1990), and hence have capacities to settle in the vicinity of their mother colonies (Richmond, 1997); but they are equally well suited to maintain their competency for extended periods of time, enabling long-distance dispersal (Richmond, 1987; Harii et al., 2002). Metamorphosis and settlement of coral larvae are influenced by a wide array of substratum characteristics, e.g. a range of chemical cues (Negri et al., 2001; Baird and Morse, 2004; Gleason et al., 2009; Lee et al., 2009), turf algal cover (Birrell et al., 2005), sediment (Hodgson, 1990; Birrell et al., 2005), and fine-scale rugosity and irregularity of available substratum (Carleton and Sammarco, 1987). Additionally, water movement not only affects dispersal distances by physically retaining or dispersing coral larvae, but may also mediate various settlement cues (e.g. the concentration of attractants), or provide a cue in itself that induces settlement behaviour (Abelson and Denny, 1997). Indeed, settlement in *in vitro* cultures of coral recruits is usually induced by a combination of chemical cues and still water (B.L. Willis, D. Abrego, A. Moya *pers. comm.*). Therefore dispersal of brooded coral

larvae is expected to happen over a wide spatial range, from meters to hundreds of kilometres, depending on cues and forcing functions at the natal site.

Accordingly, I have found high levels of geneflow over evolutionary timescales (Chapter 3), as well as evidence for substantial larval immigration and emigration in certain populations of pocilloporid corals over ecological timescales (Chapters 4 and 5), but predominantly philopatric recruitment in others (Chapters 4 and 5). These findings are in line with seemingly contradictory results of previous studies on these species. For *S. hystrix*, strong genetic structures over small spatial scales (Ayre and Dufty, 1994; Ayre and Hughes, 2000; Maier et al., 2005; Underwood et al., 2007), occasional long-distance dispersal (Ayre and Hughes, 2000; Underwood et al., 2007; van Oppen et al., 2008), and even panmixia over several kilometres (Maier et al., 2009) has been reported. Similarly, previous studies of *P. damicornis* report sufficient gene flow to maintain panmictic populations among some locations over the length of the GBR (Ayre and Hughes, 2000), but significant genetic subdivision among other populations within reefs (Ayre et al., 1997). It is possible that some of the previously observed small-scale subdivision related to habitat types is an artefact of including different cryptic species, which were undescribed at the time, in analyses (e.g. in Ayre and Dufty, 1994; Benzie et al., 1995). Nevertheless, the common factor in this growing body of empirical studies is that, on average, the dispersal kernel of pocilloporid coral larvae ranges from the scale of meters to a very long tail that extends to hundreds of kilometres, following or approximating a power function that is expected from their capacity for both immediate settlement and long-distance dispersal.

6.2.3. Patterns of connectivity in pocilloporid corals and their implications for MPA design

There is an increasing body of evidence from physiological and genetic studies that the dispersal curve of pocilloporid corals may follow power laws (see above). Power laws are emergent general characteristics of complex systems (Brown et al., 2002), and have been described from as areas as different as stock markets (Gabaix et al., 2003), the Internet (Adamic and Huberman, 2000), species-area relationships (Sizling and Storch, 2003), species extinction risks in response to climate change (Thomas et al., 2004) or

physiological processes and lifespan in birds and mammals (Lindstedt and Calder, 1981). A common feature of phenomena that follow power laws is self-similarity on various spatial scales, or a fractal-like structure (Brown et al., 2002). To reflect the power function distribution of average dispersal in pocilloporid corals, the ideal theoretical design of a network of MPAs would approximate a fractal-like design, i.e. MPAs would be represented equally at multiple levels of spatial scales. However, such a network of MPAs would be very difficult to designate and manage in practice, and furthermore, would only be ideal if the average dispersal distances are considered, and regional and inter-habitat differences in the processes that govern dispersal are ignored. Because Chapters 4 and 5 showed that there are recognizable patterns in the spatial distribution of dispersal variability, namely high rates of larval retention in the inshore Palm Island group in the central GBR, and larval dispersal around the midshelf fringing reefs of Lizard Island, it is reasonable to aim at developing MPA designs that address local specifics of dispersal, rather than “one-size-fits-all” solutions. For instance, populations around Lizard Island depend more heavily on connections with other reefs, while populations in the Palm Islands are more self-sustaining (Chapters 3, 4 and 5). Greater self-seeding in the Palm Islands is the consequence of dissimilar inter-reef connectivity in these two regions, most likely driven by differences in ocean currents (Chapters 3, 4 and 5), suggesting that larger gaps between MPAs would be acceptable in the northern midshelf region of the GBR, and a denser matrix of MPAs would be desirable in the central inshore GBR in order to achieve similar levels of population connectivity of pocilloporid corals. Nevertheless, such a conclusion needs cross-validation by complex high-resolution circulation models that include tidal forcings (e.g. Lambrechts et al., 2008). Coupling of empirical genetic data with modelled geneflow has proven to be very powerful in estimating connectivity of coral populations in the Caribbean (Baums et al., 2006; Galindo et al., 2006; Kool et al., 2010; Foster et al., 2012) and will directly assist conservation management. To maximize the benefits from this thesis for conservation management, I suggest that its results are used to parameterize larval transport models in the region.

6.3. Future research

In this thesis, I aimed to characterize genetic structures, larval dispersal distances and recruitment patterns in pocilloporid corals, to better understand population connectivity over evolutionary and ecological timescales in these important reef organisms. The results have led to several questions and hypotheses that point beyond the scope of this thesis. The main hypotheses set up by results in this thesis are:

1. Larvae of *S. hystrix* from deep (or otherwise different) habitats settle in shallow reefs, but show (near) complete post-recruitment mortality (Chapter 5)
2. Locally retained larvae of *P. damicornis* originate predominantly from spawned gametes, while brooded larvae are mainly vagabonds (Chapter 4)
3. Regional variation in clonality of populations of *P. damicornis* is related to predation pressure on larvae/eggs (Chapters 3 and 4)
4. Asexual reproduction is an important part of the life history of *Seriatopora hystrix* (Chapter 5)

One of the surprising findings of Chapter 5 was that a large proportion of *S. hystrix* larvae, especially at sites around Lizard Island, were genetically distinct from all sampled adult colonies, leading to the hypothesis that these recruits may come from deep habitats on shoals and the continental slope. The diverse communities of mesophotic reefs are relatively poorly studied (Bridge et al., 2012), but have recently gained much attention as potential refugia for shallow water reef corals from temperature-related stress and severe tropical cyclones (Riegl and Piller, 2003; Bongaerts et al., 2010a; van Oppen et al., 2011a). Vertical connectivity between *S. hystrix* populations from shallow and deep habitats has not been confirmed from the GBR (Van Oppen et al., 2011a). However, these authors inferred larval connectivity from genetic structures of adult populations, whereas if deep water larvae have near-zero survival in the shallow environment due to maladaptation, their genetic signature may be absent from the shallow water adult population. A study that compares genetic characteristics of deep water populations from shoals and the continental slope around Lizard Island with that of the recruits collected in the shallow environment would be able to tease apart larval connectivity from deep to shallow environments. Additionally,

an inverse study, where recruits are collected from settlement tiles in the mesophotic zone, could address connectivity through larval dispersal from shallow to deep habitats.

Observing high proportions of self-recruitment, but low proportions of recruits that are clones of sampled adult colonies, I hypothesized that clonal broods of *P. damicornis* lineages disperse from their natal sites, and sexually produced larvae are retained locally (Chapter 4). Spawning of *P. damicornis* colonies on the GBR has been observed for the first time only recently, and eggs were found to be negatively buoyant (Schmidt-Roach et al., 2012b), supporting the hypothesis of high retention of larvae that develop from spawned gametes. However, conclusive evidence to accept or reject this hypothesis will only be provided by a study that achieves egg fertilization *in vitro* and describes physiological and behavioural characteristics of sexually produced larvae.

In contrast to the notion that *S. hystrix* is an exclusively sexual brooder (Ayre and Resing, 1986), my results show that 1.9% of larvae are clones of adult colonies, and 3.5% of larvae are clones of each other, suggesting that asexual reproduction may be an important part of the life history of this species (Chapter 5). This observation calls for further studies on the reproduction of *S. hystrix*, including exploring the frequency of polyp bail-out (Sammarco, 1982) and its potential role as a means of regular reproduction; and re-assessing clonality of broods, including checking for potential mosaicism (Van Oppen et al., 2011c) and chimerism (Puill-Stephan et al., 2011) in the parent colonies that may bias the study (Maier et al., 2012).

One hypothesis to explain why *P. damicornis* has highly clonal populations on some reefs [e.g. Western Australia (Stoddart, 1984), degraded reefs of Tanzania and Kenya (Souter et al., 2009), Southern Taiwan (Yeoh and Dai, 2010) and Vietnam (S.S. Windahl *pers. comm.*)], but not in other places [e.g. GBR (Ayre and Miller, 2004; Sherman et al., 2005)], is that predator pressure may differ between broods and/or spawned gametes in these locations (Chapters 3 and 4). A study that compares corallivorous fish abundance relative to coral cover with the rate of clonality in *P. damicornis* populations, and quantifies the effect of predation on pre-settlement mortality of broods and spawned gametes by *in vitro* experiments, could test this hypothesis.

Tagging fish larvae in their embryonic stage using stable isotopes of barium and magnesium (Thorrold et al., 2006; Woodcock et al., 2011) enables powerful mark-recapture studies to be used to assess dispersal. Currently, the technology to tag coral larvae is not developed because coral planulae do not possess stable structures that could be used for tag recovery. Research targeted at the development of trans-generational tagging in corals would provide invaluable benefits to studies looking into dispersal of coral larvae.

And finally, the complete removal of mature colonies of branching corals from the windward sides of the Palm Islands by TC Yasi (*pers. obs.*) provides an extraordinary opportunity to study reef regeneration following complete devastation. A long-term recruitment study that takes advantage of the recently developed genetic identification assays (Chapters 2 and 5) would be able to quantify the pace of recovery, and the rate at which larval replenishment from other reefs, *versus* re-growth from surviving tissue and juveniles (Diaz-Pulido et al., 2009) contributes to it. Such a study could also provide a comparison to results of Starger et al. (2010) and analyse coral reef recovery following complete devastation on a finer temporal scale. Genetic comparison of recruits that settle on tiles, and the new cohort of corals that grow and reach maturity on natural substratum could provide interesting insights into processes of post-settlement selection. A comparative population genetic study of pre- and post-cyclone populations on the exposed side of the islands could assess stability of population genetic structures following a major disturbance, and test the hypothesis that pulses of larval packets (Siegel et al., 2008) and recruitment stochasticity can create genetic breaks on small spatial scales (Chapter 3).

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