2016-12-08

Ecological and Genomic Patterns of the Threatened Coral Acropora cervicornis

Crawford Reeves Drury

University of Miami, cdrury@rsmas.miami.edu

Follow this and additional works at: https://scholarlyrepository.miami.edu/oa_dissertations

Recommended Citation
Drury, Crawford Reeves, "Ecological and Genomic Patterns of the Threatened Coral Acropora cervicornis" (2016). Open Access Dissertations. 1777.
https://scholarlyrepository.miami.edu/oa_dissertations/1777

This Embargoed is brought to you for free and open access by the Electronic Theses and Dissertations at Scholarly Repository. It has been accepted for inclusion in Open Access Dissertations by an authorized administrator of Scholarly Repository. For more information, please contact repository.library@miami.edu.
ECOLOGICAL AND GENOMIC PATTERNS OF THE THREATENED CORAL
ACROPORA CERVICORNIS

By

Crawford Reeves Drury

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

December 2016
UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

ECOLOGICAL AND GENOMIC PATTERNS OF THE THREATENED CORAL

ACROPORA CERVICORNIS

Crawford Reeves Drury

Approved:

Diego Lirman, Ph.D.
Associate Professor of Marine Biology and Ecology

Andrew Baker, Ph.D.
Associate Professor of Marine Biology and Ecology

Marjorie Oleksiak, Ph.D.
Associate Professor of Marine Biology and Ecology

Nelson Ehrhardt, Ph.D.
Professor of Marine Ecosystems and Society

Derek Manzello, Ph.D.
Research Scientist
National Oceanographic and Atmospheric Administration
Miami, Florida

Guillermo Prado, Ph.D.
Dean of the Graduate School

Miami, Florida
The staghorn coral, *Acropora cervicornis* is an important reef-building species on Caribbean reefs that has undergone severe population declines in recent decades. As the abundance of this species has diminished, restoration efforts using the coral gardening method have become increasingly common as a method of mitigating these losses. Using a large scale outplanting experiment, we find evidence of high phenotypic plasticity and low local adaptation driven by coral genotype and the environment, which subtly interact. The flexibility of the growth and survivorship response in this species gives concrete value to genetic diversity, which is variable across regional spatial scales and concentrated on individual reefs, indicating that restoration resources can accurately recreate wild assemblages. These wild reefs are also sometimes composed of thickets, which may be important for the persistence of this species. Thickets, which are not genetically distinct, can also be constructed from nursery resources by taking advantage of the combination of sexual and asexual reproduction apparent in this species. Sexual reproduction is also critical for species recovery, but this process appears to be decoupled from genetic patterns, suggesting that sexual reproduction on modern reefs is not contributing strongly to demographics. These analyses form a cohesive unit investigating the actual and potential response of one of the Caribbean’s most important species over various time scales, integrating growth, reproductive patterns and population dynamics.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>CHAPTER 1 – INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 2 – GENOTYPE AND LOCAL ENVIRONMENT DYNAMICALLY INFLUENCE GROWTH, DISTURBANCE RESPONSE AND SURVIVORSHIP IN THE THREATENED CORAL, ACROPORA CERVICORNIS</td>
<td>8</td>
</tr>
<tr>
<td>Background</td>
<td>8</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>11</td>
</tr>
<tr>
<td>Results</td>
<td>16</td>
</tr>
<tr>
<td>Discussion</td>
<td>23</td>
</tr>
<tr>
<td>CHAPTER 3 – GENOMIC PATTERNS IN ACROPORA CERVICORNIS SHOW EXTENSIVE POPULATION STRUCTURE AND VARIABLE GENETIC DIVERSITY</td>
<td>35</td>
</tr>
<tr>
<td>Background</td>
<td>35</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>41</td>
</tr>
<tr>
<td>Results</td>
<td>45</td>
</tr>
<tr>
<td>Discussion</td>
<td>49</td>
</tr>
<tr>
<td>CHAPTER 4 – THE GENETIC COMPOSITION OF ACROPORA CERVICORNIS THICKETS AND ISOLATED POPULATIONS</td>
<td>60</td>
</tr>
<tr>
<td>Background</td>
<td>60</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>63</td>
</tr>
<tr>
<td>Results</td>
<td>70</td>
</tr>
<tr>
<td>Discussion</td>
<td>74</td>
</tr>
<tr>
<td>CHAPTER 5 – DISPERAL, CONNECTIVITY AND POPULATION STRUCTURE IN ACROPORA CERVICORNIS ON THE FLORIDA REEF TRACT</td>
<td>86</td>
</tr>
<tr>
<td>Background</td>
<td>86</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>90</td>
</tr>
<tr>
<td>Results</td>
<td>93</td>
</tr>
<tr>
<td>Discussion</td>
<td>96</td>
</tr>
<tr>
<td>CHAPTER 6 – CONCLUSIONS</td>
<td>106</td>
</tr>
<tr>
<td>Summary</td>
<td>106</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>107</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>110</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>112</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>114</td>
</tr>
<tr>
<td>Conclusions</td>
<td>115</td>
</tr>
<tr>
<td>References</td>
<td>153</td>
</tr>
<tr>
<td>APPENDIX I – CHAPTER 2 SUPPLEMENTARY FIGURES &amp; TABLES</td>
<td>173</td>
</tr>
<tr>
<td>APPENDIX II – CHAPTER 4 SUPPLEMENTARY FIGURES</td>
<td>178</td>
</tr>
<tr>
<td>APPENDIX III – CHAPTER 5 SUPPLEMENTARY FIGURES</td>
<td>181</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1.1 - Dissertation Structure ................................................................. 117
Figure 2.1 - Experimental Design and Timeline............................................. 118
Figure 2.2 - Growth of pooled corals by treatment. ........................................ 119
Figure 2.3 - Local Adaptation comparisons of growth................................. 120
Figure 3.1 – Map of collection locations. .......................................................... 125
Figure 3.2 - Discriminant Analysis of Principal Components for all populations. ... 126
Figure 3.3 – DAPC for a) Florida populations and b) Florida populations without Broward.................................................................................. 127
Figure 3.4 - Spatial Interpolation of Genetic Diversity ....................................... 132
Figure 3.5 – Expected heterozygosity as a function of number of individuals......... 129
Figure 4.1 – Map of thicket locations and picture of example landscape............. 134
Figure 4.2 – DAPC for plots and isolated colonies............................................. 135
Figure 4.3 – Map of genetic diversity interpolation at Cheetos, FL thicket.......... 136
Figure 4.4 – Diversity metrics in thickets and isolated communities as a function of sample size at Cheetos, FL ...................................................................... 137
Figure 4.5 – Percent cover and genetic diversity ................................................. 138
Figure 5.1 – Map of Release Polygons ............................................................... 145
Figure 5.2 - Network Diagram for FRT populations at 4 DTC............................ 146
Figure 5.3 – Network Diagram for FRT populations at 7 DTC............................ 147
Figure 5.4 – Dispersal densities for particles released from each region.............. 148
Supplementary Figure 2.4 ............................................................................... 173
Supplementary Figure 2.5 ............................................................................... 174
Supplementary Figure 4.6 ................................................................................................................. 178
Supplementary Figure 4.7 .................................................................................................................. 179
Supplementary Figure 4.8 – Maps of genetic diversity for Sunny Isles ................................. 180
Supplementary Figure 5.5 Normalized Probability Matrix of All Release Polygons for
4 DTC.................................................................................................................................................. 181
Supplementary Figure 5.6 Normalized Probability Matrix of All Release Polygons for
7 DTC.................................................................................................................................................. 182
LIST OF TABLES
Table 2.1 – Growth of specific genotype x environment combinations..........................121
Table 2.2 – Bleaching prevalence of specific genotype x environment combinations ...126
Table 3.1 – Sample collection locations......................................................................130
Table 3.3 – Analysis of Molecular Variance................................................................132
Table 3.4 – Patterns of genetic diversity within reefs and between nursery and wild
populations. ................................................................................................................133
Table 4.1 – Collection locations and plot arrangement..............................................139
Table 4.2 – Pairwise F_{ST} between pooled thicket and isolated colonies ................140
Table 4.3 – Pairwise F_{ST} between individual plots and isolated colonies .............141
Table 4.4 – Allelic Patterns between thickets and isolated communities...............142
Table 4.5 – Summary of Overall Results .....................................................................144
Table 5.1 – Patterns of directional dispersal between populations .........................149
Table 5.2 – Patterns of relative settlement within regions ......................................150
Table 5.3 – Pairwise Fixation Index and Nei’s Genetic Distance.............................151
Table 5.4 – Allelic Patterns of Regional Populations.................................................152
Supplementary Table 2.3..........................................................................................175
Supplementary Table 2.4..........................................................................................176
Supplementary Table 2.5..........................................................................................177
CHAPTER 1 – INTRODUCTION

Coral reefs are some of the most biodiverse communities on earth (1) and are presently facing serious threats to their survival (2, 3), with few pristine reefs remaining. Reef ecosystems only cover approximately 0.1% of the ocean floor (4), but provide invaluable services to humans and other marine ecosystems (5). Their continued survivorship (and recovery) are thus closely tied to the livelihoods of at-risk human populations that depend directly and indirectly on these ecosystems. A combination of factors including increasing temperatures, ocean acidification, eutrophication, coral disease, storm damage, and overfishing (2, 6-10) interact to threaten coral reefs. Though this ecosystem is in decline worldwide (11-13), the Western Atlantic has seen some of the most severe degradation, with a loss of nearly 80% of coral cover since the 1980s (14) and an associated phase shift form coral dominated to algal dominated habitats (15). This decline is also subject to bias due to shifting baselines, because reefs of the 1980s were likely already significantly degraded from their pristine, natural state (16).

As reef science has developed from observational to experimental, our understanding of these ecosystems has matured considerably, especially due to complex experimentation using field based frameworks. In situ manipulations represent the best available methodology for investigating questions about the ecological and evolutionary processes on reefs because they incorporate the natural suite of environmental variables and fluctuations, providing a more holistic understanding of how to conserve, protect and restore remaining resources. One framework for examining these processes is coral propagation and restoration (17), developed to restore degraded reefs and aid species recovery without continuously depleting a donor population (18). This methodology uses
in situ nurseries populated through small collections of donor colonies that are propagated and then ‘outplanted’, or returned to wild reefs or degraded sites (19). The use of nurseries facilitates an experimental paradigm to investigate the ecological processes that dictate growth, survivorship, and disturbance response in cultivated species.

The coral gardening methodology has become increasingly important in the Caribbean and is now the primary restoration technique for mitigating declines in reef cover and structure (20, 21). Coral gardening primarily focuses on branching taxa like Acropora, but recent advances in propagation techniques have expanded the types of corals kept within nurseries (22). Acropora, a fast-growing genus (23) that reproduces by fragmentation (24), is ideally suited for use with the coral gardening methodology because it can be quickly and efficiently propagated in the nursery environment (19), providing a sustainable source of corals and genetic diversity for restoration (25). In the past decade, Acropora cervicornis has become the primary focus within the Caribbean (20) because it is an important reef-builder that provides structure and habitat for many associated organisms (26), but has also undergone particularly drastic declines (27). In some areas, more than 95% of Acropora cervicornis has disappeared (28), primarily due to White Band Disease (8, 29, 30) and a range of other factors negatively influencing modern reefs. Declining abundance has prompted a shift in the population structure of this species, from thickets, formerly dominant structures composed of densely packed interlocking colonies, to sparse isolated colonies at low densities (31). Declining acroporid populations have also given way to reefs dominated by other species (32). These shifts are unprecedented in the geologic record (33) and Acropora cervicornis is now listed as threatened under the United States Endangered Species Act (34) and
considered Critically Endangered by the International Union for Conservation of Nature (35).

In response to the decline of this important reef-building genus, a community of practitioners that includes academic institutions, research labs, NGOs, and local and regional government agencies has come together to design and implement the Acropora Recovery Plan. This plan includes both management and active restoration tools for species recovery. Central to the active recovery of this species is the scientific support provided by researchers to these activities. In this dissertation, I was able to use the resources provided by the coral gardening methods to investigate the relationships between evolutionary and ecological processes in support of species recovery.

Coral nurseries serve as a common garden and can be combined with reciprocal transplantation (36), the standard for investigating ecological responses across environmental gradients [Chapter 2]. The common garden limits maternal affects, primarily through acclimatization to the same environmental conditions, helping to maximize genetic and heritable influences (36). To completely eliminate maternal effects through long term plasticity (37), at least two generations should be reared in identical conditions, however this is rarely possible with many marine species which have pelagic larvae, such as scleractinian corals (38). In species like *A. cervicornis* that can reproduce asexually, the nursery environment also allows for the creation of many genetically ‘identical’ replicates, increasing the statistical power of experimentation. This framework allows for a detailed assessment of genotype (39, 40) and environmental response (41), local adaptation (36), and phenotypic plasticity (42), critical for understanding the fate of a threatened species that has undergone a population bottleneck and may rely heavily on
flexibility to persist in rapidly changing conditions. The relationship between experimentation and the coral gardening methodology also represents a feedback loop, where scientific insights gained through active restoration can further increase the effectiveness of these practices [Chapter 2-4].

The expanding scope of active coral restoration also requires high-quality data on the evolutionary implications of intervention, primarily though genomic data on patterns of ‘wild reefs’ and the genetic resources housed within in situ nurseries [Chapter 3,4]. Without such data, achieving the goal of creating functional ecosystems via active restoration is unlikely (43, 44), so these resources are important and have practical application for several reasons. First, describing and quantifying the genetic diversity and genotypic or allelic richness can ensure that restored populations replicate natural assemblages, protecting their adaptive potential (45). Second, increased resolution of genetic data explains population structure and differences between regions (and individual nurseries), better informing the spatial units of management and describing connectivity and complex networks of genetic relatedness (46-49). These data can also describe genetic patterns on individual reefs, including differences among isolated colonies and spatial trends within thicket assemblages that dictate how management practitioners should construct thickets. Finally, genomic resources provide insight on the evolutionary implications of intervention, describing allelic patterns, inbreeding, local adaptation and founder’s effects (44). As genomic technology develops from descriptive to functional roles, the integration of evolutionary and ecological approaches can provide a stronger understanding of the past and future of Caribbean reefs through the lens of *Acropora cervicornis*. The coral gardening methodology facilitates these investigations,
but also benefits from the integration of improved understanding of genomic patterns with restoration practice.

Acroporids have long been recognized as important reef-builders in the Caribbean, where staghorn and elkhorn coral were among the most abundant species, frequently dominating shallow and mid-depth reefs. As the study of this genus has progressed, our understanding of the sexual reproduction (50-55), asexual reproduction (24, 56), the formation of thickets (31, 57-59), demographics (28, 31, 56, 60-62), clonality (63-65), symbiosis (39, 48, 66, 67), response to stressors and disease (8, 30, 32, 68, 69) and environmental gradients (70, 71) has expanded. In addition, patterns of population structure have been described at high resolution (46-49, 51, 65, 72, 73). Despite the growing body of literature, there are still significant gaps in the current knowledge of *A. cervicornis* in the context of widespread challenges to species persistence that I address in this dissertation (Figure 1.1). Little is known about the ability of individuals to survive and respond to change or stress over different temporal scales, which is key for tolerance and resilience needed to maintain reef ecosystems (74). These gaps can be roughly divided into two areas: individual flexibility in coping with change and population-level evolutionary impacts (Figure 1.1). ‘Flexibility’ encompasses intra-generational changes including phenotypic plasticity, local adaptation, symbiosis dynamics, and the influence of environment and genotype on growth and survivorship [Chapter 2]. Thickets also represent a form of flexibility because they form fundamentally different structural assemblages than isolated colonies [Chapter 4]. This area of research represents complex ecological processes that require active manipulation to parse and are thus facilitated by the use of common gardens and reciprocal transplantation during active restoration.
‘Evolutionary impacts’ include population level processes of the past and future that dictate species trajectory, but also incorporate the impacts of processes that occur in individuals and over shorter (intra-generational) timespans (Figure 1.1). This gap in understanding includes patterns of population structure and genetic diversity on multiple spatial scales, spatial components of diversity, connectivity, the relationship between nursery stocks and wild reefs, and allelic patterns that may influence the future of this species under changing conditions [Chapter 3-5]. Recent evidence suggests that the adaptive potential of individual corals is higher than previously expected (37, 75, 76), but ecological and evolutionary processes must interact for the time required to adapt to be viable for the survival of coral reefs (74, 77). These outcomes are integral to management and conservation, with implications for nursery selection and design, outplanting technique, management scale, targeted regions and corridors for protection, focal points for intervention, and the overall consequences of restoration (Figure 1.1).

To address these knowledge gaps, I used the framework of active restoration, coupled with genomic techniques and connectivity modeling (Figure 1.1). I used reciprocal transplantation [Chapter 2] to assess the hypotheses that 1) individual genotypes show phenotypic plasticity dictated by site, genotype and their interaction, and 2) local adaptation is common in A. cervicornis as in other coral species. Next, I used genomic resources to investigate wild reefs and nursery populations [Chapters 3,4] to investigate the hypothesis that 3) there is significant population structure and genetic variability within Caribbean populations, 4) nursery stocks realistically represent wild assemblages and 5) thickets represent genetically distinct assemblages which are mainly clonal. Finally, I used connectivity modeling [Chapter 5] to assess the contributions of
differential mortality and larval connectivity to Florida populations, testing the hypothesis that genetic population structure can be explained by gene flow due to larval dispersal. Overall, these hypotheses form a cohesive unit investigating the actual and potential response of one of the Caribbean’s most important and threatened species over various time scales, integrating growth, reproductive patterns and population dynamics.
CHAPTER 2 – GENOTYPE AND LOCAL ENVIRONMENT DYNAMICALLY INFLUENCE GROWTH, DISTURBANCE RESPONSE AND SURVIVORSHIP IN THE THREATENED CORAL, ACROPORA CERVICORNIS

Background

Coral reef ecosystems worldwide have experienced chronic degradation over the past century, with particularly drastic declines in recent decades. This deterioration is likely to continue as multiple natural and anthropogenic stressors interact on various spatial scales, impacting coral survivorship, growth, and reef structure and function (1). Among the most impacted regions, the Western Atlantic has lost nearly 80% of coral cover since 1970 (2), driven partially by the loss of staghorn coral, Acropora cervicornis. This species plays a critical structural role as a reef-builder, creating habitat for fish and invertebrates and consolidating loose sediments and rubble (3). A. cervicornis was formerly dominant throughout the Caribbean, but the impacts of White Band Disease and bleaching (4-6), storm damage (7) and the die-off of Diadema antillarum (8) have resulted in drastic population declines. While a few areas maintain healthy populations (9), other areas of formerly high abundance have lost > 95% of colonies (10, 11), causing an unprecedented shift in community structure (12). In response to regional declines, A. cervicornis was listed as ‘threatened’ in 2006 under the United States Endangered Species Act (13) and is considered ‘critically endangered’ by the IUCN (14).

Understanding the intrinsic capacity for acclimatization and adaptive response to stress is vital for the long-term persistence of any species facing complex environmental change (15-17). These processes are partially dependent on the influence of environment and genotype and serve to integrate their effects, which can be evaluated as phenotypic plasticity and local adaptation. Phenotypic plasticity is a characteristic of a trait (such as
growth) in response to an environmental stimulus that provides the ability to produce different phenotypes from the same genotype (18, 19), facilitating a ‘better’ potential phenotype-environment match (20) across habitats. Recent evidence suggests that phenotypic plasticity may play a role coral’s response to change over intra- and transgenerational timescales, interacting with the effects of genetic adaptation across generations (21, 22). These processes may be particularly important for long-lived individuals which must potentially cope with change over centuries (23).

Plasticity is evident in coral morphology (24-26) and is well known in plants, where it can be evolutionarily important (27, 28). However, traits that influence the fitness of an organism, such as growth and survivorship, have remained mostly unexamined in the context of plasticity, though descriptions of these trends in single environments show genotypic differences (29, 30). Phenotypic plasticity supports genetic diversity under stabilizing selection by enabling different individuals to maintain fitness by expressing advantageous phenotypes for a range of genotypes (31), which may be particularly important for sessile organisms that are unable to seek more hospitable conditions. Maintaining genetic diversity improves the potential to adapt to novel environmental changes like climate change (32) and increases resilience (33). In *A. cervicornis*, the reliance on asexual reproduction means that branches (ramets) from a single individual can be long-lived (23) and may be transported into novel reef microenvironments (34), underscoring the importance of plasticity (26). Further, during active restoration, plasticity allows corals to cope with changing or novel conditions, which may lead to expression of formerly cryptic genetic variation (35, 36). Plasticity also dictates the range of environmental conditions where a given organism can
reasonably be expected to survive and grow, critical for the persistence of threatened populations in changing climates (21, 37).

Another avenue for understanding the influence of environment and genotype is local adaptation, which is common in a variety of taxa (38) and has been examined in corals via transplantation (39, 40), laboratory experiments (41, 42), and inferred from population structure (43, 44). In marine organisms, local adaptation was historically considered unlikely because populations were thought to be demographically open, with high connectivity introducing new alleles (45). However, population-genetic studies generally suggest more closed systems (46). Restricted connectivity may be due to oceanographic patterns, larval behavior, life history, and post-settlement phenotype-environment mismatches (20, 37), which allow for divergent selection in different habitats. On the Florida Reef Tract, recent evidence shows significant population structure among *A. cervicornis* populations and high diversity within individual reefs (47). Thus, the fine-tuning of populations to the local environment via natural selection may be important for the survivorship of depleted coral populations.

In this study, we employ a common garden and reciprocal transplantation design to analyze local adaptation, phenotypic plasticity, reaction norms, and disturbance responses between genetically distinct *A. cervicornis* individuals. Local adaptation is examined here by the study of native vs. foreign populations of transplanted corals, emphasizing the comparison within individual reef habitats (48). Our research focuses on small spatial scales with distances between sites as low as 2 km and a range of reef environments, treating habitats as points along a continuum rather than focusing on specific environmental clines. We also assess phenotypic responses across various
environments using reaction norms, where comparisons can be made between individuals
to reveal potentially ‘specialist’ or ‘generalist’ genotypes (49). Using a variety of reefs
(environments) and individuals (genotypes) also enables the description of the influence
of each factor within the larger context the ecological response to active restoration in a
threatened species. We test the hypotheses that: 1) Site and Genotype have significant
effects on coral growth, survivorship and bleaching response; 2) there is a dynamic
relationship between genotype and the environment where phenotypic plasticity produces
variable reaction norms; and 3) local adaptation exhibited in native vs. foreign
comparisons favors corals returned to their original collection site over corals sourced
from other reefs. These data will increase the efficiency and efficacy of active restoration
while developing a better understanding of the roles of adaptation and acclimatization in
coral reef health and resilience through environmental and genotypic effects.

**Materials and Methods**

**Experimental Design**

We use a reciprocal transplantation experiment where donor reefs (original
collection sites) receive native and foreign corals propagated in a common garden
nursery. Under this fully crossed design, each site received every genotype from the
common garden, including corals originally collected from that site and wild controls.
The use of an *in situ* common garden serves as a genetic repository (50) and limits
maternal effects by providing a single, consistent environment during propagation,
helping to distinguish genetic differences from prior long-term acclimatization (origin
site effects) (48). During 2015, high summer temperatures caused bleaching among
experimental corals, providing the additional opportunity to examine the role of GxE in stress response. As a result, growth (March-June), bleaching susceptibility (July-August), and post-bleaching recovery (December) were analyzed separately (Figure 2.1a).

Nursery Collections, Outplanting & Monitoring

Corals were collected from ten reefs in Miami-Dade County, Florida, USA during February-March 2014 (Figure 2.1b; Appendix I). Site selection was designed to cover a large spatial range and the various habitat types of the Florida Reef Tract where *A. cervicornis* is present, including shallow and intermediate-depth sites (~2-11m depth), inshore-offshore sites, and patch reefs and consolidated hardbottom habitats (Appendix I). At each site, 10-15cm branches were collected randomly from large, healthy donor colony and maintained in an *in situ* nursery until outplanting. Corals were haphazardly distributed among floating trees within the nursery and maintained until the beginning of the experiment (12-13 months). Collections were genotyped using Genotyping by Sequencing (51) and represent 10 unique individuals (Drury, Chapter 3). In spring of 2015, ten nursery-grown corals from each genotype were fragmented from the nursery and outplanted to eight of the original collection sites (Figure 2.1b). All fragments outplanted from the nursery were approximately 10cm and only contained single branches when possible. There were no significant differences in initial size or number of branches between genotypes or sites. Two of the original collection sites were not used for outplanting due to logistical constraints (Appendix I). Plots (approximately 2.5m x 3m) were created with nails on bare substrate approximately 25cm apart and were haphazardly populated with each genotype. Each plot was also populated with ten 10cm
fragments from each of two wild controls haphazardly selected at each site. Control corals from each genotype were outplanted on fixed-to-bottom platforms within the nursery. Measurements of total linear extension (LE), number of branches, condition, and survivorship were taken for every individual at installation and after 3, 4, 5 and 8 months. Corals were collected, maintained and outplanted under Biscayne National Park Permits BISC-2014-SCI-0018 and BISC-2015-SCI-0018 and Florida Fish and Wildlife Conservation Commission Special Activities License SAL-14-1086-SCRP.

*Genetic and Physiological data*

Immediately prior to outplanting, at least 7 replicate branches were collected for initial sampling of each genotype. Scrapings (1-2 polyps) were collected haphazardly from several locations on each fragment for all samples and were preserved in SDS (1% in DNAB) for *Symbiodinium* community analysis. Samples preserved in SDS were incubated at 65 °C for two hours to prepare a RT-stable archive. This archive was sub-sampled and DNA extracted using a modified organic extraction protocol as in (52). Symbiont clade was determined with qPCR using actin primers and probes to investigate the impact of initial *Symbiodinium* community on coral growth and survivorship as in (53). The remaining skeleton and tissue were frozen at -80 °C for analyzing lipid content. Coral tissue was removed from the skeleton of each sample using an airgun with freshly filtered seawater (0.35µm), homogenized, vacuum-filtered onto a glass fiber filter (Wattman GF/A), and frozen at -80 °C for lipid extraction. Total lipid extraction followed the protocol of (54). Skeletal sample surface area and volume were calculated from 3D scans as in (55). Fragments were scanned using a white light 3D scanner (HDI Advance...
R2, 3D3 Solutions) using two 2-megapixel monochrome cameras and calibrated with a 5mm calibration board. Each sample was scanned every 20° rotation and scans were aligned and compiled into a single model using Flexscan 3D software. Models were trimmed, resampled to reduce holes in the model, and surface area and volume were measured using Leios 2 software. Skeletons were then dried overnight at 60C and weighed to calculate density.

*Environmental data*

Water temperature was recorded using logger pendants installed on a single nail within the plot (Onset Corp #UA-002-08; Massachusetts, USA) at hourly intervals. At each monitoring interval, seawater collections were made at depth in the center of the plot with a 500mL borosilicate bottle and poisoned with 200µL mercuric chloride for determination of carbonate chemistry. Temperature was measured at the time of water collections using a YSI Inc. YSI-85. Salinity was measured from water samples with a densitometer. Water samples were analyzed as in (55). Briefly, dissolved inorganic carbon (Apollo SciTech AS-C3) and total alkalinity (Apollo SciTech AS-ALK2) were determined and input into CO2SYS (56) to calculate aragonite saturation state ($\Omega_{\text{arag}}$) using the dissociation constants of (57) as refit by (58) and (59) for boric acid. In September 2015, comparative light readings were taken for several days at each of four sites using PAR loggers (WETLab ECO-PAR; Oregon, USA). The loggers were installed immediately adjacent to plots and recorded total PAR (µmol/m²/sec) at 15-min intervals. Due to logistical constraints, light data were collected during a one-week deployment at paired sites (‘Cooper’s’ and ‘Inshore’) and over a subsequent 6-day deployment at a
separate pair of sites (‘Struggle Bus’ and ‘CVFD’). Due to fouling, only the first 4 days of each deployment were used for analyses. Daytime light levels (9 am-5 pm) used in this study were averaged across all 4 days.

**Statistical Analyses**

All analyses were performed in JMP Pro 11.0.0. Coral growth over the first 3 months was converted to a daily growth rate and normalized to initial number of branches (29). Data were transformed when necessary to meet homoscedasticity and normality assumptions of parametric tests and non-parametric tests were used when assumptions were not met. Only growth data over the first three months of the experiment were used, as subsequent bleaching stress had a substantial impact on growth. A 2-way ANOVA was used to examine main factors and subsequent 1-way ANOVAs were used within sites and within genotypes. One-way ANOVA was used to test differences within genotypes (across sites) and within sites (across genotypes). To evaluate growth in the context of phenotypic plasticity, average growth rate for each genotype was transformed into rank relative to the average growth at each site (above, at, below) and each G x E treatment was classified. These values represent the reaction norm, or the spectrum of growth rates (phenotypes) for a given genotype across environments, which can vary in shape, magnitude, or both. To examine bleaching susceptibility and recovery, conditional distributions were tested using $\chi^2$ tests. Mortality was examined using a Cox-Proportional-Hazard model with Site, Genotype and interaction as factors. To test local adaptation, t-tests were used within each site with growth pooled between native corals from that site (home site and control genotypes) and corals from foreign sites (all other
genotypes; Figure 2.1b). T-tests were used to compare growth, bleaching and mortality between ‘high’ and ‘low’ light regimes and ‘warm’ and ‘cool’ temperature regimes. Bonferroni corrected p-values were used for within site, within genotype and site-specific local adaptation comparisons.

**Results**

**Overall Growth**

930 coral fragments representing ten nursery genotypes plus site controls were outplanted to eight reefs. One site (Jon’s Reef) did not receive one genotype (Cooper’s, due to limited fragment availability) or wild controls (no wild colonies were found on site). To test a fully crossed design, one site (Jon’s Reef) and one genotype (Government Cut) were eliminated from the two-way ANOVA, chosen to preserve a balance in number of treatments by site (n=7) and genotype (n=9). Growth rate (Linear Extension, LE) ranged from 0.005-0.067 cm/day across all genotypes and sites, with an experiment-wide average of 0.026 ± 0.001 cm/day (mean± 1 S.E.). A two-factor ANOVA showed a significant effect of Genotype ($F_{(8,356)}=4.743$, p<0.001) and Site ($F_{(6,356)}=18.316$, p<0.001), but no significant interaction effects ($F_{(48,356)}=1.222$, p=0.159). The ANOVA explained 41% of overall variance, with Site and Genotype explaining ~19% and ~6% respectively. Post-hoc tests identified three significantly different LE classes among the 7 reef sites (Figure 2.2a; Tukey’s HSD, p< 0.05) and two classes among the 9 coral genotypes (Figure 2.2b; Tukey’s HSD, p< 0.05).
**Growth by Site**

There were significant differences between Sites, with over a three-fold difference in LE, ranging from 0.010 ± 0.001 cm/day at Steph’s Reef to 0.044 ± 0.004 cm/day at Struggle Bus (Figure 2.2a). There were significant differences in LE between genotypes within a site at 3 of eight of reefs (Table 2.1a). There were 2 significant genotype-growth levels identified at Jon’s Reef (ANOVA, F(8,69) = 5.466, p<0.001) and 3 significant levels at Inshore (ANOVA, F(10,42) = 6.453, p<0.001) (Table 2.1a). There were also significant differences between genotypes at CVFD (Kruskal-Wallis, H=32.023, p=0.001).

**Growth by Genotype**

There were significant differences in LE among genotypes (Figure 2.2b), with growth rates ranging from 0.016 ± 0.002 (mean cm/day ± 1 S.E.) for CVFD to 0.036±0.003 cm/day for Government Cut. When each genotype’s LE was examined among sites, there were significant differences within 7 of 10 genotypes (Table 2.1b). Based on Tukey’s HSD, there were 2 significant groupings of transplant reefs based on growth rates for the Struggle Bus genotype (ANOVA, F(7,54) = 4.550, p=0.001) and 3 levels for the Coopers (ANOVA, F(7,38) = 4.825, p=0.001), Government Cut (ANOVA, F(6,53) = 4.859, p=0.001), Inshore (ANOVA, F(7,49) = 4.309, p=0.001), and Steph’s (ANOVA, F(7,43) = 4.884, p=0.001) genotypes (Table 2b). There were also significant differences in growth between transplant sites for the CVFD (Kruskal-Wallis, H=15.738, p=0.033), Site 211 (Kruskal-Wallis, H=21.863, p=0.003), and Jons Reef (Kruskal-Wallis, H=24.985, p=0.001).
Local Adaptation

LE was significantly higher in foreign than in native corals at two sites (Figure 2.3a): CVFD (t-test p=0.001) and Inshore (t-test p<0.001). Five transplant sites showed a trend of higher LE of foreign corals and three sites exhibited a trend of higher LE of native corals, though none of these differences were significant (Figure 2.3a; Appendix I). Pooled foreign genotype LE was significantly higher than LE of pooled native genotypes (Fig 2.3b; t-test p=0.021). During the initial period, mortality of native genotypes was higher than that of foreign genotypes at 3 sites, however, after three months there was no significant difference in percent survivorship among pooled native and foreign corals (89% and 85%, respectively, t-test p=0.675). At the conclusion of the experiment, there was no significant difference in survivorship of native and foreign corals (14% and 11%, respectively, t-test p=0.629). By using growth as a fitness metric, selection coefficients were calculated to express relative fitness. Negative relative fitness at five sites ranged from -0.132 to -0.444; three sites showed positive fitness coefficients ranging from 0.032 to 0.328 (Appendix I). Site CVFD showed the strongest negative relative fitness for native genotypes, partially driven by the growth of the lowest ranking genotype (Figure 2.2b).

Phenotypic Plasticity and Reaction Norms

There were three genotypes with LE at or above the median for >75% of sites, representing fast-growing corals (Government Cut, Grounding, and Struggle Bus; Table 1a). Conversely, two genotypes (CVFD and Jon’s) had LE at or below the median for at least 75% of sites, representing slow-growing corals. Regardless of fast or slow growth, genotypes were further divided into ‘generalists’, which have relatively flat reaction norms and lower variability in growth rate between sites, and ‘specialists’, which have
widely fluctuating LE dependent on the environmental conditions at a given site. For example, the Government Cut genotype has above median LE at every site, while the Struggle Bus genotype is at or above the median at all except one location (Table 2.1a). These genotypes may be successful over a wide range of sites. Conversely, some genotypes are ‘specialists’: Jon’s, Miami Beach and Inshore fluctuate above or below the median at different sites. A single genotype (Steph’s) represents especially high specificity; it is the only individual to show the highest ranked LE rate within one site (CVFD) and the lowest LE within another site (Jon’s). Importantly, relative growth rates are never conserved between genotypes across all sites and many genotypes exhibit multiple pairwise reciprocal changes in ranking between sites (Table 2.1a). For example, Jon’s and Inshore genotypes only share a relative ranking (above or below median) at a single site (CVFD). Nursery growth (Table 2.1a) is not a good predictor of growth at any site, as relative growth rank within the nursery does not match any of the outplant sites. The dependence on both site and genotype is further highlighted by differential growth rates in 90% of genotypes between sites (Table 2.1b), with at least a two-fold difference in growth rate between the fastest and slowest sites for every genotype. Faster growing coral genotypes do not have a higher range of LE ($F_{(1,6)}=1.84, p=0.2118, R^2=0.187$), however, sites with a wider range of coral growth showed a higher average LE ($F_{(1,6)}=10.175, p=0.019 R^2=0.629$).

**Physiological Data**

All coral genotypes contained *Symbiodinium* from Clade A at the time of transplantation, with no detectable background levels of clades B, C, or D. Total lipids
ranged from 1.79-2.42 mg/cm\(^2\) and were not significantly different between genotypes (ANOVA, F\(_{9,42}\)=0.7962, p=0.621). There was a weak positive relationship between lipid content and LE (r\(^2\)=0.246, p=0.144). There was a significant difference in skeletal density between genotypes, ranging from 0.631-0.848 g/cm\(^3\) (Kruskal-Wallis, H=20.802, p=0.014) and a strong negative relationship between LE and skeletal density (r\(^2\)=0.838, p=0.001).

**Environmental Data**

There were no significant differences in aragonite saturation state (\(\Omega_{\text{arag}}\)) between sites (Appendix I), or a relationship between \(\Omega_{\text{arag}}\) and LE (regression p>0.05). Light regimes were significantly different among sites (Fig. S1; Appendix I; ANOVA, F\(_{3,131}\)=23.273, p<0.001). LE for all genotypes combined was significantly higher at ‘low’ light sites (Struggle Bus and Inshore) than at ‘high’ light sites (Cooper’s and CVFD; t-test p<0.001). Average daily temperature for the growth period (March to June) ranged from 27.2-28.8 °C at different sites (Appendix I). There was a significant difference in daily average temperature based on site (ANOVA, F\(_{7,739}\)<0.001), but no temperature metrics predicted growth (regression, p>0.05). There was no significant difference in aragonite saturation state (\(\Omega\)) between sites for raw data (Kruskal-Wallis, H=6.54, p=0.477). Data was also adjusted to account for seasonal fluctuations (i.e. higher values in Spring) by taking the difference between individual saturation state values and the average within the timepoint to correct for seasonal fluctuations. These differences were averaged and showed no significance between sites (ANOVA, F\(_{7,11}\)=0.2961, p=0.941). There was no significant relationship between growth and raw \(\Omega\) (r\(^2\) = 0.026,
p = 0.699) or seasonally corrected $\Omega$ ($r^2 = 0.039$, $p = 0.637$). When skeletal density was used in combination with genotype specific growth rates to examine calcification, there were no significant differences between genotypes (ANOVA, $F_{(9,68)} = 0.389$, $p = 0.937$).

CVFD (average daytime value over 4 days = $779 \pm 56 \mu\text{mol/m}^2/\text{sec}$; depth = 1.8m) and Coopers ($600 \pm 49 \mu\text{mol/m}^2/\text{sec}$; depth = 3.4m) had significantly higher light than Inshore ($384 \pm 31 \mu\text{mol/m}^2/\text{sec}$; depth = 5.5m) and Struggle Bus ($297 \pm 27 \mu\text{mol/m}^2/\text{sec}$; depth = 10.7m) (Fig. S2). Maximum light levels ranged from $1313 \mu\text{mol/m}^2/\text{sec}$ at CVFD to $539 \mu\text{mol/m}^2/\text{sec}$ at Struggle Bus for any instantaneous reading.

Average, minimum, maximum, range, and variance of temperature were not significantly predictive of growth by site. Daily temperature range among all sites was typically around 1.5 °C, but mean temperatures were not significantly related to depth ($r^2 = 0.035$; $p = 0.656$).

**Bleaching Impacts**

Bleaching was not observed during the growth period (March-June). During July and August, average temperatures ranged from 30.6-31.1°C and were significantly different between sites (ANOVA, $F_{(7,423)} = 10.55$, $p < 0.001$), with two discrete site groupings identified based on temperature, though average differences between sites was <0.5°C. The ‘hot’ sites (CVFD, Inshore, Steph’s, and Grounding) average 2.8 m deep while ‘cool’ sites (Jon’s, Miami Beach, Struggle Bus and Cooper’s) average 5.8 m.

During July, sites experienced significantly different levels of bleaching prevalence, which ranged from 13%-47% of colonies (Table 2.2a; $\chi^2 = 37.1$, df=7, $p < 0.01$). At this time, prevalence was also significantly different based on genotype ($\chi^2 = 68.7$, df=9,
p<0.01) with 9%-53% of corals per genotype bleaching (Table 2.2a). During July, there were no significant differences in bleaching prevalence at ‘hot’ and ‘cool’ sites (t-test, p=0.529). There was higher bleaching at ‘low’ light sites than ‘high’ light sites during July (34% vs. 17%, respectively).

During August, bleaching prevalence was significantly different among sites (Table 2.2b; ²χ=25.6, df=7, p<0.05), with 63% -99% of colonies bleached within sites. Unlike in July, there were no differences in bleaching prevalence among genotypes as temperature stress intensified in August (Table 2.2b). During this timepoint, bleaching prevalence at ‘hot’ and ‘cool’ was not different (t-test, p=0.281), but bleaching was higher at ‘high’ versus ‘low light’ sites (91% vs. 67%, respectively).

Mortality

Coral mortality throughout the experiment was significantly influenced by site (Cox-Hazard, p<0.001) but not by genotype or the interaction of terms. In June, prior to the onset of bleaching, coral mortality was variable and significantly different by site (χ²=172.2, df=7, p<0.01). Mortality ranged from 0% to 42% of outplanted colonies at Steph’s and Inshore, respectively. Notably, mortality in June was >30% at three sites (Inshore, Miami Beach, Struggle Bus) and <5% at the remaining 5 sites (Cooper’s, CVFD, Grounding, Jon’s, Steph’s), indicating that some sites are more challenging habitats post-transplantation than others. Genotype was not a significant driver of initial mortality (χ²=8.1, df=7, p>0.05).

All mortality observed after the growth period was attributed to bleaching stress, though unobserved predation or disease could have contributed. At each timepoint,
bleaching mortality was significantly influenced by site but not by genotype. In July, mortality was highly variable and significantly different by site ($\chi^2=152.7$, df=7, $p<0.01$), ranging from 1% to 53% at CVFD and Inshore, respectively. In August, mortality was also site specific ($\chi^2=128.1$, df=7, $p<0.01$), ranging from 8%-65% at CVFD and Struggle Bus, respectively. Recovery (% of surviving corals exhibiting normal coloration) was significantly impacted by site ($\chi^2=82.7$, df=7, $p<0.01$) and genotype ($\chi^2=41.4$, df=9, $p<0.01$), with nearly 80% of surviving corals coming from just three sites (Cooper’s Reef, Jon’s Reef, Miami Beach). In addition, there was a distinct bimodal pattern in recovery by genotype, with 6 genotypes at ~15% and 4 genotypes with ~1% survivorship. There was significantly higher recovery at the ‘cool’ sites compared to ‘hot’ sites (24% vs. 7.5%, t-test $p=0.0327$). Neither pooled growth by site ($r^2=0.035$, $p=0.656$) nor pooled growth by genotype ($r^2=0.215$, $p=0.176$) predicted mortality. LE was not significantly different between corals that experienced subsequent morality and those that survived (Wilcoxon, $p=0.136$).

**Discussion**

*Acropora cervicornis* is an important reef-building coral in the Caribbean, forming habitat for many associated organisms (60) and structure needed for reef function (3, 61). In recent years, it has become a focus for mitigation by active restoration to limit the extensive declines in this region (62). The habitats and coral genotypes used here cover a broad environmental range of Florida reefs and expand on prior results showing differential growth among genotypes in a common garden (29, 30) and variable growth between sites (63). We show *A. cervicornis* transplanted onto a variety of reefs
exhibits variable growth and disturbance response dynamically dictated by multiple factors. Coral genotype was a significant factor in pooled growth, where environmental variability might be expected to overwhelm genotypic differences between individuals, meaning genotype is an important driver of colony success. Environment was also a significant determinant of growth, with reefs classified into three significant growth levels over a four-fold difference in average growth. We find extensive evidence of phenotypic plasticity, with individuals exhibiting a wide range of LE at different sites which likely serves to temper local adaptation, which was not observed in this study.

The most influential driver of growth and survivorship in this experiment was the environment, though the impacts of specific metrics (temperature, carbonate chemistry, light, etc.) were difficult to resolve. Very shallow sites (~2m) had the lowest LE (CVFD and Steph’s), and sites with low light levels (regardless of depth) had significantly higher LE (Struggle Bus and Inshore reefs), suggesting that light at shallow sites may be so high that it is inhibitory (64). Water chemistry and light data measured here verify known trends, suggesting the data, though limited, were generally representative. Inshore and offshore reef sites in Florida show similar diurnal patterns in light, but significant cross-shelf and seasonal differences in carbonate chemistry and light (65, 66). Given that six of the eight sites were ‘mid-channel’, it is not unexpected that there were no significant differences in Ω_{arag} between sites, as similar distance from shore suggests similar carbonate chemistry (66). Depth is a primary driver of the diurnal variability in carbonate chemistry on reefs, with variance increasing with decreasing depth (67), yet variance in Ω_{arag} did not change with depth, and was actually lowest at one of the shallowest sites. More thorough sampling of carbonate chemistry and light (e.g., time-series), particularly
the diurnal variability in $\Omega_{\text{arag}}$, is necessary to fully understand the role of these variables on growth, but were not resolvable here due to field constraints. The importance of genotype is further supported by the lack of significant relationship between LE and temperature, which is often the primary and most important environmental factor in coral growth (68). Other abiotic variables not measured (e.g., nutrients, sedimentation) may also have influenced growth and survivorship.

Environmental characteristics were the primary driver of mortality throughout the growth period and had a strong influence on bleaching. The ‘challenging’ sites identified here (Struggle Bus and Inshore) and Miami Beach, a hardbottom habitat with high turbidity, showed high mortality during the first three months of this study, prior to bleaching. However, the role that temperature and light played in site-specific patterns of bleaching prevalence were not as expected during the onset of bleaching, with warm sites with high light hypothesized to have higher bleaching prevalence (69). No significant differences were found in bleaching prevalence between ‘hot’ and ‘cool’ sites; however, there was a difference in bleaching between sites with ‘high’ and ‘low’ light levels, but this pattern changed over time. Importantly, certain sites where light regimes were significantly different but temperature was similar (e.g. Struggle Bus and Cooper’s), showed lower bleaching prevalence in the lower light environments, highlighting the importance of light as a factor in bleaching severity (69-71). Overall, temperature differences between sites were most important during recovery, where a significantly higher proportion of corals transplanted to ‘cool’ reefs survived to the end of the experiment. These small differences are crucial for understanding potential habitat refugia, especially if utilized with novel combinations of coral genotypes. Differential
growth of *A. cervicornis* has important implications for coral population dynamics under changing climate, particularly if changing population distribution involves range shifts that include marginal habitats (15, 72).

Coral genotype also has significant influence on growth rates, even when considered across a range of environments. Coral genotypes can be classified as fast or slow growing, but every individual varied >200% between the fastest and slowest growing locations, indicating broad acclimatization potential based on the host of conditions at a given site. Especially high growth differences between specific sites indicates that some individuals may be specialists that thrive only under certain conditions (e.g., cool temperatures, high turbidity, high light) and exhibit low relative growth rates elsewhere (49). In contrast, the reaction norms of some genotypes showed a somewhat more consistent growth response, indicating they may play a more generalist role and have higher fitness over a broad range of environments (48). For example, the Government Cut genotype, which had the highest average LE, can be considered a generalist, as it showed growth rates above the median at every site while maintaining one of the narrowest ranges in growth of any genotype. Conversely, Steph’s genotype represents a specialist, which is more dependent on environmental conditions and has higher growth variance; this genotype is the fastest growing coral at one site (CVFD) and the slowest growing coral at another (Jon’s). The development of these two strategies may be related to temporal environmental heterogeneity (73), where more variable environments produce generalist responses able to cope with temporal change. Generalist corals that are able to maintain growth at an even wider range of environments than those investigated here may be critical for long-term survival and recovery of diminished *A.*
*cervicornis* populations, representing valuable additions to coral propagation and restoration programs in regions facing uncertain future conditions.

In addition to linear extension, genotypic differences in skeletal density and patterns of faster linear extension in corals with larger lipid content illustrates the various ways in which individual genotypes are unique, interacting with the environment. The tradeoff between high-density skeletons and fast growth shown here is an important driver of intraspecific differentiation in fragmentation potential which may lead to distinct distributions on individual reefs, an example of phenotypic differentiation. Though no data was collected here, growth tradeoffs will influence long-term recovery trajectories, especially if reproductive capacity is compromised. Over the long-term, higher growth allows individuals to escape size specific mortality and will generally increase colony level fecundity due to size, but short term sacrifices may also be important. Reproductive investment is among the first processes to be compromised during stress (48), so a better understanding of the balance between growth and reproductive output is critical for restoration. This tradeoff is particularly important if sexual reproduction can achieve a large recruitment event, jumpstarting species recovery. Biological flexibility provides practical information for understanding impacts of future global change on restoration (16, 74) because it illustrates the balance of differential response that is unique to each genotype.

Genotype also influenced bleaching and mortality in this experiment, though more subtly than environmental conditions. Mortality was not different between genotypes until after the temperature maximum, but genotypic influence was apparent for the onset of bleaching prevalence in July. Bleaching patterns did not directly translate to
mortality, likely because high temperatures overwhelmed genotypic differences and the
ability of the holobion to cope with the intense thermal challenge. Propagation in the
common garden nursery can be expected to minimize (but not eliminate) maternal effects
from the site of origin, so differential response to heat stress is attributed mostly to
intrinsic differences in these corals and their symbionts, either through genetic
predisposition, acclimatization after outplanting (48) or fine-scale differences in
*Symbiodinium* communities and the microbiome (74). Beyond maintenance in a single
environment to minimize maternal effects, distinguishing between long-term
acclimatization (i.e., to the original home site) and genetic differences is beyond the
scope of this study. Though symbiont identity plays a critical role in growth and
bleaching response (75, 76), all genotypes in this experiment contained only clade A
upon outplanting. Any differential growth or bleaching response based on symbiont
community must therefore be based on sub-cladal differences in thermotolerance (77, 78)
or symbiont density (79).

Importantly, coral genotype was a determinant of survivorship after the bleaching
event. In this case, temperature stress resulted in two groups of genotypes, one with
approximately 15% survivorship and another with approximately 1% survivorship. The
more robust individual genotypes with higher survivorship highlight the importance of
GxE interactions and exemplify how habitat refugia may act synergistically with specific
coral genotypes, increasing their ability to survive extreme disturbances. In this
experiment, examples of GxE combinations that yielded high survivorship include
Steph’s genotype transplanted at Cooper’s Reef (70% survivorship), Inshore genotype at
Cooper’s (70%) and Inshore genotype at Jon's Reef (60%). Given that these sites
experienced significant thermal stress ($\geq 28$ days over $31^\circ C$, maximum temperature $>32^\circ C$), they represent disturbance resistant combinations. Multiple genotypes only survived at ‘foreign’ sites, illustrating how the environment can produce refugia for individual genotypes and the importance of spreading the risk of mortality of individual genets through restoration.

The integration of genotype and site affects can be seen in patterns of local adaptation, where foreign corals had higher pooled growth than native corals but no difference in survivorship, supporting the conclusion that individuals are not fine-tuned for specific habitats on the scale examined here. The lack of local adaptation observed here contrasts patterns observed in *Porites astreoides* (40). Differences in life history, including brooding reproduction, time to competency, and vertical transmission of symbionts have the potential to reduce gene flow in *P. astreoides*, promoting local adaptation in this species (40) and potentially driving interspecific differences in adaptive potential. Local adaptation with respect to temperature has been demonstrated in Pacific species (21, 39), though environmental gradients in our experiment are likely more subtle, providing better context on the local scale. This result also conflicts with genetic patterns in *A. cervicornis*, where large differentiation between sites could be a sign of local adaptation occurring over individual reefs, where genetic diversity is concentrated (47). The resolution of these conflicting concepts from different data sources may be possible using a different experimental design which focuses on multiple genotypes per site, enabling a better understanding of how genetic diversity on reefs produces plasticity and its impacts on local adaptation, which is an important avenue of future research.

Flexibility of growth rates (plasticity) acts in an antagonistic manner to local adaptation,
so observations of high plasticity and low local adaptation may indicate that selection on individual Florida reefs is relatively weak in this species. Another possible factor that would limit local adaptation is the mismatch between individual corals and their current environment, which has changed and is no longer evolutionarily relevant. The analysis of local adaptation is limited by the random nature of genotype selection at the beginning of the experiment, where a single coral is selected for the nursery. However, pooling of control and local nursery genotypes from separate individual colonies on a given reef expands the genetic diversity of local types, tempering this concern to some degree. In addition, the selection of large and healthy colonies for this experiment makes it more likely that we would find local adaptation if it existed, suggesting that our results are conservative. In addition, maintenance of corals in the common garden shared environment is designed to minimize the impacts of long-term acclimatization from the site of origin and isolate genetic affects. Though this process is imperfect, it is the best available option for many marine organisms such as corals which are difficult to breed. It is also important to note that temporal affects may influence this result. Acclimatization to a new site may mean that relative growth rates change over time as individuals adjust to the environment, so it is possible that local adaptation would be evident over longer time scales. Seasonality should also be considered as different individuals may take advantage of changing conditions throughout the year and exhibit different growth patterns, which is especially important in the context of generalists and specialist.

One interesting trend documented is the low growth of nursery controls (i.e., control corals kept within the nursery site) and most wild controls, which were in the bottom 10% of growth rates at 85% of sites. Further, growth rates were lower for nursery
controls of 80% of genotypes compared to average growth at all other sites. These trends suggest that changing environments during transplantation stimulates growth in fragmented corals, beyond the initial increase in growth rate or “pruning vigor” produced by fragmentation within a site (9). This added growth after fragmentation and transplantation may be an adaptation to improve fragment cementation and survival in a new microhabitat for a species that relies on asexual propagation for population expansion and recovery after disturbance (34).

Though the interaction term of the two-way ANOVA was not significant, data shows a dynamic relationship between specific combinations of site and genotype as would be expected during an interaction. High variability between sites and bleaching disturbance likely compromised the power to find a significant interaction if it exists, however this process is still potentially important. First, reciprocal differences in growth between sites (i.e. crossed reaction norms) for various genotypes, as shown extensively here, are evidence of GxE. Next, the absence of conserved reaction norms, which describe patterns of response across a range on conditions, provides evidence that site and genotype are flexibly interrelated, despite the non-significance of the interaction term. Lastly, ignoring interactive effects between these main factors masks the intrinsic flexibility documented here, providing a naïve explanation where site and genotype strictly dictate growth and survivorship systems rather than highlighting the subtle and complex interrelationship between factors that drives success and failure in this species.

A limitation of this study is the importance of *Symbiodinium* to the function of the holobiont, which recent evidence suggests is increasingly important. Analysis here shows that site, genotype and their interaction only explain 41% of variation in growth,
so other factors including the microbiome likely play an important role. Acroporids are commonly associated with *Symbiodinium fitti* (type A3), but differentiation in function between strains of the same type (80, 81) can impact thermal tolerance and expression level, which are contingent on an interaction with the host (82). We observe that the coral host significantly impacts growth rates and recovery after stress, however this affect is likely influenced by functional diversity of the sub-type symbiont strain, which we cannot resolve at the appropriate scale. By taking advantage of the ‘acroporid - *S. fitti* system’ (82), a better understanding of the impacts of these factors on growth and survivorship may be possible, but remains unresolved in this experiment. The microbiome may also play a functional role in growth and survivorship responses between genotypes shown here, but determining the relative contribution of these factors is difficult (78).

Phenotypic plasticity suggests that *A. cervicornis* may be able to cope with changing conditions, up to a threshold, given the current levels of genetic diversity (83). It is also possible that plasticity has helped maintain genetic diversity, which is still high in Florida (47, 84) during population declines on Caribbean reefs that might be expected to create a genetic bottleneck. Since different genotypes show unique reaction norms, emergent patterns of growth and survivorship may arise as new genotypes are matched to new environments, potentially allowing for human intervention to aid adaptation and acclimatization. The importance of cryptic genetic variation may become evident as these new combinations are discovered, pairing novel alleles and conditions leading to a range of phenotypes (35, 36). Likewise, the evidence for some generalist genotypes implies that some individuals may be successful across a range of conditions. If the range in which
generalists can grow into healthy colonies and the potential range of synergistic factors for specialists include future environmental regimes, phenotypic plasticity may spread the risk of mortality for certain genotypes across sites. By this mechanism, long-lived corals may serve as a bridge to sexual reproduction and subsequent adaptation, contributing to assisted gene flow through redistribution during restoration (85) which may help maintain diversity needed for reef survival under changing climate.

The absence of local adaptation is also relevant for restoration. The fate of outplanted corals is determined by site and coral genotype, but the absence of local adaptation indicates that individuals will not be systematically disadvantaged at new or foreign sites, at least over the spatial scales investigated here. Nursery repositories may therefore be able to supplement natural recruitment, since higher growth in foreign/immigrant corals counters some concern about particular traits that are uniquely suited to native environments. This barrier has been a concern for restoration (86), but should be tempered by our results. Maximizing genotypic (and thereby genetic) diversity should be a focal point of future outplanting efforts and will serve to produce novel GxE combinations and enhance reproductive success.

Overall, this experiment resolves a flexible system where coral genotype and environmental conditions contribute to the growth and survivorship response of a threatened species in a highly dynamic manner. Our data indicate that small environmental variation can drive ecologically important differences, especially in combination with specific coral genotypes, serving as refugia. Although population declines have been severe, phenotypic plasticity may help maintain population sizes
under changing conditions, adding to the adaptive potential for change needed for long term sustainability of coral reefs.
CHAPTER 3 – GENOMIC PATTERNS IN *ACROPORA CERVICORNIS* SHOW EXTENSIVE POPULATION STRUCTURE AND VARIABLE GENETIC DIVERSITY

**Background**

Coral reefs are among the most biodiverse and productive ecosystems on earth but are seriously threatened by a combination of natural and anthropogenic stressors (16, 134). Large-scale stressors such as climate change and ocean acidification interact over smaller spatial scales with overfishing, eutrophication, sedimentation, storm damage and disease, contributing to further reef system decline (2). In the Caribbean, declines have been particularly dramatic as historical overfishing (10), loss of the grazing sea urchin *Diadema antillarum* (79) and white-band disease in Acroporids (8) have led to the loss of nearly 80% of coral cover (14). As efforts to remedy reef decline continue, high-quality genomic data are needed to build a more holistic understanding of population structure, connectivity, diversity and adaptive potential among surviving stony corals (74, 135) that form the structural basis needed to sustain reef biodiversity and function. These data are critical from several perspectives: 1) genetic diversity is related to resilience and resistance to ongoing stress, 2) this diversity also influences interspecific diversity and ecosystem function, providing important information on reef health, and 3) coral reef restoration and management require better information about factors that dictate the spatial scale of focal areas, as well as a strong conceptual understanding of the consequences of active intervention on individual reefs.

*Acropora cervicornis*, listed as threatened under the US Endangered Species Act (34), has declined more than 95% in some parts of the Caribbean (28). This species is a major reef builder with high growth rates (23) and frequent asexual reproduction via
fragmentation (24) that binds sediments and creates essential structure for associated reef organisms. These characteristics make it ideally suited for restoration using the coral gardening technique (136, 137). Given its prominent role in reef function and potential for population modification due to active intervention, understanding genetic diversity patterns is practically and conceptually valuable because it provides a fundamental connection between ecological, evolutionary and management processes (138). A major goal of most conservation plans is to conserve species biodiversity, but a focus on intraspecific genetic variation, the most basic source of biodiversity (139), should be a focal point of conservation efforts motivated to build evolutionary resilience (45). To achieve this goal, intra- and transgenerational acclimatization and adaptation must work together over multiple time scales (37, 75, 76).

Structural species diversity is correlated with species diversity at the ecosystem level, acting through a variety of mechanisms including consumer diversification, increased resource specialization and variation due to host specialization (140). Some evidence also indicates that intraspecific diversity has important consequences at the ecosystem level (141), especially in foundation or dominant species (142, 143). Similar to species diversity, intraspecific diversity increases productivity, promotes disease resistance, structures associated communities and may lead to higher biodiversity (140, 142, 144-147). The improvement of community function occurs through selection effects (i.e. the odds of having highly productive individuals in the sample) and complementarity effects (139, 147), where niche partitioning and improved resource utilization can lead to higher average overall fitness within the assemblage (147). Although neutral genetic
markers, like those investigated here, are not expected to have ecological consequences 
*per se*, they may be correlated to functionally significant traits.

Genetic diversity also increases resistance and resilience potential when dealing 
with disturbance (92), (93, 141, 148, 149). High standing genetic variation can allow 
organisms to adapt with changing environments over various temporal scales (150) and 
form communities in which variation may still exist after disturbances occur (149), 
increasing overall resilience (93). Conservation actions should aim to develop 
evolutionary resistance, or the ability to cope with changing conditions, by increasing 
genetic variability and maintaining adaptive potential (45, 150), requiring a solid 
understanding of focal species' population structure and diversity.

Understanding *Acropora cervicornis* genetic diversity is especially important 
because active restoration has emerged as one method for mitigating ongoing declines of 
this taxon in the Caribbean (39). The coral gardening methodology, which propagates 
coral in an *in situ* nursery, minimizes impacts on donor colonies and provides a 
sustainable coral source for restoring reef structure and function (137). As the scale and 
efficiency of active intervention increase, data on 1) the genetic diversity of regional 
populations, 2) how these populations relate to coral distributions on individual reefs, and 
3) how populations vary within political regions (e.g., counties) and across broader 
spatial scales are of practical importance. Along the Florida Reef Tract (FRT), a network 
of seven coral nurseries operated by six organizations (Coral Restoration Foundation, 
Florida Fish and Wildlife Conservation Commission, Nova University, MOTE Marine 
Lab, The Nature Conservancy, and University of Miami) are responsible for *Acropora 
cervicornis* active restoration (39). These nursery populations are of critical ecological
and evolutionary importance because they represent the functional unit (i.e., source of tissue used in restoration) of the active conservation response to declining Caribbean Reefs.

Early *A. cervicornis* investigations concluded that individual Caribbean reefs harbored many colonies of single or few genotypes. Using a self-recognition assay, colonies were mapped within reefs and clonality was considered common at the scale of 10m (63). Early population genetics studies discovered reefs separated by as little as 2km showed fine-scaled genetic differentiation, but this was largely due to introgression signatures from *A. palmata* to its congener *A. cervicornis* at a single reef (72). At scales >500m, genetic studies showed significant population structure among nine regions (72). These results indicate restricted population ranges, with reefs relying on local recruitment and requiring regionally-focused management efforts, potentially with different strategies based on the populations and demographics of each region (72). The authors also found a region-wide ratio of genets/colonies sampled of 0.580, suggesting that individual reefs have diverse assemblages that are not monoclonal over the range of dozens of meters.

Spatially limited studies in the Florida Keys showed similar trends of regional scale population structure across the Caribbean and high connectivity within the FRT (n=52) (73). These studies were supported by an expanded microsatellite dataset (n=158), which found little population structure (48). Samples of *A. cervicornis* from Florida also showed the second highest nucleotide diversity of any region investigated, indicating that, despite drastic population declines (28), levels of diversity are similar to other regions of the Caribbean. Overall, these studies support a link between the western Caribbean and Florida by virtue of shared haplotypes and low population differentiation,
corresponding to significant structure between the eastern and western Caribbean in *A. palmata* (46). As the research focus shifted to smaller spatial scales, population structure was apparent at scales less than 5km in Puerto Rico (47) and on the Meso-American Barrier Reef (151). In an early implementation of Next Generation Sequencing on corals, Drury et al. (49) described population structure within the Florida Reef Tract and between Florida and the Dominican Republic, supporting previous findings of diversity localized within populations and even within reefs. As our understanding of population structure and connectivity in *A. cervicornis* has developed, a picture has emerged of reefs as more ‘closed’ systems with localized diversity which are influenced by local oceanographic conditions, variation in reef habitat, environmental heterogeneity and stochastic recruitment.

As techniques and sampling methodologies have improved and expanded, it is clear that greater resolution can offer additional information about scleractinian coral genetic patterns, especially in light of the near-significance of many regional and sub-regional comparisons of population structure in the above studies. Advances in sequencing technologies have led to an increase in the power and breadth of population genetics studies in recent years, with a focus on sampling many loci from many individuals and using this large dataset to infer demographic patterns, understand evolutionary processes, and uncover cryptic genetic variation (94, 152). Here, we use Genotyping by Sequencing (GBS) (105), a method which takes advantage of reduced genome complexity produced by restriction enzymes to produce large numbers of Single Nucleotide Polymorphisms (SNPs) for analyzing within-species diversity. GBS lowers per-sample costs through highly-multiplexed barcoding, providing orders of magnitude
more markers for the genomes of individual organisms compared to conventional methods such as sequencing individual genes or microsatellite genotyping. These factors make this technique ideally suited to investigate the diversity and structure of regional populations and wild coral communities. With multi-locus data for each individual and a better understanding of genetic diversity within reefs, concerns about inbreeding, outbreeding depression, founder effects and genetic swamping may be addressed, producing more responsible reef restoration actions (44) and better defining population structure.

We take advantage of wild and previously collected nursery corals to produce a hierarchical sampling methodology that utilizes samples from the entire Florida Reef Tract, including within regions and within reefs, to examine *A. cervicornis* genetic diversity relevant to conservation and management actions. We also analyze samples from the Cayman Islands, Dominican Republic, and Belize to contribute to the growing knowledge of Caribbean-wide population structure for this species (46, 47, 49, 72, 73, 151). We test the hypotheses that 1) there is population structure (i.e., limited gene flow) within the Florida Reef Tract, 2) collections from *in situ* nurseries in Florida represent high genetic diversity, 3) diversity among individuals from nursery stocks is comparable to genetic variation of colonies on wild reefs, and 4) genetic diversity is uneven across the span of the FRT, potentially due to environmental or habitat heterogeneity. Data presented here form a concrete link between analysis of population genetics and management efforts.
**Materials and Methods**

**Colony Collections**

Regional collections were obtained from individual reefs across the Florida Reef Tract, Cayman Islands, Dominican Republic, and Belize (Figure 3.1) with the goal of minimizing the collection of clonemates and maximizing genetic diversity. These samples are maintained *in situ* within locally managed nurseries. Rarely, duplicate samples were collected from individual reefs, but were presumed to represent non-clonemates due to distance separating colonies. Wild collections were made during 2015 from 15-25 haphazardly-selected discrete colonies on each individual reef.

**Sample Collection**

Samples were collected between June 2012 and August 2015 from nurseries and wild reefs along the Florida Reef Tract. Additional samples used as outliers/reference were collected from the Dominican Republic, Cayman Islands and Belize between 2013-2015. Approximately 0.5cm apical tips were collected with a razor blade, transferred to 250µL of chaotropic salt preservative (‘Chaos’, 4.5M guanadinium thiocynate, 2% N-lauroylsarcosine, 50mM EDTA, 25mM Tris-HCl pH 7.5, 0.2% antifoam, 0.1M b-mercaptoethanol) and stored at 4°C until processing. Samples were collected from 184 individuals across entire Florida Reef Tract for analysis of nursery stocks (Figure 3.1), with 88 additional individuals from the Cayman Islands (n= 53), Dominican Republic (n=29), and Belize (n=6). In addition, 63 samples from 3 wild reefs in Florida and 2 wild reefs in the Dominican Republic (Table 3.1) were collected for comparisons within regions of nursery and wild reef diversity.
DNA Isolation and Library Preparation

Samples (skeleton and preservative) were homogenized using silica beads in original collection tubes and extracted using a silica column protocol and a vacuum manifold modified from (153). DNA quality was evaluated using gel electrophoresis. Extracted DNA was quantified (AccuBlue\textsuperscript{TM} High-Sensitivity dsDNA Quantitative Solution) and 100ng of each sample was dried down in 96-well plates. Libraries were prepared using a modified protocol of (105). Briefly, samples were hydrated in 5\mu L water and cut with ApeKI to produce restriction fragments. ApeKI is a partially methylation-sensitive restriction enzyme, which reduces cut sites in repetitive regions of the genome and enhances cuts in lower-copy regions (105). After digestion, samples were bead-purified to eliminate small fragments; 4-9bp barcodes unique to each sample and a common adapter were ligated to fragments (see (105) for adapter sequences). Ligated samples were pooled and bead-purified to select fragments in the 100-250bp size range. Pooled samples were amplified using primers complementary to barcodes and the common adapter, which also produces an overhang complimentary to the oligonucleotides used in Illumina flow cells for ease of sequencing. PCR products were bead-purified, eluted in 10mM Tris, and fragment size distribution was analyzed on an Agilent Bioanalyzer. Samples were sequenced using single end 75bp reads on an Illumina HiSeq 2500 (Elim Biopharmaceuticals Inc., Hayward, CA).

Data Processing

Raw sequences were processed using a parsing script modified from (154) to remove reads without barcode and cut sites. These reads were trimmed using
Trimmomatic 0.32 (155) to remove low quality bases at the leading and trailing end and remove reads if a 4bp sliding window average read quality (Phred score) fell below 20. Reads were demultiplexed according to barcode using a modified script from (154) and aligned to the *Acropora digitifera* genome (156) using Bowtie2 (157). Aligned reads were processed using GATK 3.4.2 (158) following a protocol modified from existing best practices to produce the SNP dataset (159, 160). Briefly, optical/sequencing and preparation duplicates were marked with MarkDuplicates and HaplotypeCaller was used to produce initial genotypes under the cohort analysis framework. Base Quality Score Recalibration was conducted, but due to multiplexing, some samples had too few bases within read groups for adequate model convergence. Intermediate gVCF files were merged and genotyped using Genotype-gVCFs. This initial SNP matrix was recalibrated with known variants, which were bootstrapped from replicate samples. Six pairs of biological replicates (from samples not analyzed in this dataset) with the highest number of reads were used to find ‘known sites’ using filtering through TASSEL 5.2.29 (161) to detect loci that were consistently genotyped in replicate pairs. Matching calls between replicates were then used as ‘known sites’ to train Variant Quality Score Recalibration, using the annotation values of known sites to determine thresholds for accepting SNPs.

Tranche plots were examined and a truth sensitivity of 99.9 was used for recalibration, a relatively permissive setting to balance between discovery of novel variants and potential false positives.

Called SNPs were quality-filtered following (49) to produce two datasets for comparison, Regional populations and Wild populations. Separate datasets were used because independent assessment of each subset of samples allowed optimization of
number of loci per sample, allelic patterns and independent AMOVAs. Briefly, the SNP matrix was filtered iteratively using TASSEL 5.2.29 (161) to select loci called in at least 70% of individuals and individuals with at least 30% of loci called. This threshold was used to favor preservation of sample numbers across populations. Loci with excessive heterozygosity out of Hardy-Weinberg Equilibrium (p<0.01) were identified using Arlequin 3.5.1 (162) with 1,000,000 steps and removed. Loci in LD ($R^2$>0.2 or p<0.01) were identified using TASSEL in a 50bp sliding window and removed.

**Analyses**

Population differentiation, including AMOVA (163) and pairwise $F_{ST}$ was analyzed in Arlequin (3.5.1), with 10,000 permutations for significance values. DAPC was completed using the adegenet package in R (164) to visualize differentiation. Molecular diversity indices and allele patterns were analyzed in GenAlEx 6.502 (165). Isolation by distance (Mantel’s test) was evaluated within Florida using the package ade4 (166) with matrices of pairwise genetic differences calculated within TASSEL and geographic distance, using 9999 permutations. Genetic diversity within the Florida Reef Tract was visualized with ArcGIS toolbox Genetic Landscapes GIS Toolbox 10.1.3 (167) plotting pairwise differences between points and the nearest 30 samples and producing an IDW interpolation. Comparison of genetic diversity based on sample size was completed by creating random subsets of individuals from the Miami-Dade population. Samples (n=2-16,18,20,25 individuals) were each taken from the population 50 times with replacement to generate average values, since diversity metrics change based on which specific individuals might have been selected for analysis.
Results

Interpopulation differentiation

In total, 272 samples from nursery/regional populations and 63 samples from individual reefs were analyzed at 3163 and 3766 loci respectively (Table 3.1). In the regional pairwise comparisons, significant $F_{ST}$ values ($p<0.01$) occurred in 35% (16 of 45) comparisons (Table 3.2), including between Belize and every other population examined. Within Florida, significant population structure exists, although the Dry Tortugas population was not significantly different from any other population. All Caribbean populations (Caymans, Belize, Dominican Republic) were significantly different from each other but show variable relationships with populations in Florida, where the Cayman Islands are not significantly different from any nursery population and Dominican Republic populations show intermediate population structure when compared with Florida populations. $F_{ST}$ values ranged from -0.252 to 0.319, including 3 comparisons between Belize and other populations with $F_{ST}$ values greater than 0.2, typically considered to be strong population differentiation.

DAPC shows mixed population differentiation, with Broward, Lower Keys, and Middle Keys populations and Belize separating from the core cluster in the regional analysis (Figure 3.2). The Broward population shows strong separation along the first principal component from all other populations, which show little separation along this axis. Overlap between Miami-Dade, Upper Keys, Dry Tortugas, Cayman Islands, and Dominican Republic samples corresponds to some pairwise $F_{ST}$ values (Miami-Dade - Dry Tortugas, Miami-Dade - Caymans, Caymans - Upper Keys, etc.) although other patterns of structure in the $F_{ST}$ comparisons are not apparent. Among Florida populations
only, the same outliers are evident, along with strong overlap between Upper Keys, Miami Dade, and Dry Tortugas (Figure 3.3a). When Broward populations are removed for visualization, the Middle Keys population shows strong differentiation, corresponding with large $F_{ST}$ values, while the Dry Tortugas maintains overlap with all populations (Figure 3.3b), corresponding to its high connectivity in pairwise comparisons.

For regional samples, an AMOVA was structured with Florida, Dominican Republic, Cayman Islands, and Belize as groups, with populations assigned within Florida as in Table 3.1. Nearly all genetic variation is within populations, with small fractions among groups and among populations within groups (Table 3.3a). A separate AMOVA on wild samples was structured with Florida and the Dominican Republic as groups, with individual reefs treated as populations within each (3 in Florida, 2 in the Dominican Republic). Variation was still largely within populations and individual reefs showed an order of magnitude higher percent variation among groups and among populations within groups (Table 3.3) than the regional AMOV. The AMOVA for wild samples also shows relatively high percent variation among populations within groups, suggesting that structure and limited gene flow between individual reefs may exist at smaller spatial scales than captured by regional sampling.

**Intrapopulation differentiation**

The 9 regional populations examined had between 6 and 53 samples per population (although all populations except Dry Tortugas and Belize had at least 17 samples). Average allelic richness ranged from about 1 to 1.273 for all populations and closely correlates with expected heterozygosity values for all populations (Table 3.4).
Observed heterozygosity was much lower than expected for all populations. In regional populations, locally common alleles (defined as alleles with frequency >5% within a population, but frequency <5% in 75% or more of populations) varied nearly tenfold from 0.005 (Dry Tortugas) to 0.043 (Lower Keys). These alleles may be disproportionately influential and explain some of the spread in DAPC, where higher values correspond to some outlying clusters (i.e., Broward, Middle Keys, Mote, but see Belize and Miami-Dade) (Figure 3.2).

**Geographic Patterns of genetic differentiation**

There was significant Isolation by Distance within the Florida populations (Mantel Test, Correlation = 0.09, p=0.012). Spatial differentiation can also be examined on smaller scales using interpolation of local sample genetic differentiation (pairwise distance) to understand regions of high or low diversity. Figure 3.4 shows scaled values for interpolated pairwise distance among every point and its 30 closest samples. The Lower Keys, Middle Keys, and Broward regions show the highest diversity values, while the Upper Keys and the ecotonal habitats between Broward and Miami-Dade counties show the lowest diversity. The Dry Tortugas region shows moderate diversity, balancing spatial isolation with high connectivity (Table 3.2). The ‘safety valve’ area of Biscayne Bay, an area where the tidal flow of water from Biscayne Bay disrupts temperature and salinity on adjacent reefs, and areas surrounding the port of Miami may influence connectivity between Miami-Dade and Broward, leading to small-scale (10s km) depression of diversity between Miami-Dade and Broward populations. Sampling locations (Figure 3.1) covered most of the distribution of *A. cervicornis* in Florida,
although gaps between the Dry Tortugas and Key West (Lower Keys), the Upper Keys and Miami-Dade, and Palm Beach County have not been extensively sampled.

*Comparing regional nursery populations to individual reefs*

One common concern for active restoration is to determine the minimum number of coral individuals to maintain within nurseries to preserve the same or similar levels of genetic diversity found on surrounding reefs. Regional nursery populations in Florida have similar or larger values of genetic diversity than most individual wild reefs (Table 3.4). Average allelic richness and expected heterozygosities are higher in nursery populations than on wild reefs, although the values are similar and increase asymptotically with larger sample sizes (Figure 3.5). Predicted asymptotes (i.e., maxima) for expected heterozygosity for wild reefs and regional nursery populations are 0.025 and 0.026, respectively. Using expected heterozygosity as a diversity metric, an average of five individuals need to be collected and propagated within each regional nursery to match the mean heterozygosity of wild reefs (Figure 3.5). To equal maximum expected heterozygosity from single wild reefs, approximately 10-12 individuals need to be collected and kept within regional nursery populations. As the number of individuals maintained within nurseries increases beyond 12, no substantial increases in genetic diversity occur (Figure 3.5). Due to the diminishing increases in heterozygosity with sample size, 90% of asymptotic diversity is achieved with 7 or 8 samples, so comparisons of genetic diversity (heterozygosity) based on sample size should be interpreted with caution for populations with fewer than 8 samples.
Locally common alleles are nonexistent in wild populations in this dataset, although this may be attributed to the low number of ‘populations’ examined and the influence of geographic distance between the two groups (Florida and Dominican Republic). Although the percentage of variation for Among Group and Among Population was an order of magnitude higher in the Wild AMOVA than Regional, the same relative variation was maintained. Approximately 82% (Table 3.3b) of variation in wild reefs is within individual reefs. This is less than in the regional populations (99%, Table 3.3a) but much larger than the other sources of variation. As regional nursery samples were collected over broader geographic ranges, this supports the Isolation by Distance discovered in this species, because ‘groups’ (as defined by AMOVA) cover different spatial scales in regions and on individual reefs. Allelic richness tracks closely between wild populations and nursery groups as sample size increases, although exhaustive sampling of individual reefs may lead to higher diversity values.

Discussion

As genetic resolution improves, the concept of population structure and connectivity in *Acropora cervicornis* has progressed from an understanding of high connectivity within regions to the recognition that genetic diversity is concentrated within populations over small spatial scales (49, 72). This developing appreciation informs active restoration as a form of management and conservation. Since increased genetic diversity is beneficial for resistance and resilience within species (93, 141) and within communities (74, 150, 168), maintaining or increasing diversity and promoting adaptive potential are critical for management and conservation, especially in species where active
management is ongoing. In this study, we expand on prior *A. cervicornis* work, using a nested approach that shows previously unresolved population structure within the FRT and between larger Caribbean regions, highlights differences in diversity throughout the FRT, and provides practical data on restoration methodologies.

Florida populations show extensive structure, sampling at high density with an average of 1 ± 0.22 (1 SE) km between each sample and its closest neighbor. Overall, about 1/3 of pairwise comparisons between Florida populations were significant, although increased sampling effort contradicts some previously described patterns (49), including the differentiation of Miami-Dade and the Lower Keys. The Dry Tortugas showed no population structure when compared to other FRT regions, and while likely influenced by the relatively small nursery size, this relationship could potentially be due to the downstream location of this population in the Florida Current and the ‘stepping stone’ model of connectivity, where larval transport could connect different regions over multiple historical recruitment events. The Dry Tortugas also has few private and locally common alleles, supporting the connectivity of this population with the rest of Florida. Based on this framework, connectivity between the Lower Keys and northern regions (Upper Keys, Miami and Broward) could further support gene flow across the FRT originating in the Dry Tortugas. An additional candidate link supporting along-Keys connectivity in the absence of connectivity among intermediate populations is between the Upper Keys and Broward, which appears isolated from Miami-Dade, supporting previous findings (49). The Dry Tortugas and Upper Keys, which also shows lower localized diversity, should be focal points of management and restoration as they may enhance FRT-wide connectivity by acting as hubs to other populations. Additionally, the
Dry Tortugas are a critical area of importance for management as dispersal from this downstream area likely influences connectivity to all other parts of the FRT, acting as a source of new genetic material through gene flow.

The Broward population shows connectivity to other areas of the Keys, but also has unique alleles that are disproportionately influential in DAPC, separating it from all other populations in the first discriminant function. This collapse of variation is not shown in pairwise $F_{ST}$ calculations because all loci were used to examine structure, rather than those that drive the majority of variation. The Middle Keys area is also uniquely isolated, with significant population structure between every other Florida population except the Dry Tortugas; this isolation is well supported by DAPC. These patterns may be related to differential mortality due to environmental conditions across the FRT, potentially related to influx of high-salinity, high-temperature inimical waters from Florida Bay across the reefs of this area (169). Although genetic population structure in Florida has previously been demonstrated, the level of complexity at multiple spatial scales shown here provides added detail and resolution and suggests that regional scale management actions are appropriate; differences in genetic variation and patterns of connectivity mean each region has unique resources and obstacles. Under this framework, we suggest the Dry Tortugas should be protected as a highly connected community, while the Middle Keys should be a focal point for the introduction of new genetic material due to high genetic differentiation. Within Florida, genetic connectivity between distant populations but not their intermediate reefs points toward the complex forces shaping current conditions. These assemblages may previously have had more
larval connectivity, but could now show stronger differentiation due to a population bottleneck caused by reef collapse in the Caribbean in recent decades (14).

The Cayman Islands and Dominican Republic represent populations that were previously under- or unexamined with genetic or genomic techniques, providing novel information about relationships between these areas and Belize and Florida. All samples examined here fall west of the Mona Passage, considered to be a barrier to connectivity between the eastern and western Caribbean (46, 170), however connectivity within the western area of the Caribbean does not preclude fine-scale population structure, and pairwise comparisons between these populations exhibit a range of differentiation. The Cayman Islands show no significant differences with Florida populations, indicating high connectivity. Conversely, Belize shows strong population structure when compared to Florida, with FST values ~0.2 for most comparisons, supporting previous results that show Belize is significantly different from Florida and indicating large differentiation between these populations (73). Intermediate levels of structure between the Dominican Republic and Florida highlight the complexity of long-distance relationships and the fine-scale structure of populations in Florida. For the first time, we make comparisons between each Caribbean population, where pairwise FST values show significant structure, highlighting diversity and limited gene flow over large spatial scales, supporting previous findings (46, 48, 72).

The increased sampling undertaken here facilitates comparisons of diversity within populations over smaller spatial scales compared to previous studies (49), highlighting local variation in diversity levels. Florida populations fall at, or slightly above, the range of other regions for most metrics, including allelic richness (Na),
proportion of private alleles, locally common alleles and expected heterozygosity. Confirming previous findings, slightly elevated expected heterozygosity and allelic richness in Florida indicate that this region may not have suffered disproportionate loss of diversity during population declines (73), indicating that standing genetic diversity is still present, providing the raw materials for adaptation. This result is tentative, however, as simulated population declines require generations to pass before observable genetic diversity declines (171), which likely has not yet happened in _A. cervicornis_. Conversely, other Caribbean areas, particularly the Cayman Islands, have relatively lower diversity, which may contribute to the similarities between this population and Florida shown in pairwise comparisons. Although the FRT has generally elevated genetic diversity, it is uneven and values for expected heterozygosity are highest in Broward and the Lower Keys, corresponding to outlier groups in DAPC, which may be important resources as repositories of standing variation (25).

Observed heterozygosity is much lower than expected for all populations, indicating potential inbreeding among isolated _A. cervicornis_ populations and providing evidence that increased genetic diversity should be a management objective (45). Since inbreeding may already be an issue in this species, increasing genetic diversity by utilizing more diverse assemblages from nursery populations may alleviate some areas of restricted variation, however observed heterozygosity is low in nursery populations as well. Observed heterozygosity values are relatively consistent across regions, but are much lower than values found by sequencing individual genes (72, 73), which may be partially due to the biallelic nature of data used in this analysis (105). These results may also be related to pipeline artifacts and the use of a Pacific congener as reference (_A._
digitifera), where non-reference alleles are assumed to be errors unless sufficient evidence in the dataset suggests otherwise. Due to multiplexing in the laboratory protocol, reads per individual were limited, which may contribute to an underestimation of heterozygotes during read processing, however this procedure is conservative and produces a high confidence SNP dataset that should not bias relative differences between populations.

As restoration efforts mature and expand throughout the Caribbean (20), incorporating genetic data in management and restoration becomes increasingly important (44) for creating natural assemblages that will be resistant, resilient, and maintain adaptive potential. One of the major advantages of the sampling scheme utilized here is the ability to compare regional populations to individual wild reefs to provide practical information from the genetic data using various tools, including AMOVA and allelic patterns. The AMOVA for each dataset shows the same relative patterns, with diversity localized within populations. Despite the proportionality of variation between regional and wild tests, an order of magnitude more variation is found ‘Among Groups’ and ‘Among Populations within Groups’ for the wild populations, suggesting that some residual population structure may not have been captured for wild populations. The ‘Within Population’ source of variance represents spatial scales as small as dozens of meters in this example, highly localized diversity which would not be expected if asexual reproduction via fragmentation is the main source of reproduction, but only one option among healthy communities like those historically present on Caribbean reefs (65). We hypothesize that connectivity along the FRT may be a result of frequent sexual reproduction in the past which has been tempered during recent population declines and
given way to a series of dynamically interconnected populations which now rely primarily on fragmentation for reproduction. This concept reconciles the extremely rare discovery of definitively sexual recruits (23) with the continued presence of diverse assemblages on individual reefs.

The regional populations examined here are characterized by slightly elevated genetic diversity compared to individual reefs sampled in Florida and the Dominican Republic, supporting the AMOVA analysis of similarly structured populations. Expected heterozygosity of nursery populations is equal to or slightly larger than that of most wild populations and allelic richness closely tracks as sample size increases between both groups, so using more individuals can create more diverse outplanting assemblages, up to a certain point. On average, 5 samples are needed from a regional nursery population to reach mean diversity values for an individual reef during restoration. Genetic diversity of an assemblage made of individual corals varies depending on the genetic differentiation between individuals, so while 5 individuals meets the mean expected heterozygosity of a wild reef, approximately 10 are needed to ensure that sampling effects meet this limit, i.e. all randomly selected assemblages fall above the threshold. Ten individuals in an assemblage also replicates, on average, the maximum expected heterozygosity from a wild reef in this study, meaning this figure is an important target for management and conservation. Presently, all nursery programs in Florida exceed this minimum number of individuals, with some reaching well over 100 individuals. The variability shown by repeatedly subsampling corals from the nursery also illustrates how genotypes harbored in nurseries can be closely or distantly related, influencing the diversity of outplanted assemblages and suggesting that the use of non-clonemates, regardless of multilocus
genotype, can be used to achieve genetic variation and adaptive potential. Maximizing diversity of outplanted corals should be a fundamental goal of restoration as it will increase resilience and resistance, while providing the genetic variation needed to cope with changing climate (92) and support improved ecosystem function (141). Although genetic diversity increases marginally beyond 8 samples, other factors motivate the goal of maximizing within-reef ‘individual diversity’ (e.g., genotypic diversity) including reproductive compatibility (51, 52) and unforeseen growth and survivorship patterns mediated by genotype by environment interactions (Chapter 2). One limitation of this assessment is that population declines have likely decreased genetic diversity on individual reefs to some degree, leading to current estimates from wild reefs that are not reflective of historical healthy reefs. By using changes in sample size (see supplementary materials) and modeling differences in diversity within nursery populations, some concerns regarding whether small sample size accurately reflects diversity of larger assemblages can be tempered because asymptotes for expected heterozygosity are similar for wild (0.025) and regional (0.026) populations. Additionally, sample sizes here reflect the actual size of nursery populations, but wild populations with less than 7 samples were excluded. However, if population declines removed large portions of genetic variation that correlated with stress tolerance (i.e., non-hardy individuals harbored large amounts of genetic variation), current values may not be representative. The individual patch reefs in this study remain among the healthiest populations of *A. cervicornis* in Florida today, so estimates made here reflect the best current data on intraspecific diversity on healthy reefs.
Genetic distance across the Caribbean corresponds to geographic distance (49), consistent with the null hypothesis of the Mantel test and passive larval dispersal in corals. Sampling across the FRT, isolation by distance is still present, however localized areas of variability in genetic diversity produce a more complex genetic landscape. From the perspective of relatedness with nearby individuals, small-scale variation and lower genetic diversity occur in two areas: offshore of Miami Beach in Miami-Dade and in the Upper Keys. While untested, we hypothesize that the northern area of lower diversity corresponds to an ecotonal area where benthic habitat switches from patch and bank-barrier reef habitats to consolidated hardbottom, roughly corresponding to the beginning margin of habitat range and the transition to the Southeast Florida Continental Reef Tract (172). This region of low diversity also corresponds to the area of influence of the ‘safety valve’, a wide passage connecting the offshore reef habitats with shallow, urban Biscayne Bay (173, 174). Reef habitats in the area are influenced by the tidal outflow of water coming from Biscayne Bay with potentially higher temperature and salinity variability, nutrient content and pollutants (175), which could influence connectivity and local diversity. Interestingly, high diversity is found in nearby areas of Broward county, which also support dense thickets (large populations of living staghorn colonies with interlocking skeletons) that remain infrequent elsewhere on the FRT (58, 176). Based on the potential influence of inimical water quality on connectivity, we expected to see lower diversity in the Middle Keys where the influence of Florida Bay is strongest (177) and reef structure is most sparse (178), but found evidence of moderate diversity in this area and connectivity across this region, suggesting it is not a strong barrier. The other region of lowered diversity is in the Upper Keys, which has relatively abundant patch
reefs and is not proximate to land based stressors or inimical waters. It is possible that depressed local diversity in this area and near Miami-Dade is related to differential mortality during long term Caribbean reef decline or due to localized stressors such as White Band Disease, bleaching, and storm impacts. High expected heterozygosity in the Lower Keys and Broward populations also correspond to areas of high variation between individuals (as assessed by pairwise differences), highlighting the dynamic relationship between environmental characteristics (which vary spatially) and genetic relatedness. Resolving differences in diversity across small spatial scales as a result of environmental heterogeneity is an important area of further study, with valuable lessons for restoration on how the natural complexity of reef habitat and conditions influences coral assemblages before and after outplanting.

*Acropora cervicornis* diversity is consolidated within small spatial scales, where most variation exists within wild and nursery populations. This has important implications for conservation: nurseries can faithfully replicate wild populations during outplanting, an important goal for maintaining and increasing genetic diversity and ecosystem resilience (45). Despite this capability, each region of the FRT has its own resources and obstacles, making local management imperative. Setting goals to increase diversity (Miami-Dade and Upper Keys), protect hubs of connectivity (Dry Tortugas and Upper Keys) or protect unique or highly diverse areas (Lower Keys, Middle Keys and Broward) may allow for more efficient and effective conservation of an important foundation species, with cascading effects on individual reefs and eventually throughout the species range. Declining population sizes throughout the Caribbean may affect diversity, but populations still show significant structure across regional and local spatial
scales, and populations in Florida do not appear to have faced disproportionate loss of diversity; similar standing genetic variation will allow these populations the chance to cope with stress throughout their range. Although inbreeding may be a problem for this species, the infrequency of locally common or private alleles on wild reefs found here dampens concerns about co-adapted gene complexes and outbreeding depression (44), especially given that local adaptation appears rare in this species (Drury, Chapter 2).

As conservation and active restoration attempt to mitigate declining reefs, the use of high-resolution genetic techniques can be essential for determining focal points, the scale of management, and creating goals. This information can be used to better understand areas where diversity and connectivity can be treated as resources and which regions need special attention, while informing practical decisions about replicating natural, functional systems (43). Population structure and diversity also promote a better conceptualization of the role of foundation species like reef building corals play in their communities, where increased intraspecific diversity leads to enhanced ecosystem function and improved resilience and resistance. This data represents a valuable resource for building upon the links between ecology and evolution and the factors influencing species persistence while guiding our understanding the consequences of active intervention and management.
CHAPTER 4 – THE GENETIC COMPOSITION OF *ACROPORA CERVICORNIS* THICKETS AND ISOLATED POPULATIONS

*Background*

Coral reefs worldwide are under serious threat due to a combination of anthropogenic and natural stressors (3), suffering massive declines in abundance and habitat structure (16). The deterioration of coral reefs in the Caribbean has been particularly severe, with a loss of nearly 80% of coral cover since the 1970s (14). This region-wide decline was driven in part by the drastic loss of the genus *Acropora*, a formerly dominant taxon that has almost completely disappeared from some areas (27, 28, 61). Caribbean acroporids have been uniquely affected by a combination of factors including White Band Disease (8, 30), storm-damage (9, 56), bleaching (30), overfishing (10) and the die-off of the sea urchin *Diadema antillarum* (79). These stressors have induced an unprecedented shift in community structure on modern reefs (33, 179) and prompted the listing of *Acropora* species as ‘threatened’ under the United States Endangered Species Act (ESA) in 2006 (34). The protection and active restoration of Acroporids has become a focal point of management efforts throughout the Caribbean because of their ecological importance and life-history, which is amenable to nursery propagation using the coral gardening method (17).

The staghorn coral, *Acropora cervicornis*, has high growth rates (180) and propagates frequently by fragmentation (23, 78), characteristics which make it partially responsible for the accumulation of structure (and associated function) on shallow Caribbean reefs (113). The decline in abundance of this species has also resulted in the loss of dense aggregations, commonly known as thickets (31). Thickets are formed by multiple coral colonies with interlocking skeletons (Figure 1a), which can sometimes
cover hectares of reef substrate (63, 181-183). These aggregations are now uncommon and have been replaced by populations composed of discrete, isolated colonies occurring in much lower abundance (27, 28). Remaining *A. cervicornis* thickets (31, 59, 176, 184) provide significant reef structure and habitat for associated organisms and represent rare thriving populations in an otherwise declining species, so rebuilding these structures has become a key target of ongoing restoration programs.

Acroporids rely heavily on fragmentation and thickets have long been assumed to be monoclonal and contain low genetic diversity (58, 63). The improved resolution provided by recent genetic and genomic advances now allows a direct assessment of this assumption, providing an improved understanding of patterns of genetic diversity and genotypic diversity within individual reefs (49, 64, 65). In *Acropora palmata*, reefs with both monoclonal and genotypically diverse populations have been observed throughout the Caribbean, created by varying degrees of sexual recruitment and fragmentation (64, 65). Thicket or within-patch genetic studies are more limited in *A. cervicornis*, where early observations using self-recognition assays concluded clonality was common among neighbors, with an average clone diameter of 4.4m, and clonemates occasionally found up to 25m apart (63). The composition of within-reef populations is important because foundational species can influence the structure of associated communities (143, 147), confer disease resistance (146), and lead to higher resilience (93), though these effects and the consequences on ecosystem function remain largely unknown on coral reefs. These processes may also be important in monoclonal stands, where the accumulation of somatic mutations over time represents a source of standing genetic variation (76, 185),
which could be adaptively important if damaged communities recover via asexual reproduction from remnant populations (186).

*Acropora cervicornis* thicket are important components of Caribbean reefs, serving as ecosystem engineers that create habitat and directly influence the diversity and species richness of associated reef organisms (33, 34). Dense *Acropora* thicket may also increase reproductive potential, where high fecundity (50, 54) and gamete concentration can partially alleviate the alle effect in species with small population sizes (64). Thickets with large colonies and high survivorship may thus be an important resource for supporting local fertilization and subsequent recruitment, enhancing sexual reproduction (179, 187), which is a critical component of species recovery. In addition, emergent properties (188), where individual genotypes produce different phenotypes when growing within thicket than the same genotypes produce in isolated populations (139) may be an important factor in the growth and survival of these unique structures. The spatial complexity within thicket produced by dynamic fragmentation (176) can place genetically dissimilar colonies in close contact, potentially facilitating this emergent function through non-additive effects such as niche partitioning (189). This type of genotype x genotype interaction is virtually unknown in coral reef ecosystems, but may be a key to understanding why remaining thicket are thriving in the context of general species decline.

As active restoration efforts throughout the Caribbean increase in scale (20), remaining *A. cervicornis* thicket (31, 58, 59, 176, 184) represent a critical resource for understanding population dynamics of threatened coral species, the balance between sexual and asexual reproduction, emergent properties of diverse assemblages, and how
thriveing thickets relate to nearby sparse, isolated populations. The goal of creating
thickets that will be fully functional ecological units (43) using nursery-grown corals is
now possible given the recent successes in coral propagation in the Caribbean (20).
However, the successful creation of staghorn thickets requires a detailed understanding of
the genetic components that influence each structure. To address these knowledge gaps,
we sampled *A. cervicornis* thickets in Florida and the Dominican Republic, using
genotyping by sequencing (GBS) (49, 105) to provide high-resolution genomics data on
these assemblages. We used a range of plot sizes to focus on the spatial patterns of
genetic connectivity (i.e., fragmentation) within thickets, examine differences between
thickets, as well as between thickets and nearby assemblages of isolated colonies. Using
these data, we test the hypotheses that: 1) population structure exists within a site
between plots and isolated colonies, 2) isolated colonies are more genetically diverse than
colonies that form thickets, 3) thickets are largely clonally produced, 4) isolated
individuals can be used to produce functional thicket structures during restoration, with
representative genetic diversity signatures, and 5) genetic diversity influences functional
response (such as percent coral cover) within plots (Table 4.5).

**Materials and Methods**

**Sample collection**

Samples were collected from two staghorn thickets in Florida (Cheetos and Sunny
Isles) and two thickets in the Dominican Republic (Cayo Carenero and Punta Rusia)
between August 2013 and July 2014 (Figure 4.1b). Square plots were created and
samples were collected from plots and from nearby isolated colonies outside the thicket
Plots were randomly placed in areas of high *A. cervicornis* cover and sized between 4-8 meters per side depending on thicket dimensions (Table 4.1). The largest plot was sampled at Cheetos reef in Florida where a single 8m x 8m plot was used to focus on spatial analysis within the thicket and the ‘genetic construction’ of the assemblage by taking advantage of the ability to cover the majority of the coral assemblage in a single large plot. At other sites, replicate smaller plots were used to assess spatial genetic patterns and were sized based on the extent of the assemblage. Smaller, more dispersed plots were used at Sunny Isles (~10m between plots) in Florida, while Cayo Carenero plots in the Dominican Republic were larger and slightly closer together (<5m between plots).

To delineate each plot, a rebar stake was hammered into the substrate, and a mason line was used to mark the plot perimeter. A leaded line marked at 1m intervals was moved across the plot to create the 1-m grid used to guide sample collection. Flagging tape was attached to the branch closest to each sampling point within the plot. Isolated colonies were haphazardly selected from discrete individuals that were definitively beyond the boundary of the dense assemblage. These colonies were sampled from the same reef, except at Punta Rusia in the Dominican Republic, where ‘non-thicket’ individuals were not present and isolated colonies from a reef ~10km away were sampled instead. Approximately 0.5cm of each of branch tip (apical only) was collected with a clean razor blade, transferred to 250µL of chaotropic salt preservative (‘Chaos’, 4.5M guanadinium thiocynate, 2% N-lauroylsarcosine, 50mM EDTA, 25mM Tris-HCL pH 7.5, 0.2% antifoam, 0.1M b-mercaptoethanol) and stored at 4C until processing.
Landscape mosaics

To document the status of thicket assemblages and measure coral cover, landscape photo-mosaics were created for the two sites in Florida by stitching together high resolution images of each plot following (190). Briefly, after delineation of the plot and sample points, a dual downward-facing camera rig recorded photos at 5 second intervals across a ‘lawnmower’ pattern of transects of each plot, complemented by the same pattern in a perpendicular orientation until the entire area had been surveyed with overlapping photos. Photos were recorded approximately 2m above the substrate at each site and assembled into a composite landscape image following (191). To calculate percent cover within plots, 15 photos were randomly selected from each plot (except Cheetos, which used 20 photos for the single plot) and 25 random points were assessed within each photo using Coral Point Count (192). Landscape mosaics have been used in the past to assess status and trends of Acropora thickets in Florida (193), Belize (194), and Puerto Rico.

DNA isolation and library preparation

Samples consisting of skeleton, tissue and preservative were homogenized using silica beads in original collection tubes and extracted using a modified silica column and vacuum manifold protocol (153). DNA quality was verified with gel electrophoresis and each extracted sample was quantified (AccuBlue™ High-Sensitivity dsDNA Quantitative Solution). Based on DNA concentrations, 100ng of DNA was dried down and rehydrated in 5µL water. Libraries were prepared as in (49) using a modified protocol of (105).
Briefly, each library was cut with ApeKI to produce restriction fragments, which were bead purified to remove small fragments. 4-9bp barcodes unique to each sample and a common adapter were ligated to fragments (see (105) for adapter sequences) and ligated samples were pooled and bead purified to select fragments in the 100-250bp range. Pooled samples were PCR amplified for 18,20,22, and 24 cycles (49) using primers complementary to the oligonucleotides used in Illumina flow cells to facilitate sequencing. PCR products were bead purified, eluted in 10mM Tris and analyzed on an Agilent Bioanalyzer. Samples were sequenced using single end 75bp reads on an Illumina HiSeq 2500 (Elim Biopharmaceuticals Inc., Hayward, CA).

Data processing

Raw sequences were processed using a parsing script modified from (154) to remove reads without a barcode and cut site. Trimmomatic 0.32 (155) was used to remove low quality bases at the leading and trailing end of reads, and to remove reads where a 4bp sliding window average read quality fell below 20 as an initial filtration step. Reads were demultiplexed according to barcode using a modified script from (154) and aligned to the *Acropora digitifera* genome (156) using Bowtie2 (157). Aligned reads were processed using GATK 3.4.2 (158) following a protocol modified from existing best practices to produce the SNP dataset (159, 160). Briefly, duplicates were removed and intermediate gVCF files with initial genotypes were produced using HaplotypeCaller. Base Quality Score Recalibration was not performed because limited reads due to multiplexing did not provide enough bases per read group for adequate model convergence. Intermediate gVCF files were merged and genotyped using Genotype-
gVCFs following the cohort analysis framework. This initial SNP matrix was recalibrated with known variants, which were bootstrapped using TASSEL 5.2.29, where sites matching between replicates were selected as known sites from six pairs of biological replicates (samples not analyzed in this dataset). This technique uses annotation data for ‘known sites’ to produce a selection of cutoff tranches for balancing false positives with detection of novel variants. A truth sensitivity of 99.9 was used to produce the final SNP dataset.

Called SNPs were quality filtered following (49) to produce the dataset for analysis, which was split by site to produce four datasets for independent analysis, maintaining number of individuals and loci for analysis at each site. Briefly, the SNP matrix was iteratively filtered using TASSEL 5.2.29 (161) to select loci called in at least 70% of individuals and individuals with at least 30% of loci called. This threshold was used to favor maintenance of sample numbers across thickets and provide adequate replication within plots. Loci with excessive heterozygosity out of Hardy-Weinberg Equilibrium (p<0.01) were identified and removed using Arlequin 3.5.1 (162) with 1,000,000 steps. Loci in LD (R2 >0.2, p<0.01) were identified with TASSEL using a 50 SNP sliding window and removed.

An additional dataset was filtered to look at intra-individual differences compared to within-thicket diversity. Clonality would be expected to produce patterns genetic differentiation patterns similar to those found within individual colonies, so this dataset was created to produce a threshold for assessing the contributions of fragmentation by different individuals. This data included 8 biological replicates from separate apicals of one individual coral and samples from a single plot from Cheetos. Individual SNPs were
not cross-validated (195), so conclusions on the importance of intra-individual variants must be tempered. However, these variants were only used to produce a threshold for comparison within thickets.

Analyses

Population structure was assessed via pairwise differences in $F_{ST}$ between pooled thicket and isolated colonies within all sites using Arlequin 3.5.1 (162), with 10,000 permutations used to calculate significance values. Populations were then reassigned by plot and isolated colonies to examine differences between plots and between individual plots and isolated colonies. Population structure was then reassessed in the same manner. Discriminant Analysis of Principal Components (DAPC) was used to visualize differences between plots and isolated colonies (164). Molecular diversity indices and allelic patterns were analyzed in GenAlEx 6.502 (165), Isolation by Distance (Mantel’s test) was evaluated within Cheetos using the R package ade4 (166) using 9999 permutations with matrices of pairwise genetic differences calculated in TASSEL and geographic distance calculated from UTM in GenAlEx. Genetic diversity within plots was visualized with ArcGIS toolbox Genetic Landscapes GIS Toolbox 10.1.3 (167) using pairwise genetic differences between each point and its closest 5 samples. This distance cutoff was used to emphasize the importance of nearby colonies to assess clonality and the contribution of fragmentation to thicket development. Larger distance thresholds in the genetic landscape analysis did not substantively change within-plot diversity interpolations. Comparison of genetic diversity based on sample size was completed by separately creating random subsets of individuals from the Cheetos thicket plot and
Cheetos isolated colonies. Samples (between n=2 and n=50 from the thicket and n=2 to n=17 from the isolated colonies) were each taken randomly from the population 100 times with replacement to generate average values of He and Na using ade4 (166). This technique was used because diversity metrics change based on which specific individuals were selected for analysis, so average and variance are informative. Diversity and sample size were analyzed using JMP 12.0 and the best fit curve was selected using the best AIC value, predicted asymptotes were generated by the best fit curves, similar to a modified rarefaction analysis of biodiversity (197). Sample size cutoffs were developed independently for isolated colonies and thicket colonies for 90% of asymptotic values.

Comparisons of intra-plot and intra-individual differentiation were completed for Cheetos in JMP 12.0 and ArcGIS, using pairwise genetic differences between replicates of a single individual and between all samples within the Cheetos thicket. Briefly, a matrix of pairwise genetic differences was calculated using TASSEL to generate average and distribution for 1) intra-individual comparisons, 2) intra-plot comparisons, and 3) pairwise comparisons between isolated colonies. Multiple samples of the same individual varied at an average of 0.76 ± 0.003% (mean± 1SD) of loci, individuals within a plot varied at a slightly higher but significantly different rate, approximately 1.87 ± 0.013% and isolated colonies varied at significantly higher 2.42 ± 0.012% of loci (One-Way ANOVA, F(2,1499)=17.968, p<0.001). This analysis was repeated 5 times with 50 randomly selected comparisons from the thicket and isolated colonies to correct for large discrepancies in sample size (One-Way ANOVA, p<0.001). The 99.5% cutoff of the intra-individual distribution of pairwise genetic differences occurs at 1.59%, which can be used as a liberal assessment of clonality, while mean percent pairwise genetic
difference (0.76%) serves as a more conservative assessment of clonality for understanding spatial structure within a plot. Mean and 99.5\textsuperscript{th} -percentile values for intra-individual variation were used as thresholds to examine spatial distribution within the plot, which was mapped with the Genetic Landscapes GIS Toolbox 10.1.3. Connections between sampling points below the mean threshold were measured, and number of connections originating from every individual sample was computed as a proxy for concentration of clonemates within the plot, which were used in Getis-Ord Gi* (Hot Spot Analysis) in ArcGIS and to calculate dispersal distance of asexually produced fragments.

\textbf{Results}

\textit{Within-site population structure and allelic patterns}

In total, 265 samples from 10 plots at 4 sites were analyzed (Table 4.1). No significant differences were found between pooled thicket and Isolated colonies except at the Cayo Carenero site, where $F_{ST}$ between populations was 0.162 (Table 4.2, Table 4.5). This structure at Cayo Carenero was driven by significant differences between isolated colonies and plots 1 and 3, respectively (Table 4.3a). Punta Rusia, which had ~10km between thicket plots and isolated colonies, showed no overall population structure between plots and isolated populations (Table 4.2, Table 4.5). However, Plot 1 and Plot 2 at Punta Rusia were differentiated in the fine-scale analysis (Table 4.3b, Table 4.5). At Sunny Isles and Cheetos, no population structure was found at any spatial scale.

Differentiation between plots 1 and 3 and the isolated colonies at Cayo Carenero as well as separation of plots 1 and 2 at Punta Rusia are visualized by DAPC (Figure 4.2a,b). The overlap between plots and isolated colonies indicated by pairwise $F_{ST}$ at Sunny Isles
reflects particularly tight clustering of each group in DAPC (Figure 4.2c). DAPC at Cayo Carenero, Punta Rusia, and Cheetos also shows a split (or bimodal) distribution of isolated colonies, some of which heavily overlap with plots (Figure 4.2).

Expected heterozygosity was higher in isolated colonies than within thickets for all sites except Sunny Isles, which had slightly higher heterozygosity within thicket plots (Table 4.4a, Table 4.5). However, the average expected heterozygosity at Sunny Isles was half (0.007) of the value from the next lowest site, Punta Rusia (0.014) and the two lowest overall values of expected heterozygosity were both within Sunny Isles.

Conversely, percentage of polymorphic sites and allelic richness are higher within thickets than isolated communities at all sites (Table 4.4a), though this is likely related to sample size differences. Percentage of polymorphisms peak in Cayo Carenero and Cheetos thickets with values nearly twice as high as their corresponding isolated colonies (Table 4.4a). Private allele frequency was higher in all thickets than corresponding isolated colonies and is over ten times as high in the Sunny Isles thicket as in the nearby isolated colonies. Interestingly, the site with the highest number of private alleles (Cheetos) does not show significant population structure.

Expected heterozygosity is unevenly distributed among plots within sites at Cayo Carenero (Table 4.4b); but is more evenly distributed among plots at Sunny Isles and Punta Rusia (a single plot was analyzed at Cheetos, so between plot values are not available for this site). At Cayo Carenero, allelic richness also varies more between plots than at other sites and is lowest in plot 3, where values drop below those for isolated colonies despite more than twice as many samples. Allelic richness also varies depending on the assemblage considered, and although Punta Rusia has higher allelic richness in the
thicket overall (Table 4.4a), each plot contains fewer alleles than the isolated colonies when evaluated independently (Table 4.4b).

*Sample size and genetic diversity of isolated colonies and thickets*

As sample sizes increase within the Cheetos thicket and isolated colonies, expected heterozygosity increases logistically (Figure 4.4a,b), with asymptotes about 5% larger than those described in the allelic patterns (Table 4.3a). Isolated colonies have a higher overall expected heterozygosity than colonies within thickets (0.0199 vs 0.0156, respectively) even though the sample size is much smaller for the isolated colonies. This effect is due to the diminishing returns in expected heterozygosity when adding individuals; both thickets and isolated colonies near maximum diversity at 12-15 individual samples. Each group reaches 90% of the asymptotic value at 9 samples, so only ‘populations’ (thickets and isolated colonies) with at least 9 samples were used. Conversely, allelic richness is higher in the thicket colonies (asymptote = 1.228) than in the isolated colonies (asymptote = 1.149)(Figure 4.4c,d) and both are higher than projected in the overall allelic patterns. Although the logistic fit was best, the curve indicates that allelic richness will continue to increase substantially beyond the sample sizes investigated here, meaning that although heterozygosity as a metric of genetic diversity stops increasing beyond approximately 10-12 samples, allelic richness increases beyond 50 samples. During restoration, 7-10 samples from non-clonal individuals, either wild corals or those from an *in situ* nursery (Drury, Chapter 3) can replicate the expected heterozygosity seen within a thicket plot (Figure 4.4b, Table 4.5). However, replicating allelic richness requires substantially more than 50 samples.
**Spatial structure and clonality**

Evaluating the spatial structure within the Cheetos plot shows no significant isolation by distance (Mantel test, p=0.147), indicative that thickets are not consistently expanding in a single or simple directional manner. Each plot shows variable ‘genetic construction’ composed likely of fragmentation (low diversity, lighter colors) of multiple presumed non-clonemates (higher diversity, darker colors) (Figure 4.3a, Appendix II). A large signature of low genetic diversity is seen at the left of the plot, and areas of high diversity between neighboring corals occur in at least 5 locations. Spatial mapping shows several identifiable clusters of diversity (i.e., neighboring non-clonemates) in every plot examined. Differentiated individuals are apparent within plots regardless of the size of the plot (Figure 4.3a, Appendix II), indicating that these assemblages are rarely monoclonal (Table 4.5).

Within the Cheetos plot, which has 57 samples and 3192 possible pairwise links between samples (i.e., combinations of relatedness), 60.1% of sample combinations have a pairwise difference less than the 99.5th percentile threshold, indicating each sample is a possible clonemate. Of the total comparisons, 8.7% have pairwise differences at or below the mean threshold, indicating likely clonemates (Table 4.5). When the conservative cutoff of 0.76% pairwise differences is used to identify likely clonemates, the samples are an average of 5.3m apart and have a maximum distance of 10.8m, indicating that fragments from the same clonal individual are likely distributed throughout thickets. The number of ‘connections’ (count of links between individuals with less than mean intra-individual pairwise differences) can be assigned to each point in the plot and used as a proxy for relatedness and fragmentation likelihood. Using the connections metric, no
significant clustering of high values within the plot is identified with Getis-Ord Gi* (p>0.8).

Coral cover

Percent cover of live *A. cervicornis* tissue was significantly different between each site (One-Way ANOVA, F(3,105)=35.255, p<0.001), varying from 41.4 ± 2.7% at Punta Rusia to 73.8 ± 2.2% at Cayo Carenero. Percent coral cover at Cheetos was 63.2 ± 2.7%  (Figure 4.3b). Live coral cover was significantly positively correlated with genetic diversity (Figure 4.5, Table 4.5; Linear regression, r²=0.15, p<0.001), which was calculated from multiple images of each plot. Though sample size and plot diversity are correlated, the flattening of the curve around 10-12 samples and corresponding lack of change in genetic diversity suggests that plot size is unlikely to be impacting the genetic diversity regressor because all plots had at least 14 samples.

Discussion

Thickets are rare but important structures that represent valuable resources for understanding the past and future of *A. cervicornis*. We present spatial genomics data on thickets and isolated colonies of *A. cervicornis*, for the first time investigating the genetic composition of these assemblages in Florida and the Dominican Republic. Allelic patterns, spatial mapping, and clustering analyses support the conclusion that staghorn thickets are rarely monoclonal and are not typically genetically distinct from nearby isolated colonies. Though the exact number of colonies within each assemblage is variable and unknown, multiple individuals contribute to thicket formation via dynamic
asexual reproduction, which may also contribute to the fluidity of shape and size of modern thickets. We also found evidence of ecological impacts of genetic diversity, where more diverse assemblages had higher coral cover, suggesting that functional properties may be related to intraspecific diversity in this foundational coral species.

*Within-site population structure and allelic patterns*

Only one of four sites (Cayo Carenero) showed significant population structure between thicket and isolated colonies at the same site, which was driven by comparisons between the isolated colonies and plots 1 and 3, respectively. Importantly, plot 1 has the highest genetic diversity (He) of any plot examined except Cheetos reef (which is nearly twice as large). Conversely, plot 3 at Cayo Carenero, which is also significantly different than the isolated colonies, has 35% lower expected heterozygosity than plot 1. This difference is the largest between any plots at the same site, and suggests that high and low diversity can each contribute to population structure between these plots and the isolated population. It is also possible that Cayo Carenero plots 1 and 3 captured areas of relatively high or low asexual reproduction via fragmentation, which combined with differences in allele frequencies indicate populations that are genetically structured. DAPC suggests that a sampling artifact may also play a role in this differentiation, as a split distribution of discriminant functions shows some samples from the isolated community closely align with the thicket plot samples (Figure 4.2), suggesting a common origin. No other sites had significant population structure between the thickets and isolated colonies at the same site, meaning that thicket and isolated colonies represent single populations with adequate connectivity and that genetic composition is not driving
the difference in physical structure between the two. DAPC supports this conclusion via
the overlap of some portion of isolated samples with thicket plots, indicating that these
groupings are not distinguishable.

Comparisons at a finer spatial scale between plots within a site show little
population differentiation except at Punta Rusia. We hypothesize that the uniqueness of
the Punta Rusia community is due to its recent disturbance history that may have resulted
in differential mortality at this site. When examined for the first time in 2010, recent
tissue mortality was only 5% (31), but by 2014 nearly 50% of cover within plots was
composed of dead coral skeleton illustrating the large declines of this population in the
prior four years (estimated at >90%, Schopmeyer personal communication), likely due to
sedimentation and eutrophication likely caused by coastal development (Drury, personal
observation). The population structure between plots 1 and 2 at Punta Rusia may thus be
an artifact of the mortality experienced at the site and not indicative of connectivity or
patterns of asexual reproduction on this thicket. If this is the case, further examination of
this thicket may focus on loci under selection that could be influential in the survivorship
of remaining individuals. At other sites, the lack of differentiation between plots can be
interpreted in two ways: 1) entire thickets lack population structure because they are
produced by extensive fragmentation of single individuals or 2) single plots within a
thicket are not significantly different because the scale of asexual propagation is smaller
than the area of a plot, meaning each plot contains multiple individuals reproducing via
fragmentation which could be shared among plots within the same thicket. We favor the
second interpretation due to allelic patterns, mapping of pairwise differences, and
comparisons of change in expected heterozygosity with sample size in both plots and
isolated colonies. These results suggest that thickets in most cases are likely composed of single, connected populations on the scale of ~50m (maximum distance between plots at Sunny Isles) and are not genetically distinct from isolated populations at the same site. Thus, the creation of thickets is not determined by the general genetic composition of individual corals, though the impacts of emergent properties in assemblages may be important. These patterns illustrate the dynamic nature of branching and fragmentation patterns (78, 176), which may serve to ‘scramble’ the genetic distribution over time at multiple spatial scales (63), presenting an ‘evenly heterogeneous’ genetic surface across thickets.

Though significant structure between thickets and isolated colonies is only apparent at Cayo Carenero, 3 of the 4 sites sampled have elevated expected heterozygosity in isolated colonies, which would be expected if these colonies represent discrete individuals with little or no contribution of clonality. Sunny Isles, which is the only site with higher expected heterozygosity within the thicket, has the lowest overall diversity and the largest sampling footprint of any site, with an average heterozygosity across all sites less than one half of the next lowest diversity value (0.006 and 0.014, respectively). The regional diversity near Sunny Isles is genetically depauperate (Drury, Chapter 2) when compared to other regions of the Florida Reef Tract, which may explain the deviation from the trend of higher diversity in isolated colonies. In addition, distances up to ~50m between plots at this site may lead to higher diversity captured in thickets, which would be expected under the null hypothesis of isolation by distance if fragmentation is an important component of thicket reproductive processes. If this
hypothesis is correct, it indicates that second order spatial structure, (i.e., between plots) is an important component of thicket diversity that needs further research.

Contrary to expected heterozygosity, allelic richness is higher in all thicket populations than corresponding isolated colonies. The best-fit curve of allelic richness by sample size shows logarithmic growth and supports a higher asymptote in thickets than in isolated colonies at Cheetos (1.228 and 1.149, respectively). This indicates that samples collected here have not adequately captured allelic richness, and this value continues to increase as more individuals are examined, meaning that higher allelic richness interpreted as genetic diversity is more closely related to sample size. Allelic richness is more dependent on the presence and absence of rare alleles, which can be adaptively important in a given population, especially over long time scales (198), but we believe the relative sample-size independence of expected heterozygosity makes it a more relevant measure of the genetic diversity between thicket and isolated populations, more accurately reflecting the potential contributions of fragmentation to thicket development relative to its role in isolated populations.

The influence of private alleles is also important, as they occur at a frequency 3-10x higher in thickets than in corresponding isolated colonies, except at Punta Rusia. If private alleles contain adaptive genetic diversity, this signature could be indicative of genetic differentiation in functional traits that is not captured in the broader-scale analysis of population structure. Other results, including the lack of population structure between thickets and isolated colonies at most sites, indicate that typical thickets are not genetically unique (when compared to nearby isolated colonies) and that the construction of these rare assemblage is not predetermined by the genetic material of the individual
corals. However, the frequency of private alleles suggests that thickets contain many more unique alleles that are not shared with isolated colonies than vice versa, which could contribute to their structural role.

Though surviving thickets represent mostly single, connected populations, variation between plots on a smaller spatial scale shows some differentiation, particularly in allelic richness and private allele frequency. Every individual plot has some private alleles that are not shared by other plots within the same site. Although genetic diversity is relatively consistent between plots for both allelic richness and expected heterozygosity, plot 3 at Cayo Carenero is one example of within-site differentiation. In this plot, depleted heterozygosity and allelic richness indicate lower genetic diversity, which may be a signature of higher prevalence of asexual propagation. This plot also has fewer private alleles, further supporting lower connectivity and higher fragmentation relative to other plots at the same site. Relatively consistent distribution of expected heterozygosity between plots within sites is another signature that suggests thickets are single populations, supporting the pairwise analysis between plots for all sites but Punta Rusia. This distribution, where differences in expected heterozygosity between sites are much more pronounced than within sites, highlights the importance of regional-scale differentiation and supports previous results that show diversity is concentrated within populations (49) and varies over sub-regional spatial scales such as the Florida Reef Tract (Drury, Chapter 2). For example, the Sunny Isles thicket has only half the expected heterozygosity within thickets as the Cheetos thicket, suggesting potential differences in genetic construction which may be related to regional demographic trends (64) that differ over the scale of ~40km.
Spatial patterns of thicket construction

Only Cheetos was selected for an extensive analysis of spatial structure because it was the largest plot (8m x 8m) and covered the majority of the A. cervicornis thicket on which it was placed. In addition, this site was generally representative of the distribution of diversity and allelic patterns for all sites and did not have multiple plots at varying distances, which may confound analysis because of lack of data on distances between plots. Within the Cheetos thicket, isolation by distance was not significant, showing that any contribution of asexual propagation to create thickets is dynamic and multidirectional. In this context, isolation by distance would likely be due to the accumulation of somatic mutations of long-lived corals that had fragmented across the landscape over time. Maps of spatial structure on each plot can be interpreted based on the presence or absence of differences between neighboring samples, where low values are likely indicative of fragmentation. At Cheetos, a large signature of fragmentation is seen at the left of the plot (Figure 4.3a), indicating probable occurrence of clonemates radiating from this point. Areas of relatively higher genetic diversity occur in at least 5 locations throughout the plot (darker areas) and are indicative of neighboring corals that are unlikely to be clonemates. This interpretation indicates that each plot includes multiple individuals, each of which contributes to population spatial structure through fragmentation within the plot. Interestingly, areas of high diversity are concentrated around the borders of the plot at Cheetos, where the sampled area encompassed most of the thicket. This signature could indicate that the thicket began as several isolated non-clonemates and has filled in the gaps through fragmentation, or that the thicket structure has influenced sexual recruitment. If the latter is true, this influence could be an artifact.
of self-recruitment, showing that thickets form a biologically viable reproductive community, but this trend may also be related to physical structure arresting the transport of asexual fragments from nearby discrete colonies. The close alignment in DAPC of some isolated colonies at each site with plot distributions supports the latter hypothesis.

Intra-individual variation found here is higher than values found using SNPs in *Orbicella faveolata* (195). However, polymorphisms have not been cross-validated and should only be used as a theoretical threshold. Support for the existence of intra-individual variation in Pacific corals shows that single colonies can be mosaics or chimeras composed of several multi-locus genotypes (199, 200). This mosaicism may not be directly applicable to the branching morphology of *A. cervicornis*, so intra-individual SNP variants are likely due to some combination of actual mutations, which we anticipate will be low, and artifacts of sequencing (201). The intra-individual variation found here (~0.7% of loci) translates to a mutation rate of approximately $1.4 \times 10^{-4}$ per base pair (by dividing pairwise differences by average number of bases per SNP), which is a viable theoretical mutation rate for somatic tissue in corals (185), but is 4 orders of magnitude larger than recent empirical rates for *Orbicella faveolata* (195). We caution any use of these values other than to determine thresholds for examining thickets within this dataset, which we assume suffers from the same systematic biases due to sequencing and library prep. Using the most liberal cutoff, the 99.5th percentile of intra-individual variation, approximately 60% of the samples in the plot could be clonemates, indicating relatively high contributions of fragmentation to plots. Conversely, using the most conservative cutoff, the mean pairwise differences for intra-individual differentiation indicate that less than 8% of the thicket is constructed by fragmentation. Given the patterns of mapping
spatial structure within these plots, the realistic contribution of fragmentation to thicket structure falls somewhere between the liberal and conservative cutoffs, meaning that 8-60% of a thicket is created by fragmentation, which corresponds to prior lattice network analysis of self-recognition reported for this species (63). The conservative cut-off also indicates an average of 5.3m and a maximum distance of >10m between ‘clonemates’, indicating that fragmentation can widely distribute fragments, however, the number of breakage and dispersal events involved remains unknown. In cogen A. palmata, the contributions of fragmentation and sexual recruitment varied substantially between nearly complete clonality over distances greater than 10m to genotypically diverse populations (64). This difference was partly attributed to regional differences in the frequency and efficacy of sexual recruitment in different parts of the Caribbean, which suggests that these estimates may only be generalizable to the Western Caribbean, where sexual recruitment is hypothesized to be low in Acropora (64). Results presented here show that thickets fall between these two extremes. Using the ‘connections’ metric, we found a lack of clustering, representing a dispersed pattern of fragmentation that may be indicative of the discrete nature of initial colonies, many of which have expanded unevenly across space from their origin. This process also likely contributes to the fluid nature of thicket size and shape (176).

Impacts of genetic diversity on survivorship

The long-term viability of Acropora cervicornis and coral reefs in general is closely related to resilience and resistance to climate change and other anthropogenic stress (2, 134). The trend of increased coral cover in more diverse assemblages
documented here shows links genetic diversity to coral reef function for the first time. This finding is especially relevant for conservation and management efforts for several reasons. First, it extends concepts of increased resilience in diversified communities in other ecosystems to coral reefs (93). Second, the effect of higher live coral cover as diversity increases sheds light on the impacts of variable genetic diversity on regional scales (Drury, Chapter 3), where differences in genetic composition may dictate functional response to ongoing stress. Finally, this finding directly supports the use of more diverse assemblages during active restoration, suggesting diverse individuals can enhance the long-term viability of restoration activities. Although a threshold of genetic diversity representative of wild reefs can be achieved at relatively low numbers of individuals during restoration (Drury, Chapter 3), further increases in number of individuals may supplement the survival of these populations.

The impact of genetic diversity of producers or foundational species on ecosystem function is poorly understood in coral reef communities, but may prove to be a critical avenue for better understanding reef decline and recovery (202). In other ecosystems, intra-specific diversity has impacts comparable to species-level biodiversity, including increased primary production, disease resistance, more efficient nutrient cycling, and resilience after stress (140, 141, 146, 147, 203). If these patterns apply to coral reefs, surviving high-diversity populations can help develop a better understanding of disturbance response and help identify targets for enhanced protection (43, 45). While the impact of genetic diversity on growth remains unresolved, intra-specific competition does limit growth and branching in tightly-spaced assemblages of outplanted corals (204).
which suggests a trade-off between growth and survivorship in thickets as crowding increases.

Implications for management, conservation, and restoration

Thickets investigated here rely on both sexual and asexual reproduction for their formation and maintenance, with the influence of fragmentation likely growing over time if sexual recruitment remains low (63, 176). Spatial mapping, patterns of diversity relative to isolated colonies, intra-individual variation, and allelic patterns support the contribution of multiple individuals to these structures. Equipped with this information and the modified rarefaction analysis of sample size, restoration practitioners can recreate thickets that will be functional units of the ecosystems of which they are a part. Starting with an assemblage of non-clonal individuals (i.e. colonies), either from isolated colonies or in situ nurseries, the diversity of thickets can be replicated, on average, with 7-10 individuals, and consistently surpassed with 15 individuals. These corals should be arranged haphazardly throughout plots to allow for the contribution of fragmentation, because individuals do not appear to be clustered in the plots investigated here. The number of individuals used for restoration activities should be maximized for reproductive compatibility, due to unforeseen genotype x environment interactions (Drury, Chapter 2), and most importantly because higher diversity supports survivorship in A. cervicornis. Though private alleles suggest some contributions of unique alleles at individual loci to the success of thickets, these assemblages are generally not genetically distinct from their neighboring isolated colonies, meaning that source corals from other reefs are likely adequate to replicate these structures, even if they do not have the same
allele frequencies and polymorphisms. On a larger scale, management should afford additional protection to reefs, regions, or countries with particularly diverse assemblages (Drury, Chapter 3), as these may have higher survivorship and represent more resilient communities.

**Conclusions**

As our understanding of population structure in *A. cervicornis* has evolved, genetic diversity concentrated over smaller spatial scales has been recognized (47, 49, 72). Here, we show that individual reefs harbor substantial genetic diversity in both dense thickets and isolated colonies, but that these structures are not genetically differentiated within sites. Thickets are composed of multiple individuals which contribute to structure via asexual propagation, which may also facilitate retention of fragments and create important self-reinforcing capacity that supplements high survivorship and provides increased resilience. Thickets remain important communities on modern reefs and although they are uncommon, they offer unique opportunities for understanding the past and future of wild populations, managed populations, and the relationships between the two, facilitating function on modern reefs through appropriate structure.
CHAPTER 5- DISPERAL, CONNECTIVITY AND POPULATION STRUCTURE IN ACROPORA CERVICORNIS ON THE FLORIDA REEF TRACT

Background

The long-term persistence of coral reef ecosystems under threat from natural and anthropogenic stressors depends on multiple, interacting factors influencing resistance and resilience to disturbance (3). Among the most important of these factors is connectivity (134, 205), the demographic linking of local populations through the dispersal of individuals, primarily as recruits or larvae (206). Most sessile or demersal reef organisms rely on some form of active or passive movement of larvae during their reproductive cycles. Dispersal patterns from these static populations dictate demographics, genetic connectivity, and ecological connections across various spatial scales for many marine species (207), including reef-building corals (208). Scleractinian corals are uniquely important in coral reef ecosystems, creating the reef structure (113, 209) that influences ecological processes throughout the community (210). Drastic global declines in coral cover and abundance in recent decades (11, 12, 14) represent a critical threat to the persistence of reef ecosystems (16). In the context of these declines, sexual reproduction and successful recruitment are critical for maintaining population size and distribution (205, 206, 211), recolonizing depleted areas (50, 212, 213), and maintaining genetic diversity critical for resistance and resilience (139, 214).

Corals produce planktonic larvae via internal or external fertilization (38), utilizing different strategies that may also include asexual reproduction via fragmentation or budding (38). The recruitment of juvenile corals into the population depends on the success of several complex steps, including gamete production, fertilization, larval dispersal, settlement, and post-settlement survival (215). During dispersal, the density of
larvae arriving and settling at a particular site determines the potential recruitment strength and represents connectivity between the site of origin and settlement, with the spatial scale of connectivity varying depending on reproductive strategy and physical factors. Local retention of gametes or larvae produced by the brooding reproductive strategy (internal fertilization) can lead to inbreeding, or the homogenization of genetic variation and decrease of heterozygosity (216). Conversely, broadcast spawning corals (external fertilization) have longer pelagic larval durations (38) and may shift to outbreeding, where most fertilization occurs between unrelated individuals relatively far from the site of gamete release, especially in species which have prezygotic isolating mechanisms preventing self-fertilization or fertilization of related gametes (52). Larval dispersal and population structure are thus intimately linked, and detection of gene flow between populations based on genetic markers is critical for understanding large-scale demographic patterns of dynamically interconnected populations (217). Sexual reproduction and long-distance dispersal are especially important as a natural recovery mechanism after disturbance (212), where communities can be supported by healthier populations from remote unaffected source sites.

Massive declines in the genus Acropora contributed significantly to declining coral cover in the Caribbean, where these formerly dominant reef-builders nearly completely disappeared from some areas (27, 48, 61) due to a combination of stressors, including White Band Disease (8, 29). In response to these declines, the staghorn coral, Acropora cervicornis was listed as threatened under the United States Endangered Species Act (ESA) in 2006 (34). While its abundance has decreased, Acropora cervicornis remains an important resource for understanding ecological patterns a
connectivity on Caribbean reefs, particularly as active management, propagation and restoration contribute to the recovery of this species (20). One of the main goals of active species recovery for this species is to facilitate sexual reproduction (and natural recovery) by establishing populations of nursery-reared corals to bridge large spatial gaps in extant distribution, counteracting the allee effect where sparse population densities limit fertilization due to the diffusion of gametes (218, 219). In addition to the management and conservation implications, *A. cervicornis* is also a valuable study species because it reproduces asexually through fragmentation (23, 24), so patterns of local-scale dispersal (i.e. within-reef) are likely influenced by both sexual and asexual reproduction. Recent evidence indicates that genetic diversity in this species is concentrated over scales as small as individual reefs (49), suggesting that past sexual reproduction had significant impacts on population genetic structure and demographics in this species. However, the current near-absence of sexual recruits (63, 78) suggests very low present levels of recruitment (63, 187). While *A. cervicornis* is presently thought to rely almost exclusively on fragmentation for propagation, sexual and asexual reproduction can be viewed as complimentary rather than mutually exclusive (41).

Though dispersal is only one component of the coral reproductive cycle (215), the assumption that larvae are passively transported in the water column until competent to settle facilitates the use of biophysical models to understand patterns of dispersal and connectivity. A description of *A. cervicornis* dispersal in Florida is currently unavailable, so basic understanding of connections between populations, source/sink dynamics, and potential barriers to dispersal would provide novel and key information for management, incorporating a stronger understanding of different life-history stages into conservation
decisions. Importantly, the ability to describe regional-scale population connectivity is significantly more valuable given the genomic resources currently available (220). These resources enable comparative link- and node-based approaches (221) to help parse ecological and evolutionary factors relevant for the recovery of this important species under stress scenarios that include local and climate change factors (221).

Genetic population structure among *A. cervicornis* regions of the Florida Reef Tract (FRT) has been assessed at varying resolutions (48, 73), including the recent use of next generation sequencing (49), showing significant genetic structure among populations over spatial scales as small as 50km. These populations are the functional units of active restoration in Florida, composed of genetically diverse corals housed in *in situ* nurseries that represent assemblages collected from within each region and propagated within a common garden (Figure 5.1). Here, we utilize next generation sequencing data for these *A. cervicornis* individuals (n=188) throughout the FRT to compare population structure and demographic patterns to larval dispersal, examined via the Connectivity Modeling System (CMS) (222). CMS is a biophysical individual-based model that integrates physical oceanographic parameters including currents, turbidity and bathymetry to examine the transport of individual particles, representing larval dispersal (222). This model enables a direct comparison of observed patterns of genetic population structure and connectivity, describing the potential impacts of broad-scale ecological processes such as recruitment potential and gene flow on population dynamics in this threatened species. Here, we describe connectivity within Florida and test the hypotheses that: 1) variable levels of connectivity are apparent between regional populations of the FRT, 2) observed patterns of genetic population structure within the FRT are correlated to the
connectivity network developed using CMS, 3) areas with higher genetic diversity result from proportionally higher larval input from more diverse areas of the FRT, and 4) potential signatures of inbreeding relate to areas of higher self-recruitment. The data presented here form an important link between genomics, connectivity, and management by highlighting source/sink dynamics, areas of particularly broad dispersal potential, and regions that should be focal conservation and restoration targets due to particularly low larval input from other areas.

**Materials and Methods**

*Larval Dispersal*

The Connectivity Modeling System (CMS) is a biophysical, individual-based model that uses physical oceanography to simulate the dispersal of particles. Bathymetry, currents, turbulence, and individual behavior such as time-to-settlement are coupled and can be used to understand the dispersal of passive planktonic larvae, such as those of scleractinian corals (38). We used the South Florida Hybrid Coordinate Ocean Model (HYCOM) (223) to study dispersal within the Florida Reef Tract (FRT). Known *A. cervicornis* locations were plotted onto reef habitat polygons (224) (Figure 5.1) and equal numbers of particles (n=1000) were released from each polygon (n=281) on each night 4-7 days after the July full moon for 2012, 2013 and 2014. Release dates correspond with observed spawning times of Acroporids throughout the Caribbean (50-52, 55). Larval competency was established at 4 and 7 days after spawning to bracket the range of time to competency observed in this species (225, 226). No larval behaviors for swimming or vertical migration were included in the simulation. After competency, settlement was
recorded by reef polygon (224), which were classified into seven regional populations corresponding to sample locations used in (Drury, Chapter 3) (Figure 5.1). Briefly, each regional boundary was established at the midpoint between the collection locations of genetic samples from separate regions. An additional region of known *A. cervicornis* presence was defined for the Marquesas, although no genetic samples were obtained from this region in this study. The raw output from the model provides the number of particles transiting between any two polygons combined for all years.

For the purposes of examining link-based connections between regions in Florida, the polygon distribution of habitat is ideally representative of potential spawning and settlement activity. However, this distribution introduces artifacts at both ends of the FRT. Given the strongly northeastward currents in this area, the Broward population has inflated values for local-retention because no settling particles were transported to the south and no habitat was available in this model simulation to the north. Similarly, the Dry Tortugas has no habitat polygons to the west or south, so all settlement here is defined as local retention or ‘downstream’ settlement.

*Genetic relatedness*

Genetic relatedness values are from (Drury, Chapter 3). Briefly, Genotyping by Sequencing (GBS) (105) was used to produce a series of Single Nucleotide Polymorphisms (SNPs) for *A. cervicornis* individuals throughout the Florida Reef Tract. In total, 188 samples from six Florida populations were analyzed at 3163 loci. Fixation index (*F*<sub>ST</sub>), pairwise distances, expected heterozygosity (*He*), Nei’s genetic distance, and allelic patterns were calculated as in (Drury, Chapter 3) using Arlequin 3.5.1 (162),
TASSEL 5.2.29 (227), and GenAlEx (196). Nei’s Genetic distance was calculated for examining relatedness in populations (228) using GenAlEx (196)

Analyses

Output of CMS was converted to a matrix of source-sink connections using MATLAB 9.0. Raw output was converted to probabilities by dividing by the total number of particles in the matrix and polygons were pooled into regions as defined above and used for analysis. Directional migration was calculated and proportional recruitment by source region was created for each region. The primary direction of currents around the Florida Keys is to the north and east, which is termed ‘downstream’ in this analysis, while ‘upstream’ transport represents flow to the south and west. Self-recruitment was defined as a percentage of particles settling in region of origin out of non-local particles settling in that region. Local retention was defined as a percentage of particles settling in region of origin out of all particles released from that region. Source-sink dynamics were defined as [probability of particles released from a region/probability of particles settling in region], with values >1 indicating sources and values <1 indicating sinks. A mantel test for correlation between two matrices was used for regional level correlations for two genetic distance metrics: average pairwise differences and Nei’s genetic distance. Each of these matrices was compared to dispersal values for 4 and 7 DTC, with p-values generated from 9999 repetitions. Network structure was analyzed in R using the package iGraph (229). Values in network diagrams represent probability of particles originating in one region and settling in another. Network visualizations use the square root of scaled
values for relative display purposes. Matrices represent probability of transit between origin and settlement polygon, scaled by total probability from each polygon of origin.

Results

Dispersal patterns at 4 days to competency

With larval competency beginning at 4 days post-spawn (4 days to competency, DTC), local retention was 19.7% to 92.9% in the Dry Tortugas and Marquesas, respectively. However, 100% retention was recorded in Broward (Table 5.1a). This high value is an artifact of the settlement polygon distribution, where northward currents swept all particles from Broward that did not settle in this region out of the study area, and indicates that no southward flow of larvae occurred from this population (Figure 5.1). Percentage of larvae going ‘upstream’ (i.e. to the south or west along the FRT, in the opposite direction of the prevailing Florida Current) was highly variable, between 0% in Broward and the Upper Keys and 63.0% in the Lower Keys (Table 5.1a). Regions in the northern extent of the Florida Reef Tract (Upper Keys, Miami, Broward) had much lower ‘upstream’ settlement than regions in the southern half (not including Dry Tortugas, which could not have upstream settlement because of polygon distribution) (Table 5.1a; Figure 5.2a, Appendix III). ‘Downstream’ settlement (i.e. to the North and East of spawning region) was between 1.2% of larvae in the Marquesas and 60.2% in the Upper Keys. However, another artifact of the polygon distribution indicates 80% of larvae from Dry Tortugas settled ‘downstream’, because no settlement polygons existed ‘upstream’ of this population (Figure 5.11). Source/sink ratio ranges from 2.930 in the Lower Keys to 0.147 in Broward, indicating strong larval sources and sinks respectively. In the Lower
Keys, nearly 3 times as many larvae are exported to other regions as settle within, regardless of source (Table 5.1a). Self-recruitment was between 15% and 84% in Broward and the Lower Keys, respectively (Table 5.1a). All regions received larval input from between 3 and 5 source regions (Table 5.2a), which was related to distance between these populations. Not all patterns were related to distance, as Miami and Broward both received more input from the more distant Dry Tortugas than from the Marquesas or Lower Keys (Table 5.2a). Dispersal distances are notably short for Broward, Miami, and the Upper Keys (Figure 5.3a), partially due to the polygon distribution artifact and northward currents in this area. The Dry Tortugas, the most remote population, has the longest dispersal due to a peak of settlement in the Middle and Upper Keys (Figure 5.3a). The Middle Keys, Marquesas, and Lower Keys all have ‘upstream’ dispersal, reflective of transits between these regions and the Dry Tortugas (Table 5.1a, Figure 5.3a).

*Dispersal patterns at 7 days to competency*

At 7 DTC, local retention values (excluding Broward) drop for every region to between 4.5% in the Middle Keys and 79.7% in the Marquesas, indicating that later competency time allows farther dispersal (Table 1b). ‘Downstream’ settlement percentages were higher after 7 DTC than 4 DTC in all regions except the Marquesas and showed larger increases in the northern regions of the FRT (Upper Keys, Miami, Broward) (Table 5.1b; Figure 5.2b, Appendix III). Percentage of upstream settlement changed little between 4 and 7 DTC, except for the Marquesas and Lower Keys. Patterns of source/sink ratios are generally more extreme at 7 DTC, ranging from 0.016 in Broward to 4.907 in the Lower Keys (Table 5.1b). Self-recruitment was between 2% in
Broward and 93% in the Lower Keys, and decreased in every region except the Lower Keys when compared to self-recruitment at 4 DTC (Table 5.2b). Importantly, longer time to competency changes patterns of larval transport from all regions, but indicates that the Dry Tortugas is a net sink only during the longer time to competency. Dispersal distances are similar at 4 and 7 DTC, with an increase in distance for the Middle Keys at 7DTC (Figure 5.3b). In addition, upstream transport from the Lower Keys, Marquesas, and Middle Keys to the Dry Tortugas increases at 7DTC (Figure 5.3b).

**Genetic relatedness**

Significant genetic population structure occurs between approximately 1/3 of pairwise comparisons within Florida (Table 5.3a). The Dry Tortugas are not significantly different from any other FRT population, while the Middle Keys have significant population structure compared to every other population except the Dry Tortugas. Miami-Dade and Broward are significantly different, as are Broward and the Lower Keys (Table 5.3a). Nei’s genetic distance was high between the Dry Tortugas and other populations and ranged from 0.002 to 0.031 (Table 5.3b). Expected heterozygosity ranges from 0.032 in the Lower Keys to 0.016 in the Dry Tortugas, a nearly two-fold difference. Allelic richness, another metric of genetic diversity that heavily weighs unique alleles, is between 1.273 in the Lower Keys and 1.009 in the Dry Tortugas. Observed heterozygosity is between 0.00152 and 0.00341 and is much lower than expected heterozygosity for all populations (Table 5.4).
Larval dispersal and genetic connectivity

Overall, pairwise genetic differences and dispersal structure (relative larval transit) were not significantly correlated for either 4 DTC (Mantel test, 0.258, p=0.353) or 7 DTC (Mantel test, 0.162, p=0.368). In addition, Nei’s genetic distance was not significantly correlated with dispersal at 4 DTC (Mantel test, -0.267, p=0.678) or 7 DTC (Mantel test, -0.302, p=0.836). No significant relationship exists between observed heterozygosity and percent or absolute self-recruitment or local retention (linear regression, p>0.05). Likewise, no significant relationship exists between expected heterozygosity or allelic richness and percentage recruitment from other regions, absolute recruitment from other regions, or number of regions from which recruits are derived (regression, p>0.05). Particularly strong larval connectivity between Miami and Broward is contradictory to the significant genetic population structure observed between these regions. Likewise, strong larval connectivity between the Middle Keys and Upper Keys does not correlate with the significant population structure observed. Conversely, the Dry Tortugas shows not genetic population structure with other regions of the FRT, supported by larval connectivity with each region (Figure 5.2a,b). The Dry Tortugas is the only region which exports larvae to all other regions, though this input is typically less than 10% of larvae that each region receives (Table 5.2a,b).

Discussion

Larval dispersal is a critical component of the life history of sessile marine organisms such as corals, but remains poorly resolved over local scales (206). We simulate this process for Acropora cervicornis on the Florida Reef Tract and compare the
results to genomic patterns. Simulation output showed regional scale variation in *A. cervicornis* dispersal across the Florida Reef Tract (FRT), with highly asymmetrical transfers of larvae moving predominately to the north and east with the Florida Current. We also show that time to competency in this species can dictate the dispersal direction and local retention patterns of coral larvae.

The direction of flow and complexity of local current patterns on the FRT can be divided broadly between regions north and south of the Upper Keys. Dispersal in the Upper Keys, Miami, and Broward shows more unidirectional transport of larvae to the north, with very strong connectivity from the Upper Keys to Miami, Miami to Broward, and the Upper Keys to Broward. Little to no larval transit occurs to the south in these areas, except for a small proportion of larvae produced in Miami and settling in the Upper Keys, which is orders of magnitude smaller than the downstream transport in this area. Conversely, dynamic multi-directional flow leads to up- and downstream transport of particles that originate south of the Upper Keys. In this area, the tidal flux of water from Florida Bay likely influences dispersal patterns (177), leading to a much more complex network of interconnections between the Dry Tortugas, Marquesas, Lower Keys, and Middle Keys than found further to the north. For example, ‘upstream flow’, defined as particle movement against the Florida Current, accounts for approximately 30% to 70% of particles released from the Middle Keys and Lower Keys, respectively. This trend is consistent with previous observations in this area (224) at both competency times. Illustrating this pattern is the low dispersal from the Marquesas to the nearby Lower Keys, indicating that in some areas beyond the extent of the main Florida Keys (i.e. west of Key West) larvae may not be frequently transported ‘downstream’, but mostly return
to the Dry Tortugas. In the same way, connectivity between the Middle Keys – Dry Tortugas and Lower Keys – Dry Tortugas supports the westward flow of larvae.

Proportion of self-recruitment is highest in the central regions of the FRT, mostly in the Lower and Upper Keys, with a smaller value in the Middle Keys. Farther from this central region, self-recruitment drops, especially at the longer competency period, when more time is available for larvae to be transported away from their region of origin. In the context of complex larval movement in the southern half of the FRT high self-recruitment in the Lower Keys makes sense, because oceanographic patterns do not disperse larvae in a uniform manner, so more are available to settle in their home region. However, this result is somewhat surprising in the Upper Keys, where most larvae are advected to the north. Though self-recruitment is high, larval retention increases in the northern areas of the FRT because of the limits of habitat but also due to the mostly northward flow, which removes larvae from the system. These trends complement the pattern of asymmetrical connectivity among populations in Florida. Only the Dry Tortugas - Marquesas and Lower Keys – Middle Keys have similar values of larval transport in both directions, while much stronger values typically occur between all other populations and their downstream regions. Because of this trend, the Dry Tortugas transports larvae to each downstream region, and although the absolute number of larvae are low in some instances, this is an important influence on the connectivity of these regions shown in the genetics data. The Dry Tortugas is also the only region which shows variable source/sink patterns based on dispersal time, because longer dispersal leads to more import from nearby regions at 7 DTC. Conversely, the Lower Keys is the largest relative source region for staghorn larvae in the FRT, with between 3 and 5 times as
many larvae exported from the region as settling there, dependent on time to competency. The Upper Keys and Middle Keys are also strong sources of larvae at both time competency periods, which is likely independent of the release concentrations because nearly every polygon in these regions contained colonies.

Patterns of larval dispersal relate poorly to coral genetic population structure in the Florida Reef Tract, contrary to other studies in different species (230, 231). The Middle Keys are relatively well connected in both upstream and downstream directions, exporting larvae to and receiving larvae from the Upper Keys, Lower Keys, and Dry Tortugas. However, strong population structure exists between each of these regions, except the Dry Tortugas, suggesting that modern populations in the Middle Keys are not reflective of current or historical larval connectivity. We hypothesize that the influx of inimical waters from Florida Bay (177, 232), which can be hypersaline, turbid, and show high temperature variability (233), serves as a barrier to settlement on reefs in this region, which is not reflected in the overall dispersal patterns. Though connectivity between the Middle Keys and Dry Tortugas is relatively low, the Dry Tortugas has larval connectivity with every region, which may reflect the low population structure between this area and all others. Connectivity between the Dry Tortugas and Upper Keys may also serve as a stepping stone to other populations, where relatively higher magnitude dispersal from the Upper Keys to the northern extent of the FRT supplements dispersal directly from the Dry Tortugas and promotes gene flow, enhancing connectivity directly from the Dry Tortugas.

One area of particularly strong disagreement between larval transport and genetic population structure is the Miami -Broward transition, where strong northward larval
transport occurs. Miami may serve as a stepping stone between the Upper Keys and Broward, but significant genetic differentiation occurs between Miami and Broward populations. This contrast may be partially due to the asymmetrical nature of larval transport at the northern end of the FRT and the limitations of polygon distribution, but strongly indicates that larval dispersal is not driving population differentiation in this region. This area is an ecotonal region (172) of low genetic diversity (Drury, Chapter 3), where the unidirectional nature of larval transport may serve to create genetically similar populations over very small spatial scales that show significant population structure when grouped as regions because of barriers to dispersal or patterns of population structure occur over smaller scales than those observed here. Though some regional links supported population structure patterns for individual comparisons, the lack of correlation between pairwise differences, Nei’s genetic distance, and dispersal density indicates that a significant mismatch between dispersal and genomic patterns has occurred. Conversely, few localized areas of larval connectivity correspond to genetic patterns. One such example is high connectivity between the Upper Keys and Broward, which show no genetic differentiation, though given the complexity of population structure between these regions and the intermediate Miami population, this result should be interpreted with caution.

Using a node-based approach allows the genetic diversity and allelic patterns of each region to be compared to various larval dispersal parameters such as self-recruitment and local retention, potentially supporting or refuting link-based assessments of population structure and the larval dispersal network. We hypothesized that higher self-recruitment or local retention would influence observed heterozygosity, but these
factors were unrelated. When observed heterozygosity is significantly less than expected (as in all regions investigated here), it can be a signal of potential inbreeding, where like individuals breed and increase the amount of homozygosity. Similarly, expected heterozygosity, a general measure of genetic diversity, would be expected to be higher in regions with a higher proportion or relative number of settlers from other regions, but we found no correlation. Allelic richness, another measure of genetic diversity that is highly influenced by unique alleles also shows no correlation to input from other regions, relative or absolute, which is important because this metric is sensitive to the input of rare alleles that might be introduced during recruitment from distant populations. Overall, the most genetically diverse populations are the Lower Keys and Broward. The Lower Keys are well connected in a ‘subnetwork’ of the surrounding populations due to the oceanographic patterns in the southern Florida Keys, which provide a much more dynamic level of larval dispersal that would be expected to homogenize populations. While Broward, which is also relatively very diverse, receives heavy input from Miami-Dade and the Upper Keys, this connectivity is mostly unidirectional, and one-way gene flow would need to be exceedingly strong to drive population homogenization. The conflicting nature of connectivity in these two high diversity areas supports the lack of correlation between the dispersal simulation and patterns of genetic structure.

The simulation used here has two types of limitations, spatial and biological. Spatial limitations clearly impact some results of this study, because the primary direction of nearshore flow in the Florida Current is to the north and east along the Florida Keys. The Dry Tortugas receive relatively little simulated input because we did not simulate connectivity further upstream, where larvae from more distant Caribbean
populations may originate (46, 234). Likewise, values for local retention and dispersal distance are heavily skewed for Broward, because the direction of flow mandates that larval particles originating here settle in Broward or are advected from the study area. Given these limitations, values for both endpoints of the FRT shown here should be interpreted with caution. The second, biological limitation relates to an oversimplification of the complex processes involved in coral spawning and gamete fertilization in this simulation. Larval dispersal only accounts for one component of successful reproduction and recruitment (215), while adult demographics, fecundity, parental diversity, larval survivorship, settlement success and juvenile survivorship all contribute to connectivity. These factors integrate gametes into an existing population, but many are poorly documented for *A. cervicornis*, thus this is an important area for evaluation in future simulations.

Another area of uncertainty is the importance of sexual reproduction in this species, which may have changed over time. *Acropora cervicornis* has long been thought to reproduce almost exclusively via fragmentation (23), an assumption which has been historically based on the absence of sexual recruits recorded in visual surveys or settlement studies (63, 78). However, modern populations still undergo gamete development and spawning throughout their range (52-54, 235) once colonies reach a certain size (50), so the potential for sexual reproduction is apparent and may be important for the recovery and persistence of this species. In addition, patterns of genetic diversity on individual reefs (Drury, Chapter 3) indicate that neither thickets, the consolidated structures of interlocking skeletons that can cover large areas of the bottom (31), nor isolated colonies are produced exclusively by fragmentation. This evidence, in
addition to the consolidation of genetic diversity over small spatial scales such as individual reefs (Drury, Chapter 3) suggests that sexual reproduction has historically been an important component driving the current distribution of genetic diversity, though it may have a reduced contribution on modern reefs. Acroporid decline due to White Band Disease (8, 29) and other stressors has been severe in this area (27, 28), and could influence remnant populations as a selective pressure and through stochastic genetic drift acting on smaller populations. We hypothesize that differential mortality during the past several decades has influenced these patterns, selecting for more disease resistant populations, presenting an energetic tradeoff that influences the resources devoted to sexual reproduction. The uncertainty about the importance of sexual reproduction may explain the difference in results between this study and a similar investigation of *Orbicella annularis*, where sexual reproduction is common and dispersal patterns closely relate to population structure (230). The Middle Keys likely provides the best illustration of this hypothesis because of low reef development and *A. cervicornis* density, which is presumably influenced by the tidal influx of inimical waters from Florida Bay (232). High diversity in this region and moderate larval connectivity through the southern half of the FRT indicate that genetic connectivity should be moderate, but differential selection of certain individuals able to withstand the stress of this environment could drive the strong population structure observed between the Middle Keys and most other regions.

Source-sink dynamics, patterns of connectivity and genetic diversity suggest that different regions of the Florida Reef Tract are important to conservation and management for diverse reasons. All populations in this study (except Broward) are afforded some
level of protection through the Florida Keys National Marine Sanctuary, Dry Tortugas National Park and Biscayne National Park, in addition to the general restrictions of the Endangered Species Act (34). Increased protection through no-take zones, fishing regulations, mooring buoys, ‘research-only’ areas and stronger enforcement (236) further conserves vulnerable populations (237) and contributes to recovery of these areas and other regions beyond their boundaries (207, 217, 238). Dispersal potential is thus an important resource for conservation and management to design Marine Protected Areas and networks (102, 239). Based on the results presented here, we recommend increased protection of several regions for various reasons. First, the Dry Tortugas are an important hub connected to all other areas of the FRT and contributing to low population structure. This region also likely incorporates upstream diversity from elsewhere in the Caribbean into Florida populations by acting as a stepping stone over multiple recruitment events, so its function for supplementing existing populations is extremely valuable. Second, the corridor between the Dry Tortugas and Lower Keys may be important for protecting genetic diversity and larval dispersal across the entire extent of the FRT, making this an important focal point and highlighting the importance of spatial configuration of reserves (240). Next, the Lower Keys is highly connected to several other regions in the southern ‘subnetwork’, has the highest capacity for larval dispersal and is the most diverse population, so further restrictions on use in this area may help conserve valuable diversity important for recovery and resilience (93, 142). Finally, active intervention through coral restoration (20, 39) can also aid some areas where connectivity translates very poorly to genetic diversity, such as the Middle Keys and Upper Keys via the introduction of new colonies from other regions may help to boost diversity (Drury, Chapter 3).
Data presented here provide an important link among genomics, connectivity, conservation, restoration, and management in a threatened coral species. Patterns of dispersal in the Florida Keys are highly dynamic, with large-scale differentiation near the Upper Keys. To the south of this midpoint, complex dispersal patterns to the upstream and downstream directions link most populations to each other, though the relative connections vary in magnitude. Conversely, in the Upper Keys and northern regions of the FRT, spawning colonies release larvae that are transported almost exclusively to the north, where limited habitat availability may influence these populations by restricting settlement. Highly asymmetrical dispersal along the FRT suggests that some regions are disproportionately important sources of larvae which should be the focal points of increased protection, representing important areas of larval export that may be able to seed depleted areas elsewhere and assist with recovery. The apparent decoupling of larval connectivity and genetic population structure may be a result of post-dispersal processes including settlement and juvenile survivorship, but also points to the possibility that reproductive viability has declined in recent decades. This information is critical for ongoing efforts to restore A. cervicornis, and should be further investigated to maximize the efficiency of management efforts to ensure the persistence of this spec
CHAPTER 6 – CONCLUSIONS

Summary

*Acropora cervicornis* is an important reef-building coral that creates structure and habitat for many reef organisms, contributing to reef function in the Caribbean (78). Its abundance has declined significantly in recent years (28), and efforts to mitigate these losses have become increasingly common (20), especially through the coral gardening methodology (17, 19). This technique facilitates manipulative experimentation that is a powerful tool for resolving ecological processes, but also requires genomic data for responsible intervention (43, 44). These data are interrelated and form the foundation for ongoing persistence of *A. cervicornis* and reefs in general, through flexibility and adaptation over the short- and long-term. I find extensive flexibility in growth and survivorship dictated by environment and coral genotype, which could serve as the bridge from intra- to intergenerational resistance and resilience. This plasticity can maintain fitness and survivorship under some range of conditions, providing the raw materials for adaptation in the following generations. This raw material, genetic diversity, is variable across the Florida Reef Tract and Caribbean, leading to significant population structure over previously unresolved spatial scales. Thickets represent the smaller spatial scales investigated here, where clonality is common but not absolute. In addition, thickets are rarely genetically unique from isolated colonies. Local scale patterns of staghorn assemblages produce a hierarchy of genetic variation that resembles nursery populations, with most genetic diversity concentrated within the smallest spatial scales. These data suggest that restoration can be adequately used to replicate genetic diversity seen in remnant wild populations and point toward an influential role of sexual reproduction in
creating the current assemblages found on modern reefs. Contrary to this result, genetic differentiation within Florida does not relate closely to larval connectivity. These findings integrate ecological and evolutionary processes, describing how flexibility of individual colonies can maintain diversity, which is variable at many spatial scales and creates population structure, providing feedback for conservation, management and active restoration.

Chapter 2

One critical component of the survival of species under changing conditions is the ability to acclimatize within a generation (37, 74). Manipulative studies of Acropora cervicorns have focused mostly on the response to specific stress and environmental parameters (68, 70), but previous field experiments have also shown that within a site coral genotype can produce significant variation in growth rates (39, 40). Thus, the coral gardening methodology has begun to provide the foundation for examining higher order ecological processes that, in some cases, have been poorly examined in coral reef ecosystems. I used a common garden and controlled reciprocal transplant to investigate patterns among 10 genotypes at 8 reefs. For the A. cervicornis examined here, site significantly impacts growth and survivorship and individual genotypes have growth rates that vary up to 5-fold among sites. Coral individuals can be classified as ‘generalists’ and ‘specialists’ (104), because some exhibit high or consistent growth across most environmental conditions, while others have high variability, suggesting that they are more specialized for particular environments. The distinction between strategies is critical for the long-term persistence of this species, because each provides a different avenue to growth and survivorship, maintaining the raw materials needed for adaptation.
First, generalists may be able to survive and prosper under a wider range of conditions than those investigated here, potentially including circumstances developing under climate change. Second, specialists may take advantage of specific niches, especially if they are acclimatized or adapted to high light, high temperature environments.

The plasticity found here may also serve to temper local adaptation. Flexibility allows an individual to produce a beneficial (or at least non-harmful) phenotype under a range of conditions (82), so plasticity decreases selection pressure on an individual and can contribute to genetic diversity (91). Local adaptation was not apparent in the *A. cervicornis* individuals examined here, which contrasts to typical patterns in many marine organisms (36). Though previous results have found some local adaptation over larger spatial scales (241), the use of a continuum of environments provides a better model for evaluating home-site fidelity in this species (42). No sites showed significantly higher growth of local genotypes and when all results were pooled across sites, foreign corals grew significantly more quickly. This finding may be impacted by the duration of the experiment, where acclimatization is still underway, but indicates that the transplantation of corals between environments is not systematically biased against ‘foreign’ individuals. The use of a controlled transplantation model also showed that corals which were not moved to a different environment (nursery controls, site controls) tended to have lower growth, suggesting that some change in environmental cues dictate growth in addition to the ‘pruning vigor’ observed in *A. cervicornis* after fragmentation (19).

Despite the lack of local adaptation, site is an important driver of coral growth and survivorship, explaining a large proportion of the overall growth variance. The use of many site-genet combinations provides a more conclusive examination of the
influence of environment than in previous studies (41), revealing significant differences between sites and a large range of growth rates. Survivorship and disturbance response were also dramatically affected by the environmental conditions. For example, small differences in environmental conditions allow some habitats to serve as refugia and certain genotypes only survived outside of their site of origin. Temperature differences as small as 0.5°C led to significantly higher recovery following a mass bleaching event, producing a limited set of site x environment combinations that had low relative mortality. The influence of site also subtly interacts with coral genotype, highlighting the complex relationship between environmental and genetic factors that dictate growth and survivorship outcomes. Though lack of power did not enable us to find a significant interaction term, reciprocal changes in growth rate between sites for various genotypes indicates that both factors dynamically influence growth. The complexity observed here also has important implications for restoration, suggesting the nursery environment is a poor predictor of performance on reefs, so diversity should be maximized to produce potentially beneficial genotype x environment interactions, some of which create differences in growth and survival which serve to spread mortality risk for a given genotype. The result of this complexity, high plasticity, and low local adaptation also temper concerns about coral transplantation, indicating managers can responsibly redistribute genetic material across environments. This potential redistribution would influence population-level genetic patterns through the introduction of new alleles, so an intimate description of current genetic patterns is an important prerequisite. These data can suggest focal areas and resources, each of which should be targeted to increase resistance and resilience by enhancing the diversity of local assemblages.
Chapter 3

The presence of functional differences between genotypes provides direct evidence for the importance of genetic diversity, illustrating the connection between individuals and populations. This diversity can be quantified by reef, thicket, nursery, region, and even over larger spatial scales. By sequencing many loci of many individuals (152) our results enable a link-and-node based approach (242) to compare population structure between populations and examine absolute differences between those regions. These results support previous findings of regional scale population differentiation (46, 48, 49, 72, 73) between Florida, The Dominican Republic, the Cayman Islands, and Belize, adding samples from previously unrepresented areas to the developing understanding of spatial genetic patterns in this species. Long distance (>500km) gene flow is low but variable, resulting in different trends between the more densely sampled Florida populations and other Caribbean populations. For example, the Dominican Republic has significant structure for half of the Florida populations in pairwise comparisons, suggesting a mosaic of processes is dictating current relationships. This complexity is evident within Florida, where various populations (nurseries) have unique properties. In this region, the Middle Keys are isolated, while other regions like the Dry Tortugas and Upper Keys are more closely related to their neighbors along the FRT. For example, the Dry Tortugas, which lies upstream of all populations in the Florida Current, is not significantly differentiated from other populations. This technique shows higher resolution than previous efforts to examine Florida’s A. cervicornis (48, 73), providing updated information for management efforts. Certain areas of high diversity, such as the Lower Keys, can also be identified and used to develop conservation goals and
hypotheses to better understand how community level genomic patterns may contribute to healthy coral populations. Conversely, previously unrecognized areas of low diversity, including the Upper Keys and the ecotonal boundary between Miami and Broward populations, should be focal points of restoration and transplantation, potentially incorporating corals from more distant populations to bolster genetic diversity. Lastly, Broward represents a particularly unique population with rare alleles, which may be due to its location at the northern end of the FRT, making it a valuable asset for understanding populations living near the boundary of a species range.

The genomic data shown here have important implications for individual corals within an assemblage, especially when those assemblages are created by restoration efforts. Through the analysis of population patterns created by assembling cohorts of individuals, thresholds can be determined to ensure realistic, functional assemblages are developed through conservation (43-45). For example, the average genetic diversity of a wild reef can be achieved through the transplantation of only 5-7 individuals from a nursery. Similarly, the maximum observed diversity on a wild reef requires only using 10-12 nursery individuals because regional populations harbored in nurseries tend to have similar but slightly elevated genetic diversity compared to individual reefs. Both sampling strategies present similarly structured variance components (AMOVA), where most variation is found within populations, meaning nurseries can capture the genetic variation present within a region. This result also suggests that genetic diversity is concentrated on individual reefs, supporting the developing understanding of reefs as less genetically homogenous units which can be composed of many genotypes (49, 64). The flexibility and differences in growth and survivorship between individual genotypes best
illustrate the implications of population level differentiation and changes in genetic diversity. First, more diverse regional populations are valuable because they contain more raw material for short term responses like genotype X environment interactions, which are likely to be unpredictable. This process ensures genetic diversity needed for potential adaptation is present due to survivorship resulting from plasticity. Second, the variation between regional diversity and ability of nurseries to reproduce realistic assemblages supports the use of the maximum amount of genetic material during active restoration. This increases the potential for sexual reproduction (outcrossing). Genetic diversity also supports the link from individuals to communities because it has effects similar to species biodiversity (141, 144). Although these impacts remain mostly unstudied in coral reefs, they include important processes for threatened ecosystems including increased resilience, disease resistance, improved production, improved nutrient cycling, structuring affects on associated communities, and increased species-level diversity (140, 142, 145-147).

Chapter 4

Resolving the importance of genetic diversity and population structure over larger spatial scales provides important context for more spatially restrictive analyses. This approach facilitates the comparison of smaller components of A. cervicornis populations (thickets and isolated colonies) and highlights the relationships between them. Occasionally, significant structure between thicket plots and individual colonies exists, but this may be more illustrative of dynamic genetic and physical patterns within thickets than evidence that the two different structural products are genetically different. I also show that thicket plots (subsets) are rarely differentiated from each other and in some
instances this may be a signature of a spatial component of differential mortality. One intriguing trend is the presence of private alleles in thickets that are rarely shared with isolated colonies. Though the broad-scale population structure shows little differentiation between these two groups, private alleles may be rare but important instances of genes that promote the creation of thickets instead of isolated colonies. The relationship between individuals and population dynamics is another important component of thickets, representing another layer of ‘flexibility’ like phenotypic plasticity because thickets are fundamentally different structures created in situ without substantial evidence of genetic differentiation. In addition, the role of single individuals is magnified in thickets due to asexual reproduction, which appears to be an influential but not dominant component of thicket formation.

The clonality of thickets has long been assumed (58, 63), but I show for the first time that these structures are not made up of single individuals and vegetative reproduction. Instead, several individuals contribute to the thicket formation in a dynamic manner over time and space, producing ‘evenly heterogeneous’ structures that are not monoclonal but remain less diverse than discrete colonies. Despite this unexpected diversity, some contribution of clonality is still present, illustrated by the lower overall diversity of thickets as compared to isolated colonies. Examining intraindividual variation enables the creation of thresholds due to somatic mutations, which show that some differentiation in thickets can be attributed to clonality but areas composed of discrete, unique individuals are also present. The current level of resolution indicates that between 10-60% of a 100m² thicket is produced asexually, but these patterns are not significantly clustered spatially. The relative importance of sexual and asexual
reproduction highlights the role of individual colonies in creating a population, which varies depending on spatial scale, and whether a thicket is formed or colonies remain sparse and discrete.

**Chapter 5**

Reproductive flexibility in *A. cervicornis* is an influential determinant of genetic structure and diversity from individual reefs to meta-populations. *A. cervicornis* may rely heavily on asexual fragmentation for localized distribution (24), but the lack of local adaptation and concentration of genetic diversity over small spatial scales suggest that sexual reproduction is also an viable strategy for this species. I use the connectivity modeling system (CMS) (222) to examine patterns of larval dispersal in *A. cervicornis* for the first time, comparing these results to genetic differentiation and population structure from the Florida Reef Tract. North of the FRT midpoint near the Upper Keys, simple and nearly unidirectional transport to the north advects larvae from throughout the Florida Reef Tract to the north. South of this midpoint a much more complex system of interconnections is present (224), likely influenced by eddies and connectivity to Florida Bay (177). The Dry Tortugas is a particularly important example of connectivity, exporting particles to all other regions of Florida and likely incorporating gene flow from other upstream areas of the Caribbean (46) into FRT populations, suggesting that modeling is a valuable tool for determining the relative importance of different regions and how protection should be organized over certain areas and corridors.

Contrary to expectations (230), genetic patterns were poorly correlated with larval dispersal. Although this mismatch could be an artifact of modeling capabilities (222) and the complex biological processes involved with connectivity (in addition to dispersal (38,
fecundity, settlement potential, post-larval survivorship, etc.), it may also be indicative of a complex biological reality where sexual reproduction is not a major component of modern *A. cervicornis* population connectivity. In addition, expectations such as high inbreeding and low diversity in areas with high self-recruitment and a positive relationship between diversified input and genetic diversity were not resolved here, further supporting the mismatch between sexual reproduction and population structure. Genetic patterns on individual reefs indicate that sexual reproduction is important for *A. cervicornis* and present populations are fecund across the species range (50, 55), however, the infrequency of sexual recruitment (187) and mismatch between dispersal and genetic connectivity suggest contradicting patterns. I hypothesize that the population bottleneck undergone by *A. cervicornis* has selected an energetic tradeoff that disfavors sexual reproduction, making this strategy much less influential than in the past. If this hypothesis is correct, it emphasizes the importance of the individual during active restoration to impact and create populations. Management should focus on supporting a return to sexual viability through bridging spatial gaps, increasing population sizes on individual reefs, protecting regions with high larval export, diversifying specific areas to facilitate fertilization, and individual-based analyses to select for reproductively successful individuals.

*Conclusions*

The persistence of threatened ecosystems under climate change and increasing anthropogenic stress hinges on the ability of individual species to survive and reproduce. On coral reefs, scleractinian corals are the most important organisms contributing to overall structure and function (113), so their response dictates the fate of the entire
ecosystem. This stress response can occur within an individual generation through plasticity and acclimatization (74), which can be transferred to subsequent generations (37). This mechanism provides the raw materials for adaptation needed to sustain long-term survival (75, 76, 92). Here I present data on the threatened staghorn coral, *Acropora cervicornis*, showing that phenotypic plasticity is an important factor that influences growth and survival in this species, which is also determined by site, genotype, and subtle interactions between these two factors. This process tempers local adaptation and can maintain genetic diversity, which is critical for the potential changes in allelic patterns needed to cope with change. The overall patterns of diversity and population structure show signatures of differentiation potentially due to past declines, but also highlight areas needing targeted restoration and regions that should be treated as resources for conservation as adaptation proceeds within current populations. As the pressures affecting coral reefs become more severe, increasingly active management may play an important role in their persistence and recovery. Results presented here may alleviate some limitations restricting these actions, help focus areas of need, illustrate the feasibility and requirements of realistic restoration, and highlight the importance of maintaining ecological flexibility and genetic diversity. The resulting concepts are an important early step toward integrating multiple levels of biological organization, from the individual to meta-population, to help protect and restore coral reefs.
FIGURES AND TABLES

**Figure 1.1 Dissertation Structure**

Structural diagram showing the flow of information and chapter subjects.
**Figure 2.1 Experimental Design and Timeline.**

(a) Timeline for collection, propagation, outplanting and monitoring. (b) Fragments were collected from individual colonies at each collection site (n=10 sites/genotypes) and transported to the nursery. After the nursery propagation phase, all genotypes were taken to 8 of 10 original collection sites (Government Cut, Site 211 excluded for logistical constraints), so that each site received its original collection, plus 9 other genotypes from each other reef. At each outplanting site, two colonies were collected as wild controls and installed in the plot with nursery transplants. Colors represent different genets coming from each reef, only 4 are presented here as an example.
Figure 2.2 Growth of pooled corals by treatment.

(a) Average LE (cm/day +/- 1 S.E.) at each site with all genotypes pooled. Due to logistical constraints, the fully crossed design did not include the site Jon’s Reef, which is included for representation (hashed) but was not included in 2-way ANOVA analysis. Letters represent significant difference levels in log transformed data (Tukey's HSD, p<0.05), but untransformed data is presented here. Jittered points represent the growth of each genotype within that site and are color coded by genotype. (b) Average LE (cm/day +/- 1 S.E.) of each genotype, pooled across all sites. Due to logistical constraints, the fully crossed design did not include the genotype Government Cut, which is included for representation (hashed) but was not included in 2-way ANOVA analysis. Letters represent significant difference levels in log transformed data (Tukey's HSD, p<0.05), but untransformed data is presented. Jittered points represent the growth at each site for that genotype and are color coded by site.
Figure 2.3 Local Adaptation comparisons of growth.

Average growth (cm/day +/- 1 S.E.) of local and foreign corals at each site. Significant differences between local and foreign genotypes within a given site are denoted by *(t-test p<0.05). Higher growth in foreign corals suggests lack of local adaptation among native genotypes at any given reef. (b) Average growth of native and foreign corals pooled across all sites, significant differences between native and foreign are denoted by *(t-test p<0.05). Local corals were wild controls plus corals originally collected from each site before propagation in the nursery. Foreign corals are genotypes collected from all other sites.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cooper's</th>
<th>CVFD</th>
<th>Grounding</th>
<th>Inshore</th>
<th>Jon's</th>
<th>Miami Beach</th>
<th>Steph's</th>
<th>Struggle Bus</th>
<th>Nursery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>0.017</td>
<td>0.009</td>
<td>0.024</td>
<td>0.012</td>
<td>NA</td>
<td>0.020</td>
<td>0.006</td>
<td>0.018</td>
<td>NA</td>
</tr>
<tr>
<td>Control 2</td>
<td>0.049</td>
<td>0.015</td>
<td>0.025</td>
<td>0.010</td>
<td>NA</td>
<td>0.016</td>
<td>0.007</td>
<td>0.067</td>
<td>NA</td>
</tr>
<tr>
<td>Cooper's</td>
<td>0.023</td>
<td>0.012</td>
<td>0.037</td>
<td>0.056</td>
<td>NA</td>
<td>0.036</td>
<td>0.012</td>
<td>0.029</td>
<td>0.031</td>
</tr>
<tr>
<td>CVFD</td>
<td>0.024</td>
<td>0.013</td>
<td>0.009</td>
<td>0.026</td>
<td>0.025</td>
<td>0.023</td>
<td>0.006</td>
<td>0.015</td>
<td>0.014</td>
</tr>
<tr>
<td>Govt Cut</td>
<td>0.038</td>
<td>0.026</td>
<td>0.053</td>
<td>NA</td>
<td>0.053</td>
<td>0.029</td>
<td>0.016</td>
<td>0.042</td>
<td>0.022</td>
</tr>
<tr>
<td>Grounding</td>
<td>0.031</td>
<td>0.023</td>
<td>0.030</td>
<td>0.067</td>
<td>0.031</td>
<td>0.024</td>
<td>0.015</td>
<td>0.039</td>
<td>0.012</td>
</tr>
<tr>
<td>Inshore</td>
<td>0.018</td>
<td>0.013</td>
<td>0.030</td>
<td>0.030</td>
<td>0.030</td>
<td>0.022</td>
<td>0.008</td>
<td>0.045</td>
<td>0.025</td>
</tr>
<tr>
<td>Jon's</td>
<td>0.034</td>
<td>0.013</td>
<td>0.023</td>
<td>0.036</td>
<td>0.024</td>
<td>0.025</td>
<td>0.009</td>
<td>0.037</td>
<td>0.011</td>
</tr>
<tr>
<td>Miami Beach</td>
<td>0.023</td>
<td>0.017</td>
<td>0.027</td>
<td>0.044</td>
<td>0.021</td>
<td>0.022</td>
<td>0.009</td>
<td>0.042</td>
<td>0.017</td>
</tr>
<tr>
<td>Site 211</td>
<td>0.031</td>
<td>0.024</td>
<td>0.041</td>
<td>0.046</td>
<td>0.021</td>
<td>0.022</td>
<td>0.009</td>
<td>0.064</td>
<td>0.019</td>
</tr>
<tr>
<td>Steph's</td>
<td>0.031</td>
<td>0.039</td>
<td>0.027</td>
<td>0.023</td>
<td>0.017</td>
<td>0.022</td>
<td>0.010</td>
<td>0.064</td>
<td>0.019</td>
</tr>
<tr>
<td>Struggle Bus</td>
<td>0.036</td>
<td>0.019</td>
<td>0.023</td>
<td>0.039</td>
<td>0.027</td>
<td>0.026</td>
<td>0.017</td>
<td>0.067</td>
<td>0.018</td>
</tr>
</tbody>
</table>

**Table 2.1 – Growth of specific genotype x environment combinations**

Average LE (cm/day) of each genotype (row) within each site (column). Significant differences in genotypes within sites are denoted with *(One-Way ANOVA) or §(Kruskal-Wallis) at the bottom of each column using p-values with a Bonferroni correction. Significant differences occurred at 3 of 8 sites. Colors reflect values above (green), at (yellow) or below (red) median LE within each site. Control corals were collected at the time of outplanting from each site independently, thus they do not represent the same genotype across columns. (b) Average LE (cm/day) of each genotype (row) at every site (column). Significant differences between sites (excluding nursery) for a given genotype are denoted with *(One-Way ANOVA) or § (Kruskal-Wallis) to the right of the row using p-values with a Bonferroni correction. Significant differences...
between sites occur in 7 of 10 genotypes. Colors reflect values above (green), at (yellow) or below (red) median LE within each genotype. Lack of significance of Miami Beach corals is likely due to lower sample size at some sites (high mortality). Control corals were collected at the time of outplanting from each site independently, thus they do not represent the same genotype across columns and no averages are presented. Range represents only outplanted corals nursery excluded.
Table 2. Bleaching prevalence of outplanted corals.

Percent bleaching prevalence of live corals of each genotype (row) at each site (column) for (a) July and (b) August. Blank cells represent combinations of site and genotype with 100% mortality (i.e. no live corals for bleaching percentage calculations). Color scales represent bleaching prevalence, with darker cells representing higher bleaching. Average column (far right) represents the average bleaching for each genotype (row). Average row (bottom) represents average bleaching for each site (column). In July, both site and genotype were significantly different from even distribution of bleaching. In August, only site was significant, as temperatures were so high they had...
likely overwhelmed any genetic differences. Control corals were collected at the time of outplanting from each site independently, thus they do not represent the same genotype across columns and no averages are presented.
Figure 3.1 – Map of collection locations.

Large panel shows regional collections from Belize, The Cayman Islands, The Dominican Republic and The Florida Reef Tract. Inset shows locations of Florida collections, color coded by regional population. ‘Wild’ collections within Florida are the locations multiple samples were collected from individual reefs within Florida.
Figure 3.2 - Discriminant Analysis of Principal Components for all populations.

Colors assigned by population: Belize, Cayman Islands, Dominican Republic and within Florida.
Figure 3.3 – DAPC for a) Florida populations and b) Florida populations without Broward.

Colors assigned by population for Broward, Miami-Dade, Upper Keys, Middle Keys, Lower Keys, Dry Tortugas
Figure 3.4 – Interpolation of genetic diversity across the FRT.

Values are interpolations using the nearest 30 neighbors’ pairwise distance values as a diversity metric. Interpolation is corrected to a standardized scale (0 to 1) and represents relative differences in genetic diversity.
Figure 3.5 – Expected heterozygosity as a function of number of individuals.

Miami-Dade regional population (n=48) was subsampled randomly 50 times for each number of individuals. Mean wild expected heterozygosity and maximum wild expected heterozygosity are gray reference lines. Blue line is logistic best fit.
**Table 3.1 – Sample collection locations.**

Number of samples processed from each population, including regional collections and wild collections, where multiple colonies were collected from individual reefs.

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>Nursery</th>
<th>Wild</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>Broward</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Miami-Dade</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>48</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Middle Keys</td>
<td>17</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lower Keys</td>
<td>38</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>9</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cayman Islands</td>
<td>53</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>29</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Belize</td>
<td>6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>272</strong></td>
<td><strong>63</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broward</td>
<td>Miami-Dade</td>
<td>Upper Keys</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Broward</td>
<td></td>
<td>0.055</td>
<td>-0.005</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>0.000</td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>0.971</td>
<td>0.763</td>
<td></td>
</tr>
<tr>
<td>Middle Keys</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Lower Keys</td>
<td>0.022</td>
<td>0.946</td>
<td>0.999</td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>0.999</td>
<td>0.998</td>
<td>0.953</td>
</tr>
<tr>
<td>Belize</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Cayman Islands</td>
<td>0.974</td>
<td>0.960</td>
<td>0.094</td>
</tr>
<tr>
<td>Dom. Republic</td>
<td>0.106</td>
<td>0.007</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3.2 – Pairwise $F_{ST}$ values between populations.
Pairwise $F_{ST}$ values calculated by Arlequin (3.5.2) above the diagonal, with p-values calculated from 10,000 permutations. Red shading represents significance at $p<0.01$ and yellow shading represents significance at $p<0.05$. CI = Cayman Islands, DR = Dominican Republic.
Table 3.3 – Analysis of Molecular Variance.

AMOVA for a) regional populations and b) wild populations. Groups in regional analysis were Florida, Cayman Islands, Dominican Republic and Belize, with populations assigned within Groups as in Table 1. Groups in wild analysis were Florida and the Dominican Republic, with 4 populations assigned within Florida and 2 populations assigned within the Dominican Republic.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>Variance</th>
<th>Percent Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Regional Populations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Groups</td>
<td>82.32</td>
<td>0.05</td>
<td>0.28</td>
</tr>
<tr>
<td>Among Populations within Groups</td>
<td>116.20</td>
<td>0.11</td>
<td>0.66</td>
</tr>
<tr>
<td>Within Populations</td>
<td>8959.07</td>
<td>16.75</td>
<td>99.06</td>
</tr>
<tr>
<td>Total</td>
<td>9157.58</td>
<td>16.90</td>
<td>100</td>
</tr>
<tr>
<td>b) Wild Populations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Groups</td>
<td>117.03</td>
<td>1.28</td>
<td>6.47</td>
</tr>
<tr>
<td>Among Populations within Groups</td>
<td>257.67</td>
<td>2.19</td>
<td>11.03</td>
</tr>
<tr>
<td>Within Populations</td>
<td>2095.60</td>
<td>16.37</td>
<td>82.5</td>
</tr>
<tr>
<td>Total</td>
<td>2470.30</td>
<td>19.84</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3.4 – Patterns of genetic diversity within reefs and between nursery and wild populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Group</th>
<th>n</th>
<th>Na</th>
<th>Na Freq&gt;5</th>
<th>nPrivate</th>
<th>nLocal</th>
<th>He</th>
<th>Ho</th>
<th>Poly loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>Regional</td>
<td>24</td>
<td>1.178</td>
<td>1.128</td>
<td>0.040</td>
<td>0.038</td>
<td>0.030</td>
<td>0.002</td>
<td>18.6%</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>Regional</td>
<td>48</td>
<td>1.248</td>
<td>1.076</td>
<td>0.077</td>
<td>0.031</td>
<td>0.025</td>
<td>0.003</td>
<td>25.2%</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>Regional</td>
<td>48</td>
<td>1.175</td>
<td>1.054</td>
<td>0.061</td>
<td>0.021</td>
<td>0.018</td>
<td>0.002</td>
<td>17.8%</td>
</tr>
<tr>
<td>Middle Keys</td>
<td>Regional</td>
<td>17</td>
<td>1.145</td>
<td>1.138</td>
<td>0.041</td>
<td>0.028</td>
<td>0.032</td>
<td>0.002</td>
<td>14.7%</td>
</tr>
<tr>
<td>Lower Keys</td>
<td>Regional</td>
<td>38</td>
<td>1.273</td>
<td>1.109</td>
<td>0.084</td>
<td>0.043</td>
<td>0.032</td>
<td>0.003</td>
<td>27.7%</td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>Regional</td>
<td>9</td>
<td>1.009</td>
<td>1.009</td>
<td>0.013</td>
<td>0.005</td>
<td>0.016</td>
<td>0.002</td>
<td>4.6%</td>
</tr>
<tr>
<td>Belize</td>
<td>Regional</td>
<td>6</td>
<td>1.015</td>
<td>1.015</td>
<td>0.006</td>
<td>0.005</td>
<td>0.010</td>
<td>0.001</td>
<td>2.5%</td>
</tr>
<tr>
<td>Cayman Islands</td>
<td>Regional</td>
<td>53</td>
<td>1.128</td>
<td>1.053</td>
<td>0.043</td>
<td>0.016</td>
<td>0.016</td>
<td>0.001</td>
<td>13.0%</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>Regional</td>
<td>29</td>
<td>1.128</td>
<td>1.108</td>
<td>0.042</td>
<td>0.016</td>
<td>0.022</td>
<td>0.002</td>
<td>13.0%</td>
</tr>
<tr>
<td>Cayo Carenero</td>
<td>Wild</td>
<td>7</td>
<td>1.051</td>
<td>1.051</td>
<td>0.029</td>
<td>0.000</td>
<td>0.019</td>
<td>0.002</td>
<td>5.4%</td>
</tr>
<tr>
<td>Punta Rusia</td>
<td>Wild</td>
<td>8</td>
<td>1.034</td>
<td>1.034</td>
<td>0.012</td>
<td>0.000</td>
<td>0.012</td>
<td>0.002</td>
<td>3.5%</td>
</tr>
<tr>
<td>Cheetos</td>
<td>Wild</td>
<td>13</td>
<td>1.074</td>
<td>1.072</td>
<td>0.035</td>
<td>0.000</td>
<td>0.019</td>
<td>0.003</td>
<td>7.4%</td>
</tr>
<tr>
<td>Miami</td>
<td>Wild</td>
<td>20</td>
<td>1.115</td>
<td>1.099</td>
<td>0.065</td>
<td>0.000</td>
<td>0.022</td>
<td>0.004</td>
<td>11.5%</td>
</tr>
<tr>
<td>Tavernier</td>
<td>Wild</td>
<td>15</td>
<td>1.117</td>
<td>1.110</td>
<td>0.071</td>
<td>0.000</td>
<td>0.025</td>
<td>0.003</td>
<td>11.6%</td>
</tr>
</tbody>
</table>

n= number of samples, Na = allelic richness, NaFreq>5 = allelic richness greater than frequency 5%, nPrivate = percentage private alleles, nLocal = percentage locally common alleles (greater than 5% frequency within population but <5% in at least 75% of populations), He = expected heterozygosity, Ho = observed heterozygosity, Poly morphic loci = percentage of loci that are polymorphic. Line separates different sampling methodologies, regional
Figure 4.1 – Map of thicket locations and picture of example landscape

Example (a) of typical thicket assemblage taken from Cheetos, FL, b) Location of thicket sites in Florida and Dominican Republic used in this experiment.
Figure 4.2– DAPC for plots and isolated colonies

Discriminant Analysis of Principal Components for individual plots compared to isolated community. **a**) Cayo Carenero, DR  **b**) Punta Rusia, DR  **c**) Sunny Isles, FL  **d**) Cheetos, FL. Only the distribution of the first discriminant function is included for Cheetos because this site only contained a single plot (i.e. 2 classes).
Figure 4.3 – Map of genetic diversity interpolation at Cheetos, FL thicket

a) Map of pairwise genetic differences between all samples, interpolated across plot. Black dots represent analyzed samples. Represents scaled genetic diversity over local spatial scales by interpolating network of pairwise differences between samples across all points. See supplemental materials for mosaics and maps of other sites. b) Mosaic map of Cheetos, FL plot.
**Figure 4.4 – Diversity metrics in thickets and isolated communities as a function of sample size at Cheetos, FL**

a) Expected heterozygosity of isolated community samples, b) expected heterozygosity of thicket samples c) Allelic richness of isolated community samples, d) Allelic richness thicket samples. a) and b) contain reference lines for the corresponding thicket or isolated community at Cheetos and are plotted on the same axes. c) and d) are plotted on the same axes.
**Figure 4.5 – Percent cover and genetic diversity**

Genetic diversity as a function of percent cover for all sites. Individual points represent randomly selected photos scored for percent cover live *A. cervicornis* using CPCe. Colors vary between sites.
**Table 4.1 – Collection locations and plot arrangement**

Location, number of samples, plot size and distribution of samples in plots for all sites. Plots = number of plots per site, nPlots = number of samples within plots at each site, nPlot(x) = number of samples in each plot.

<table>
<thead>
<tr>
<th>Site</th>
<th>Group</th>
<th>n</th>
<th>Plot Size (m)</th>
<th>Plots</th>
<th>nISO</th>
<th>nPlots</th>
<th>nPlot1</th>
<th>nPlot2</th>
<th>nPlot3</th>
<th>nPlot4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cayo Carenero</td>
<td>DR</td>
<td>76</td>
<td>6 x 6</td>
<td>3</td>
<td>23</td>
<td>65</td>
<td>25</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Punta Rusia</td>
<td>DR</td>
<td>40</td>
<td>4 x 4</td>
<td>2</td>
<td>11</td>
<td>29</td>
<td>17</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Cheetos</td>
<td>FL</td>
<td>76</td>
<td>8 x 8</td>
<td>1</td>
<td>19</td>
<td>57</td>
<td>57</td>
<td>14</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Sunny Isles</td>
<td>FL</td>
<td>73</td>
<td>4 x 4</td>
<td>4</td>
<td>4</td>
<td>62</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>
### Table 4.2 – Pairwise $F_{ST}$ between pooled thicket and isolated colonies

Pairwise $F_{ST}$ and p-value for all comparison between thicket samples (all plots pooled) and isolated colonies. Significant values are in red.

<table>
<thead>
<tr>
<th>Site</th>
<th>Group</th>
<th>$F_{ST}$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cayo Carenero</td>
<td>DR</td>
<td>0.162</td>
<td>0.001</td>
</tr>
<tr>
<td>Punta Rusia</td>
<td>DR</td>
<td>-0.018</td>
<td>0.779</td>
</tr>
<tr>
<td>Cheetos</td>
<td>FL</td>
<td>0.032</td>
<td>0.098</td>
</tr>
<tr>
<td>Sunny Isles</td>
<td>FL</td>
<td>-0.029</td>
<td>0.879</td>
</tr>
</tbody>
</table>
**Table 4.3 – Pairwise F\textsubscript{ST} between individual plots and isolated colonies**

Pairwise F\textsubscript{ST} above diagonal, p-value below diagonal for all comparison between individual plots and isolated communities. Significant values are in red. **a)** Cayo Carenero, DR **b)** Punta Rusia, DR **c)** Sunny Isles, FL. No data for Cheetos because of single plot design.
Table 4.4 – Allelic Patterns between thickets and isolated communities

<table>
<thead>
<tr>
<th>Site</th>
<th>a) Site</th>
<th>n</th>
<th>Na</th>
<th>NaFreq &gt;5</th>
<th>nPriv</th>
<th>Ho</th>
<th>He</th>
<th>Polymorphic</th>
<th>Average He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cayo Carenero, DR</td>
<td>Thicket</td>
<td>65</td>
<td>1.105</td>
<td>1.043</td>
<td>0.071</td>
<td>0.002</td>
<td>0.013</td>
<td>10.51%</td>
<td>0.014</td>
</tr>
<tr>
<td>ISO</td>
<td>11</td>
<td></td>
<td>1.056</td>
<td>1.056</td>
<td>0.022</td>
<td>0.002</td>
<td>0.018</td>
<td>5.57%</td>
<td></td>
</tr>
<tr>
<td>Punta Rusia, DR</td>
<td>Thicket</td>
<td>29</td>
<td>1.056</td>
<td>1.054</td>
<td>0.040</td>
<td>0.002</td>
<td>0.013</td>
<td>5.62%</td>
<td>0.014</td>
</tr>
<tr>
<td>ISO</td>
<td>11</td>
<td></td>
<td>1.052</td>
<td>1.052</td>
<td>0.036</td>
<td>0.004</td>
<td>0.016</td>
<td>5.24%</td>
<td></td>
</tr>
<tr>
<td>Cheetos, FL</td>
<td>Thicket</td>
<td>57</td>
<td>1.172</td>
<td>1.043</td>
<td>0.124</td>
<td>0.003</td>
<td>0.016</td>
<td>17.14%</td>
<td>0.017</td>
</tr>
<tr>
<td>ISO</td>
<td>19</td>
<td></td>
<td>1.098</td>
<td>1.091</td>
<td>0.05</td>
<td>0.003</td>
<td>0.02</td>
<td>9.80%</td>
<td></td>
</tr>
<tr>
<td>Sunny Isles, FL</td>
<td>Thicket</td>
<td>63</td>
<td>1.065</td>
<td>1.021</td>
<td>0.056</td>
<td>0.001</td>
<td>0.008</td>
<td>6.48%</td>
<td>0.007</td>
</tr>
<tr>
<td>ISO</td>
<td>10</td>
<td></td>
<td>1.014</td>
<td>1.014</td>
<td>0.005</td>
<td>0.001</td>
<td>0.005</td>
<td>1.45%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>b) Site</th>
<th>n</th>
<th>Na</th>
<th>NaFreq &gt;5</th>
<th>nPriv</th>
<th>Ho</th>
<th>He</th>
<th>Polymorphic</th>
<th>Average He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cayo Carenero, DR</td>
<td>Plot1</td>
<td>23</td>
<td>1.069</td>
<td>1.065</td>
<td>0.021</td>
<td>0.002</td>
<td>0.014</td>
<td>6.94%</td>
</tr>
<tr>
<td></td>
<td>Plot2</td>
<td>17</td>
<td>1.056</td>
<td>1.054</td>
<td>0.018</td>
<td>0.002</td>
<td>0.013</td>
<td>5.70%</td>
</tr>
<tr>
<td></td>
<td>Plot3</td>
<td>25</td>
<td>1.045</td>
<td>1.039</td>
<td>0.012</td>
<td>0.002</td>
<td>0.009</td>
<td>4.49%</td>
</tr>
<tr>
<td></td>
<td>ISO</td>
<td>11</td>
<td>1.056</td>
<td>1.056</td>
<td>0.022</td>
<td>0.002</td>
<td>0.018</td>
<td>5.57%</td>
</tr>
<tr>
<td>Punta Rusia, DR</td>
<td>Plot1</td>
<td>14</td>
<td>1.029</td>
<td>1.029</td>
<td>0.022</td>
<td>0.001</td>
<td>0.010</td>
<td>3.04%</td>
</tr>
<tr>
<td></td>
<td>Plot2</td>
<td>15</td>
<td>1.029</td>
<td>1.029</td>
<td>0.016</td>
<td>0.002</td>
<td>0.010</td>
<td>2.78%</td>
</tr>
<tr>
<td></td>
<td>ISO</td>
<td>11</td>
<td>1.052</td>
<td>1.052</td>
<td>0.036</td>
<td>0.004</td>
<td>0.016</td>
<td>5.24%</td>
</tr>
<tr>
<td>Sunny Isles, FL</td>
<td>Plot1</td>
<td>17</td>
<td>1.033</td>
<td>1.032</td>
<td>0.015</td>
<td>0.001</td>
<td>0.008</td>
<td>3.32%</td>
</tr>
<tr>
<td></td>
<td>Plot2</td>
<td>14</td>
<td>1.021</td>
<td>1.020</td>
<td>0.005</td>
<td>0.001</td>
<td>0.006</td>
<td>2.08%</td>
</tr>
<tr>
<td></td>
<td>Plot3</td>
<td>15</td>
<td>1.030</td>
<td>1.029</td>
<td>0.012</td>
<td>0.001</td>
<td>0.008</td>
<td>2.99%</td>
</tr>
<tr>
<td></td>
<td>Plot4</td>
<td>17</td>
<td>1.025</td>
<td>1.024</td>
<td>0.008</td>
<td>0.001</td>
<td>0.006</td>
<td>2.45%</td>
</tr>
<tr>
<td></td>
<td>ISO</td>
<td>10</td>
<td>1.014</td>
<td>1.014</td>
<td>0.005</td>
<td>0.001</td>
<td>0.005</td>
<td>1.45%</td>
</tr>
</tbody>
</table>

Allelic patterns by a) site and b) plots and isolated colonies. N = number of samples, Na = Allelic Richness, NaFreq >5 = number of alleles at >5% frequency, nPriv = proportion private alleles, Ho = observed heterozygosity, He = expected heterozygosity, Polymorphic = proportion polymorphic alleles.
= percentage of loci polymorphic within population, Average He = weighted site-wide average expected heterozygosity. No data for Cheetos in b) because of single plot design.
Table 4.5 – Summary of Overall Results

Results and interpretation of each main hypothesis. Pop. Structure = Population Structure, Na= Allelic Richness, He = Expected Heterozygosity, GD = Genetic Diversity, defined here as Expected Heterozygosity except for Hypothesis 2.

*Allelic Richness is biased toward rare alleles, so higher Expected Heterozygosity in Isolated Communities is more indicative of 'population' level diversity as it is not as sensitive to sample size.
Figure 5.1 – Map of Release Polygons

Map of release polygons color coded by region.
**Figure 5.2 – Network Diagram for FRT populations at 4 DTC**

Network of FRT regional populations. Nodes are color coded based on source/sink, with red nodes representing net sinks and turquoise nodes representing net sources. Nodes are sized based on relative self-recruitment, corrected for visualization. Links are weighted by relative larval transit between pairwise regions, corrected for visualization. Links below the arc are ‘downstream’ transit to north and east, links below the arc are ‘upstream’ transit to the south and west.
**Figure 5.3 – Network Diagram for FRT populations at 7 DTC**

Network of FRT regional populations. Nodes are color coded based on source/sink, with red nodes representing net sinks and turquoise nodes representing net sources. Nodes are sized based on relative self-recruitment, corrected for visualization. Links are weighted by relative larval transit between pairwise regions, corrected for visualization. Links below the arc are ‘downstream’ transit to north and east, links below the arc are ‘upstream’ transit to the south and west.
Figure 5.4 – *Dispersal densities for particles released from each region*

Dispersal densities are color coded by region for a) 4 DTC and b) 7 DTC.
Table 5.1a

<table>
<thead>
<tr>
<th>Source</th>
<th>Local Retention</th>
<th>Upstream</th>
<th>Downstream</th>
<th>Source/Sink Ratio</th>
<th>Self-Recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>100.0%</td>
<td>0.0%</td>
<td>NA</td>
<td>0.147</td>
<td>15%</td>
</tr>
<tr>
<td>Miami</td>
<td>50.6%</td>
<td>0.2%</td>
<td>49.2%</td>
<td>0.932</td>
<td>47%</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>46.5%</td>
<td>0.0%</td>
<td>60.2%</td>
<td>1.754</td>
<td>70%</td>
</tr>
<tr>
<td>Middle Keys</td>
<td>30.1%</td>
<td>33.5%</td>
<td>36.4%</td>
<td>1.696</td>
<td>51%</td>
</tr>
<tr>
<td>Lower Keys</td>
<td>28.8%</td>
<td>63.0%</td>
<td>8.2%</td>
<td>2.930</td>
<td>84%</td>
</tr>
<tr>
<td>Marquesas</td>
<td>92.9%</td>
<td>6.0%</td>
<td>1.2%</td>
<td>0.235</td>
<td>22%</td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>19.7%</td>
<td>NA</td>
<td>80.3%</td>
<td>1.705</td>
<td>34%</td>
</tr>
</tbody>
</table>

Table 5.1b

<table>
<thead>
<tr>
<th>Source</th>
<th>Local Retention</th>
<th>Upstream</th>
<th>Downstream</th>
<th>Source/Sink Ratio</th>
<th>Self-Recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>100.0%</td>
<td>0.0%</td>
<td>NA</td>
<td>0.016</td>
<td>2%</td>
</tr>
<tr>
<td>Miami</td>
<td>26.9%</td>
<td>0.2%</td>
<td>72.9%</td>
<td>1.173</td>
<td>32%</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>41.4%</td>
<td>0.0%</td>
<td>77.0%</td>
<td>2.105</td>
<td>48%</td>
</tr>
<tr>
<td>Middle Keys</td>
<td>4.5%</td>
<td>35.6%</td>
<td>59.9%</td>
<td>4.306</td>
<td>19%</td>
</tr>
<tr>
<td>Lower Keys</td>
<td>19.0%</td>
<td>71.8%</td>
<td>9.2%</td>
<td>4.907</td>
<td>93%</td>
</tr>
<tr>
<td>Marquesas</td>
<td>79.7%</td>
<td>20.3%</td>
<td>0.0%</td>
<td>0.225</td>
<td>18%</td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>17.7%</td>
<td>NA</td>
<td>82.3%</td>
<td>0.841</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 5.1 – Patterns of directional dispersal between populations

Relative dispersal percentages from each regional a) 4 DTC and b) 7 DTC. Local retention is percentage of settlers in region of origin/total particles from that region, self-recruitment is percentage of particles settling in region of origin/total particles settling in that region. Source/sink ratio is number of particles released in a region/number of particles settling in that region, regardless of source. Downstream is with the prevailing direction of the current (North and East) while upstream is against (South and West).
### Table 5.2a

<table>
<thead>
<tr>
<th>Source</th>
<th>Settlement Region</th>
<th>Broward</th>
<th>Miami</th>
<th>Upper Keys</th>
<th>Middle Keys</th>
<th>Lower Keys</th>
<th>Marquesas</th>
<th>Dry Tortugas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>15%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Miami</td>
<td>64%</td>
<td>47%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>20%</td>
<td>48%</td>
<td>70%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Middle Keys</td>
<td>1%</td>
<td>2%</td>
<td>17%</td>
<td>51%</td>
<td>11%</td>
<td>7%</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>Lower Keys</td>
<td>0%</td>
<td>0%</td>
<td>3%</td>
<td>29%</td>
<td>84%</td>
<td>68%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>Marquesas</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>1%</td>
<td>22%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>1%</td>
<td>2%</td>
<td>10%</td>
<td>20%</td>
<td>4%</td>
<td>3%</td>
<td>34%</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5.2b

<table>
<thead>
<tr>
<th>Source</th>
<th>Settlement Region</th>
<th>Broward</th>
<th>Miami</th>
<th>Upper Keys</th>
<th>Middle Keys</th>
<th>Lower Keys</th>
<th>Marquesas</th>
<th>Dry Tortugas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Miami</td>
<td>62%</td>
<td>32%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>34%</td>
<td>55%</td>
<td>48%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Middle Keys</td>
<td>1%</td>
<td>7%</td>
<td>30%</td>
<td>19%</td>
<td>5%</td>
<td>10%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Lower Keys</td>
<td>0%</td>
<td>1%</td>
<td>8%</td>
<td>73%</td>
<td>93%</td>
<td>69%</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>Marquesas</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>18%</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>1%</td>
<td>5%</td>
<td>13%</td>
<td>7%</td>
<td>2%</td>
<td>4%</td>
<td>15%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.2 – Patterns of relative settlement within regions**

Relative settlement percentages from each regional source within each settlement region, which sum to 100% within settlement region. Gray boxes represent relative self-recruitment for a) 4 DTC and b) 7 DTC.
## Table 5.3a

<table>
<thead>
<tr>
<th></th>
<th>Broward</th>
<th>Miami-Dade</th>
<th>Upper Keys</th>
<th>Middle Keys</th>
<th>Lower Keys</th>
<th>Dry Tortugas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>0.000</td>
<td>0.055</td>
<td>-0.005</td>
<td>0.081</td>
<td>0.025</td>
<td>-0.238</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>0.971</td>
<td>0.763</td>
<td>0.005</td>
<td>0.115</td>
<td>0.002</td>
<td>-0.115</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.076</td>
<td>-0.087</td>
<td>-0.025</td>
</tr>
<tr>
<td>Middle Keys</td>
<td>0.022</td>
<td>0.946</td>
<td>0.999</td>
<td>0.000</td>
<td>0.061</td>
<td>-0.133</td>
</tr>
<tr>
<td>Lower Keys</td>
<td>0.999</td>
<td>0.953</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
<td>-0.253</td>
</tr>
</tbody>
</table>

## Table 5.3b

<table>
<thead>
<tr>
<th></th>
<th>Broward</th>
<th>Miami-Dade</th>
<th>Upper Keys</th>
<th>Middle Keys</th>
<th>Lower Keys</th>
<th>Dry Tortugas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>0.009</td>
<td>0.002</td>
<td>0.007</td>
<td>0.007</td>
<td>0.005</td>
<td>0.029</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>0.007</td>
<td>0.024</td>
<td>0.023</td>
<td>0.023</td>
<td>0.029</td>
<td>0.024</td>
</tr>
</tbody>
</table>

### Table 5.3 – Pairwise Fixation Index and Nei’s Genetic Distance

Pairwise $F_{ST}$ values and p-values for each combination of regions in the Florida Reef Tract. $F_{ST}$ values are above the diagonal, p-values are below the diagonal. Red values are significant at $p<0.01$, yellow values are significant at $p<0.05$. Nei’s Genetic distance for each combination of regions in the Florida Reef Tract.
Table 5.4

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>He</th>
<th>Ho</th>
<th>Lcomm</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broward</td>
<td>24</td>
<td>0.03092</td>
<td>0.00233</td>
<td>0.038</td>
<td>1.178</td>
</tr>
<tr>
<td>Miami</td>
<td>48</td>
<td>0.02553</td>
<td>0.00275</td>
<td>0.031</td>
<td>1.248</td>
</tr>
<tr>
<td>Upper Keys</td>
<td>48</td>
<td>0.01866</td>
<td>0.00152</td>
<td>0.021</td>
<td>1.175</td>
</tr>
<tr>
<td>Middle Keys</td>
<td>17</td>
<td>0.03204</td>
<td>0.00207</td>
<td>0.028</td>
<td>1.145</td>
</tr>
<tr>
<td>Lower Keys</td>
<td>38</td>
<td>0.03217</td>
<td>0.00341</td>
<td>0.043</td>
<td>1.273</td>
</tr>
<tr>
<td>Dry Tortugas</td>
<td>9</td>
<td>0.01605</td>
<td>0.00152</td>
<td>0.005</td>
<td>1.009</td>
</tr>
</tbody>
</table>

Table 5.4 – Allelic Patterns of Regional Populations

Genetic diversity and allelic patterns of each regional population. n= number of samples analyzed, He = expected heterozygosity, Ho = observed heterozygosity, Lcomm = locally common alleles not present in >25% of regions, Na= allelic richness. He, Ho are not sample size dependent at >10 individuals, Na is sample size dependent (Drury Chapter 3).
References


dynamics of the threatened Caribbean staghorn coral Acropora cervicornis: influence of
host genotype, symbiont identity, colony size, and environmental setting. PLoS One.
2014;9(9):e107253.

40. Lohr KE, Patterson JT. Intraspecific variation in phenotype among nursery-reared
staghorn coral Acropora cervicornis (Lamarck, 1816). Journal of Experimental Marine

41. Forrester GE, Taylor K, Schofield S, Maynard A. Colony growth of corals
transplanted for restoration depends on their site of origin and environmental factors.


diversity–ecosystem function research to ecological restoration. Journal of Applied

44. Baums IB. A restoration genetics guide for coral reef conservation. Molecular

45. Sgro CM, Lowe AJ, Hoffmann AA. Building evolutionary resilience for
conserving biodiversity under climate change. Evolutionary Applications. 2011;4(2):326-
37.

46. Baums IB, Miller MW, Hellberg ME. Regionally isolated populations of an

47. Reyes JG, Schizas NV. No two reefs are created equal: fine-scale population
structure in the threatened coral species Acropora palmata and A. cervicornis. Aquatic

48. Baums IB, Johnson ME, Devlin-Durante MK, Miller MW. Host population
genetic structure and zooxanthellae diversity of two reef-building coral species along the

variation among populations of threatened coral: Acropora cervicornis. BMC Genomics.


Supplementary Figure 2.4

PAR values for four sites. Light readings represent hourly averages for four days at each site, collected consecutively in pairs (see main text)
Supplementary Figure 2.5

Average temperature at all sites during the experiment. Black line is 30°C reference, green bars represent monitoring timepoints.
Table 1 - Site Description

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth [m]</th>
<th>Habitat</th>
<th>Inshore/Offshore</th>
<th>Collection/Outplant</th>
<th>PAR</th>
<th>Average (°C)</th>
<th>Average (°C)</th>
<th>Maximum (°C)</th>
<th>Days &gt;31°C</th>
<th>Average (°C)</th>
<th>Maximum (°C)</th>
<th>Days &gt;31°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooper’s Reef</td>
<td>1.4</td>
<td>High Relief Patch Reef</td>
<td>Midchannel</td>
<td>Both</td>
<td>3.89 ± 0.23</td>
<td>27.4 ± 1.4</td>
<td>30.7</td>
<td>0</td>
<td>30.6 ± 0.5</td>
<td>32.3</td>
<td>36</td>
<td>27.2 ± 1</td>
</tr>
<tr>
<td>CVFD</td>
<td>1.8</td>
<td>Low Relief Patch Reef</td>
<td>Midchannel</td>
<td>Both</td>
<td>3.85 ± 0.19</td>
<td>27.7 ± 1.3</td>
<td>31.9</td>
<td>4</td>
<td>31.0 ± 0.4</td>
<td>33.4</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Grounding</td>
<td>1.8</td>
<td>Consolidated Hardbottom</td>
<td>Midchannel</td>
<td>Both</td>
<td>4.10 ± 0.07</td>
<td>28.5 ± 1.2</td>
<td>31.4</td>
<td>5</td>
<td>30.9 ± 0.5</td>
<td>32.8</td>
<td>43</td>
<td>-</td>
</tr>
<tr>
<td>Inshore</td>
<td>5.5</td>
<td>High Relief Patch Reef</td>
<td>Inshore</td>
<td>Both</td>
<td>3.93 ± 0.17</td>
<td>27.9 ± 1.6</td>
<td>31.8</td>
<td>7</td>
<td>31.0 ± 0.4</td>
<td>32.8</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>Jon’s Reef</td>
<td>3.7</td>
<td>Low Relief Patch Reef</td>
<td>Midchannel</td>
<td>Both</td>
<td>3.88 ± 0.33</td>
<td>27.4 ± 1.4</td>
<td>30.7</td>
<td>0</td>
<td>30.6 ± 0.5</td>
<td>32.3</td>
<td>28</td>
<td>27.4 ± 0.8</td>
</tr>
<tr>
<td>Miami Beach</td>
<td>5.5</td>
<td>Consolidated Hardbottom</td>
<td>Midchannel</td>
<td>Both</td>
<td>3.77 ± 0.23</td>
<td>28.8 ± 1.3</td>
<td>31.8</td>
<td>9</td>
<td>30.7 ± 0.4</td>
<td>31.8</td>
<td>37</td>
<td>27.2 ± 1.3</td>
</tr>
<tr>
<td>Sligh’s Reef</td>
<td>2.1</td>
<td>Low Relief Patch Reef</td>
<td>Midchannel</td>
<td>High (nd)</td>
<td>3.95 ± 0.18</td>
<td>27.2 ± 1.4</td>
<td>31.9</td>
<td>2</td>
<td>31.1 ± 0.4</td>
<td>33.4</td>
<td>70</td>
<td>27.5 ± 1.2</td>
</tr>
<tr>
<td>Struggle Bus</td>
<td>10.7</td>
<td>Low Relief Patch Reef</td>
<td>Offshore</td>
<td>Both</td>
<td>3.81 ± 0.15</td>
<td>27.4 ± 1.3</td>
<td>30.6</td>
<td>0</td>
<td>30.7 ± 0.4</td>
<td>31.8</td>
<td>42</td>
<td>27.5 ± 0.9</td>
</tr>
<tr>
<td>Site 211</td>
<td>6.1</td>
<td>High Relief Patch Reef</td>
<td>Midchannel</td>
<td>Collection Only</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Government Cut</td>
<td>7.6</td>
<td>High Relief Patch Reef</td>
<td>Midchannel</td>
<td>Collection Only</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Royal Nursery</td>
<td>5.8</td>
<td>Fixed-to-bottom Platforms</td>
<td>Offshore</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Supplementary Table 2.3**

Environmental conditions and site summary for all outplanting sites.
**Supplementary Table 2.4**

Average growth rate for foreign and native corals and relative fitness calculations. See Figure 3a.

<table>
<thead>
<tr>
<th>Site</th>
<th>Local Growth (cm/day ±1S.E.)</th>
<th>Foreign Growth (cm/day ±1S.E.)</th>
<th>Relative Fitness (S)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coopers</td>
<td>0.031 ± 0.005</td>
<td>0.030 ± 0.002</td>
<td>0.032</td>
<td>0.8525</td>
</tr>
<tr>
<td>CVFD</td>
<td>0.012 ± 0.002</td>
<td>0.021 ± 0.002</td>
<td>-0.444</td>
<td>0.0012*</td>
</tr>
<tr>
<td>Grounding</td>
<td>0.027 ± 0.003</td>
<td>0.030 ± 0.003</td>
<td>-0.132</td>
<td>0.8063</td>
</tr>
<tr>
<td>Inshore</td>
<td>0.017 ± 0.003</td>
<td>0.043 ± 0.004</td>
<td>-0.647</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Jons</td>
<td>0.019 ± 0.005</td>
<td>0.029 ± 0.002</td>
<td>-0.357</td>
<td>0.0757</td>
</tr>
<tr>
<td>Miami Beach</td>
<td>0.029 ± 0.008</td>
<td>0.025 ± 0.002</td>
<td>0.156</td>
<td>0.4321</td>
</tr>
<tr>
<td>Stephens</td>
<td>0.008 ± 0.001</td>
<td>0.011 ± 0.001</td>
<td>-0.353</td>
<td>0.0518</td>
</tr>
<tr>
<td>Struggle Bus</td>
<td>0.055 ± 0.010</td>
<td>0.041 ± 0.004</td>
<td>0.328</td>
<td>0.874</td>
</tr>
</tbody>
</table>
### Supplementary Table 2.5

Water chemistry data for each outplanting site.

<table>
<thead>
<tr>
<th>Site</th>
<th>n</th>
<th>Salinity (ppt)</th>
<th>TA (μmol kg⁻¹)</th>
<th>DIC (μmol kg⁻¹)</th>
<th>pCO₂ (μatm)</th>
<th>Ωarag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooper’s Reef</td>
<td>4</td>
<td>34.03 ± 0.65</td>
<td>2373.3 ± 19.5</td>
<td>2034.1 ± 38.2</td>
<td>408.3 ± 36.8</td>
<td>3.89 ± 0.23</td>
</tr>
<tr>
<td>CVFD</td>
<td>5</td>
<td>34.20 ± 0.64</td>
<td>2364.8 ± 7.2</td>
<td>2027.4 ± 14.8</td>
<td>410.4 ± 24.3</td>
<td>3.85 ± 0.19</td>
</tr>
<tr>
<td>Grounding</td>
<td>4</td>
<td>34.40 ± 0.84</td>
<td>2357.6 ± 7.2</td>
<td>2027.4 ± 14.8</td>
<td>410.4 ± 24.3</td>
<td>4.10 ± 0.07</td>
</tr>
<tr>
<td>Inshore</td>
<td>4</td>
<td>34.07 ± 0.76</td>
<td>2368.8 ± 18.6</td>
<td>2024.6 ± 29.8</td>
<td>402.5 ± 40.9</td>
<td>3.93 ± 0.17</td>
</tr>
<tr>
<td>Jon’s Reef</td>
<td>5</td>
<td>34.04 ± 0.86</td>
<td>2370.3 ± 21.8</td>
<td>2029.2 ± 51.7</td>
<td>408.8 ± 83.6</td>
<td>3.88 ± 0.33</td>
</tr>
<tr>
<td>Miami Beach</td>
<td>5</td>
<td>33.98 ± 1.14</td>
<td>2358.6 ± 18.6</td>
<td>2028.5 ± 26.9</td>
<td>418.5 ± 54.5</td>
<td>3.77 ± 0.23</td>
</tr>
<tr>
<td>Steph’s Reef</td>
<td>5</td>
<td>34.28 ± 0.67</td>
<td>2359.2 ± 8.8</td>
<td>2012.8 ± 16.1</td>
<td>399.9 ± 20.2</td>
<td>3.95 ± 0.18</td>
</tr>
<tr>
<td>Struggle Bus</td>
<td>5</td>
<td>34.14 ± 0.68</td>
<td>2367.1 ± 7.4</td>
<td>2033.9 ± 13.7</td>
<td>425.6 ± 28.0</td>
<td>3.81 ± 0.15</td>
</tr>
</tbody>
</table>
Supplementary Figure 4.6

Map of pairwise genetic differences between all samples, interpolated across plot for Cayo Carenero, DR a) Plot 1 b) Plot 2 c) Plot 3
**Supplementary Figure 4.7**

Map of pairwise genetic differences between all samples, interpolated across plot for Punta Rusia, DR a) Plot 1 b) Plot 2 c) Plot 3
**Supplementary Figure 4.8 – Maps of genetic diversity for Sunny Isles**

Map of pairwise genetic differences between all samples, interpolated across plot for Sunny Isles, FL a) Plot 1 b) Plot 2 c) Plot 3 d) Plot 4
Supplementary Figure 5.5 Normalized Probability Matrix of All Release Polygons for 4 DTC
Supplementary Figure 5.6 Normalized Probability Matrix of All Release Polygons for 7 DTC

Dry Tortugas
Marquesas
Lower Keys
Middle Keys
Upper Keys
Miami
Broward

Settlement Location

DT Mar LK MK UK Miami Broward

0.0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1

0