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GENETIC STATUS OF THE CARIBBEAN REEF-BUILDING CORAL,

ACROPORA PALMATA

By

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This dissertation describes the genetic population status of the threatened Caribbean hard coral, *Acropora palmata*. Specifically, I determined the genotypic diversity within local populations of this coral, and the extent to which geographically isolated populations were genetically similar, information that is essential for future conservation and recovery efforts. To achieve this I developed highly polymorphic genetic markers (microsatellites) and tested their inheritance patterns via controlled crosses. Application of these markers to 1051 colonies sampled throughout the Caribbean identified an eastern and a western phylogeographic subregion for *Acropora palmata*, with little or no recent gene exchange between them. The split most likely occurs along a north-south running line between the Mona Passage to the Peninsula de Guajira, Colombia. Larval dispersal within each subregion is asymmetrical, with some reefs serving as larval sources while others are sinks. Larval sources are largely self-recruiting. The discovery of subdivision in an organism with a long planktonic larval stage within an area marked by strong current flow challenges the notion of open local populations in Caribbean broadcast spawning corals. Across the sampled region, sexual reproduction contributed to about 50% of the population structure. Within provinces, sexual reproduction was more important in the east than the west. The Florida Keys, part of the western subregion, showed the highest degree of clonal replication, following predictions on the rising importance of asexual reproduction in marginal habitats. Both within the western and the eastern subregion, denser stands were dominated by asexual reproduction while less dense stands were more sexual. Clonal structure was unpredictable on smaller spatial scales. This study provided data addressing the current debate about

open or closed marine populations. New molecular tools and statistical methods as well as a broad geographical sampling design were essential in describing both gene flow patterns and local modes of reproduction.

*To my family,
with love*

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CHAPTER 1: INTRODUCTION AND SIGNIFICANCE OF STUDY

GENE FLOW IN THE CARIBBEAN

Shallow-water marine organisms often have a bipartite life cycle. Adults are relatively sedentary while their larvae are planktonic, with limited capacity to influence their dispersal by ocean currents. Based on these observations, scientists for the last few decades believed marine populations to be demographically open on local scales such that recruits originate from a common larval pool but seldom from the natal reef (Heck and McCoy 1978; Veron 1995; Caley et al. 1996; Scheltema et al. 1996). This view is often accompanied by the assumption that the traits enabling long distance dispersal of larvae (such as small size) are adaptive (reviewed in Strathmann et al. 2002). Alternatively, long distance dispersal of a few propagules could be an accidental byproduct of having separate adult and juvenile habitats (reviewed in Strathmann et al. 2002). Resource partitioning might be an effective strategy in a largely oligotrophic environment. Recent evidence suggests that larvae have many behavioral adaptations that enable them to influence their dispersal to some extent (Cowen et al. 2000; Swearer et al. 2002) and enhance recruitment to their natal reef. While the majority of propagules might be retained close to their source, some will inevitably be dispersed to distant populations and so maintain genetic exchange between populations.

Determining the degree to which a species' populations are open or closed is essential to the discovery of ecological and evolutionary processes influencing a species' survival. The term "population" is used in a practical sense here, referring to individuals living in close proximity to one another, for example on one reef. Within closed populations, selection can lead to adaptation since offspring from successful adults return to the natal reef. In contrast, open populations experience a continuous influx of larvae originating from exogenous sources and so locally advantageous adaptations might be lost. For conservation questions, larval exchange rates between local populations on ecological timescales need to be known (Cowen et al. 2000). Should a local population go extinct, reseeding from exogenous larval sources is the only natural means of repopulating that area.

In the Caribbean basin, on the basis of strong currents and limited expanse of open water, local populations of most organisms, especially those with planktonic larval stages, were expected to be well connected throughout their range (Veron 1995; Roberts 1997). Throughout this dissertation, when referring to the Caribbean, the Bahamas and Florida are included. Several coral reef organism such as queen conch (Mitton et al. 1989; Campton et al. 1992) and the spiny lobster (Silberman et al. 1994) follow this prediction. However, marine fishes reveal a gradient of genetic similarity in Caribbean populations that is weakly

correlated with the larval life span (Shulman and Bermingham 1995). Recently, Taylor and Hellberg (2003) presented genetic evidence for strong differentiation among Caribbean populations of cleaner gobies (*Elacatinus evelynae*). Some populations within color forms were even reciprocally monophyletic, suggesting thousands of generations of isolation despite modest geographic separation and larvae with a three-week pelagic duration. While this study suggested that there may be phylo- or biogeographic breaks present in the Caribbean, similar in principal to the biogeographic break found in the area of Cape Canaveral in Southeastern Florida (Aulsebrook 1996), the generality of this finding is not known. In contrast to free spawning invertebrates such as many scleractinian corals, cleaner gobies attach their eggs to the substrate prior to fertilization, so that they are benthic during the first six days of their ontogeny (Valenti 1972). By the time cleaner goby larvae are pelagic, their ability to control dispersal is likely more advanced than for species with purely planktonic larvae. Here I examine genetic population structure in a Caribbean broadcast spawner to which these caveats do not apply: the elkhorn coral *Acropora palmata*.

THE ELKHORN CORAL, *ACROPORA PALMATA*

Acropora palmata Lamarck is one of the major reef building corals in the Caribbean, contributing to coastal protection and island formation and providing essential fish habitat and biodiversity (Gladfelter et al. 1978; Lirman 1999). Historically, it dominated the high-energy environments of the reef crest and the shallowest depth of the fore reef (0-5 m) termed the ‘palmata zone’ (Goreau 1959). *A. palmata* is a branching coral (Fig 1.1) with fast growth rates of 5 –10 cm/yr (Gladfelter et al. 1978; Bak 1979).

Asexual reproduction by breakage and reattachment of branches, facilitated by its capacity for lesion repair (Gladfelter et al. 1978; Bak 1979; Highsmith 1982), can be the predominant mode of recruitment for *Acropora palmata* (Highsmith 1982). Thus, this species is potentially long-lived. The high acroporid cover of Caribbean reefs prior to the 1980s resulted from the combined effects of fragmentation and high growth rates (Shinn 1966; Gilmore and Hall 1976; Tunnicliffe 1981; Highsmith 1982).

In contrast to asexual reproduction, sexual recruitment appears relatively limited (Dustan 1977; Bak 1979; Bak and Engel 1979; Hughes and Jackson 1980; Rylaarsdam 1983; Rosesmyth 1984; Hughes and Jackson 1985). *Acropora palmata* releases sexually produced gametes into the water column once a year after the August full moon in a synchronized spawning effort (Szmant 1986). Each hermaphroditic colony simultaneously releases eggs and sperm in bundles that float to the surface where they break apart and mix with gametes from other colonies (acroporids are poor self-fertilizers, Fukami et al. 2003).

Pelagic larvae become competent to settle within 5 days, but can remain planktonic for up to two weeks (Szmant pers. comm., pers. observ. Baums and Miller).

Acropora palmata populations showed a significant Caribbean-wide decrease in the 1980s together with its congener *A. cervicornis* and both species are still in a depressed state (Aronson and Precht 2001b; Bruckner 2002). White Band Disease, a disease specific to acroporids (WBD, Antonius 1981; Gladfelter 1982; Peters 1993) may be the primary cause of the recent mortality observed in wide areas of the Caribbean (Rogers 1985; Wells 1988; Sheppard 1993; Aronson and Precht 2001a). Additionally, acroporids are particularly susceptible to hurricane breakage (Stoddart 1963; Glynn 1964; Shinn 1976; Woodley et al. 1981; Shinn et al. 1989; Curran et al. 1994; Steneck 1994) and have undergone major bleaching events in the last decade (Porter and Meier 1992; Winter et al. 1998). Physical damage occurs from divers, boat anchors and ship groundings in highly visited areas such as the Florida Keys (Causey et al. 2002). Additional threats include predation by the gastropod *Coralliophila abbreviata* (Brawley and Adey 1982; Hayes 1990; Knowlton et al. 1990; Bruckner et al. 1997; Baums et al. 2003a; Baums et al. 2003b) and the polychaete *Hermodice carunculata* (Ott and Lewis 1972). The spotlight parrotfish *Sparisoma viride* (Bruckner and Bruckner 1998) bites *A. palmata* tissue on occasion. The three-spotted damselfish *Stegastes planifrons* contributes to tissue loss through the establishment of algal lawns on *Acropora palmata* colonies (Potts 1977).

The depressed state of acroporids in the Caribbean has lead to their listing as candidate species under the U.S. Endangered Species Act (Diaz-Soltero 1999) and formal review of their status is underway (Bruckner 2002). *Acropora palmata* is likely the most well-known of the Caribbean reef-building corals. It grows in shallow water, and is easily observed by snorklers and even boaters on calm days. Its brown-yellow color and branching morphology facilitate recognition. Since it provides habitat and shelter to many colorful reef fish and invertebrates, colonies are a common photographic motif. Interest from both the lay and scientific communities culminated in the submission of a formal petition for listing all Caribbean *Acropora* species as endangered under the U.S. Endangered Species Act (March 2004, Center For Biological Diversity, Oakland, CA).

Knowledge of the population structure of *Acropora palmata* is both essential to assessing potential conservation measures and potentially informative as to the generality of proposed phylogeographic breaks in the Caribbean. This dissertation tests the null hypothesis of Caribbean panmixia in *A. palmata*. The expectation of no population structure is based on the presumed long larval life spans of this coral and the strong surface currents running through its range.

ASEXUAL REPRODUCTION AND ITS CONSEQUENCES

Genetic estimates of population connectivity rely critically on estimating the frequency of genotypes within different populations. These frequencies can be skewed by local asexual reproduction of a few dominant genotypes in species like *Acropora palmata* that are capable of clonal reproduction. Asexual reproduction can create an assemblage of genetically identical colonies that can function and survive on their own (Hughes 1989; Carvalho 1994), called a clone. Cloning, therefore, is the process of producing genetically identical offspring. Each multiplied genotype is called a genet (Harper 1977). The colonies belonging to a genet are termed ramets (Kays and Harper 1974). The survivorship and reproductive output of the ramets will define the success of the clone. Note that the unit of selection in clonal organisms is not the ramet but the genet. Asexuality *per se* has no effect on allelic or genotypic frequencies in populations. It does not allow for genetic segregation and recombination, however, and so preserves the effects of selection, genetic drift, or founder effect on the genetic diversity. Bak (1983) hypothesized that high asexual reproduction rates led to low genotypic diversity so that acroporids were more susceptible to disease than non-branching species.

The dominance of asexual reproduction combined with broadcast spawning of sexual propagules has implications for the recovery potential of declining *A. palmata* populations. The effective population size, that is the number of breeding individuals, reaches its maximum if all genets (not ramets) contribute to the next generation. Once colonies become rare, the distance between them might limit fertilization success (Allee effect, Knowlton 1992; Kojis and Quinn 1994; Levitan 1995; Levitan and Petersen 1995). Populations with small effective population sizes are far more prone to extinction due to demographic stochasticity, reduction in gene diversity, or accumulation of deleterious mutations (Ellstrand and Elam 1993; Grosberg and Cunningham 2000).

Both empirical (Ayre 1985; Hunter 1993) and theoretical studies (Sebens and Thorne 1985) have suggested that genotypic diversity at a local scale might decrease over time through elimination of genets by intraspecific competition or stochastic effects. In contrast, genotypic diversity might remain high if sexual recruits, however rare, have a long life span after successful establishment (Burnett et al. 1995; McFadden 1997). In either case, interpopulation differences can be maintained (Hoffman 1987; McFadden 1997).

The interplay of sexual recruitment, physical disturbance and intraspecific competition is expected to cause a mosaic of clonal structure patterns. Broad variation in reproductive mode is observed in many clonal plants and may have significant consequences for their ecology, genetics and evolution (Dorken and Eckert 2001). In clonal plants, within species genotypic diversity and sexual reproduction is often lower at the geographical limit of the

species' range (reviewed in Eckert 2002). Eckert (2002) suggests that ecological factors such as extreme temperatures might first reduce sexual recruitment. Over time ecological sterility might lead to genetic sterility ("don't use it, you lose it" hypothesis). Since sex is produced through the interplay of many genes, it presents a broad target for mutation and so is easily disrupted when strong selection pressures are absent. It is evident that a study of clonal structure in *Acropora palmata* needs to be carried out over several spatial scales across the species' range.

This dissertation tests the null hypothesis that local populations of *A. palmata* are predominantly sexual and thus show high genotypic variation. Alternatively, highly asexual populations with low genotypic variation might be observed. Patterns of clonal structure might vary over several spatial scales with the amount of sexual recruitment and physical disturbance factors that influence fragmentation patterns. Asexual reproduction is expected to be more important in marginal habitats.

APPROACH

Assessment of the genetic status of *Acropora palmata* involves identifying gene flow patterns as well as describing clonal structure of local populations. Studies of clonal structure have to solve the fundamental problem of distinguishing colonies that are the product of sexual reproduction but still share a genotype by chance (identical in state) and colonies that arose through asexual reproduction and are identical by descent. The resolving power of any marker system depends on the number of possible "states". In the context of diploid genetic markers, these "states" are the number of alleles at each locus (polymorphism). Confidence in the identification of clones rises with increasing numbers of markers and their degree of polymorphism. In addition, scleractinian corals harbor intra-cellular algal symbionts (zooxanthellae, order *Symbiodinium*) adding specificity of the marker system to the coral host to the list of requirements. Zooxanthellae are present in the adult tissues of *Acropora palmata* and their DNA is not easily separated from the coral DNA. The only algal-free tissue source for *A. palmata* DNA is gametes. Lastly, the marker system should be able to describe gene flow patterns. A central assumption underlying population genetic models estimating gene flow is that markers are inherited in a predictable manner. For a diploid organism this means that the maternal and the paternal allele can be distinguished (i.e. they are co-dominant) should they differ in state (i.e. the individual is heterozygous at that locus).

Several avenues have been pursued to detect clonal identity in Cnidaria. The first studies utilized self-recognition analyses (Neigel and Avise 1983). However, the genetic

basis of tissue compatibility has since been challenged by studies showing fusion of electrophoretically distinct ramets (Heyward and Stoddart 1985; Willis 1985). Analysis of protein (allozyme) and DNA markers (DNA fingerprints and intron sequence data) show a wide variety of asexual reproduction patterns in Cnidaria ranging from highly clonal (Ayre and Willis 1988; Adjeroud and Tsuchiya 1999; Ayre and Hughes 2000) to mainly sexual (Ayre and Dufty 1994; Benzie et al. 1995; Ayre et al. 1997; Ayre and Hughes 2000). Since allozymes are generally co-dominant markers, the data obtained can easily be used to estimate gene flow. However, heterozygosity of allozyme markers is limited and thus their power to resolve all genotypes is restricted (Stoddart 1983b). The availability of an essentially unlimited amount of anonymous DNA markers (DNA fingerprints, Coffroth et al. 1992) allows for high resolution of clonal structure but their application is complicated by their inability to distinguish between host and symbiont DNA and their dominant inheritance patterns. A new class of nuclear, single-copy markers have become available for the study of coral genetic structure termed microsatellites (Lopez et al. 1999; Maier et al. 2001; Miller and Howard in press; Severance et al. in press; Shearer in press). Microsatellites are tandemly repeated DNA sequences of 1-6 bases (e.g. AC, AT, AAG, AAT, ATAG). They are highly polymorphic and inherited in a mendelian fashion so that they can be both applied to estimates of gene flow between populations and studies of clonal structure. For the development of microsatellites a zooxanthellae-free tissue source is required. Once primers are obtained their specificity to the coral DNA relieves the need for separating coral and algal DNA for all subsequent samples. In short, microsatellites combine the co-dominant nature of allozymes with the variability of DNA fingerprints while being coral-specific and thus should prove useful for the study of genetic structure in scleractinian corals.

SUMMARY

This dissertation describes the genetic population status of the threatened Caribbean hard coral, *Acropora palmata*. Specifically, I determined the genotypic diversity within local populations of this coral (Chapter 4), and the extent to which geographically isolated populations were genetically similar (Chapter 3), information that is essential for future conservation and recovery efforts. To achieve this I developed highly polymorphic genetic markers (microsatellites) and tested their inheritance patterns via controlled crosses (Chapter 2). Application of these markers to 1051 colonies sampled throughout the Caribbean identified an eastern and a western phylogeographic subregion for *Acropora palmata*, with little or no recent gene exchange between them. The split most likely occurs

along a north-south running line between the Mona Passage to the Peninsula de Guajira, Colombia. Larval dispersal within each subregion is asymmetrical with some reefs serving as larval sources while others are sinks. Caribbean-wide, sexual reproduction contributed to about 50% of the population structure and was more important in the western subregion than the eastern subregion. Following predictions, *Acropora palmata* was more clonal in marginal habitats like the Florida Keys in the western subregion. Within each of these provinces, denser stands were dominated by asexual reproduction while less dense stands were more sexual. Clonal structure was unpredictable on smaller spatial scales.



A



B



C



D

Fig 1.1 The elkhorn coral, *Acropora palmata*. Colony morphologies range from branching (A, most common) to encrusting (B, rare). Sometimes bowl-shaped forms (C) are observed. *Acropora palmata* can form extensive thickets generated by fragmentation (D).

CHAPTER 2: MENDELIAN MICROSATELLITE LOCI FOR THE CARIBBEAN ELKHORN CORAL, *ACROPORA PALMATA*

ABSTRACT

The genus *Acropora* constitutes the most species-rich clade of hermatypic corals, and its members are important reef builders throughout their broad tropical range. In the Caribbean, acroporid populations have declined due to disease, hurricanes, predation, and bleaching episodes over the last two decades, and some are now subjects of conservation efforts. Genetic estimates of population connectivity and clonal structure should be part of these efforts, but such studies have been hampered by low levels of mitochondrial DNA variation in corals and by an apparent dearth of variable single-copy, nuclear markers. Developing microsatellite markers in *Acropora* has proven especially difficult. We used Southern blotting to reveal that, indeed, many microsatellite motifs (AAC, AAG, AC) are rare in the genome of the Caribbean species *Acropora palmata*. However, repeats with the motif AAT are both abundant and variable. We developed eight such polymorphic microsatellite markers for *A. palmata*, and performed crosses to confirm co-dominant inheritance patterns. Five of the eight markers tested show simple Mendelian inheritance (mean observed heterozygosity = 0.88, mean number of alleles per locus = 8.8). Along with outcrossed sexual larvae, individual egg donors also produced triploid and selfed larvae that developed normally and survived for 80 hrs, when the experiment was terminated. The markers reveal variation among three Florida populations of *A. palmata* and among clones within populations. Seven of the markers amplify DNA from *A. cervicornis* and eight from the hybrid *A. prolifera*. These markers should prove to be valuable tools for developing conservation strategies for Caribbean acroporid species.

INTRODUCTION

Understanding of the evolution and population ecology of hard corals has been hampered by a scarcity of suitable population genetic markers (van Oppen et al. 2000b). Anthozoan mitochondrial DNA lacks the high levels of intraspecific variation that have facilitated population and low-level phylogenetic studies in other animals (Shearer et al. 2002; Hellberg subm). rDNA ITS-sequences have been used to study reticulate evolution, specifically in the genus *Acropora*, however the extremely high levels of intra- and inter-individual diversity of these multi-locus markers complicate their interpretation in a

population genetic context (Marquez et al. 2003). Sequences from a limited number of introns have been used to address speciation and hybridization questions (mini-collagen: Hatta et al. 1999; Pax c 47/48 intron: van Oppen et al. 2001; mini-collagen, calmodulin and mitochondrial putative control region: Vollmer and Palumbi 2002), but intraspecific variation in these markers is so low that they would prove impractical for studies of clonal structure and gene flow. Allozyme studies have provided data on population structure and the contribution of asexual reproduction in cnidarians (Jokiel et al. 1983; Stoddart 1984; Johnson and Threlfall 1987; Ayre and Willis 1988; Brazeau and Harvell 1994; Hellberg 1994; McFadden 1997; Ayre and Hughes 2000; Ridgway et al. 2001), however the need for preserving fresh tissue in liquid nitrogen makes its use impractical considering the often remote settings of coral reefs. Thus, there is a need for single copy, variable, nuclear markers

Microsatellite markers have proven useful for population genetic questions in many organisms due to their high degree of polymorphism and the relatively low cost of genotyping many individuals (reviewed in Jarne and Lagoda 1996; Schlotterer 2000). Mair et al. (2001) reported development of microsatellite markers for the coral *Seriatopora hystrix* (Pocilloporidae). Microsatellites have been developed subsequently in two Pacific faviids (Miller and Howard in press) and in the Caribbean faviid *Montastraea annularis* (Severance et al. in press). Shearer (in press) developed microsatellite markers for *Montastraea cavernosa* and the poritid *Porites porites*. Chen et al (2002) reported the use of a multi-gene microsatellite repeat located in the ribosomal intergenic spacer region (IGS) to investigate clonal population structure in a gorgonian. Multiple attempts to develop microsatellites in acroporids, however, have failed (Marquez et al. 2000). Three different lab groups tried to obtain microsatellite markers for the acroporid corals using five methodological approaches (Marquez et al. 2000). Beyond lack of variability, population genetic analyses are hindered when multiple paralogous loci are present in the genome (Harris and Crandall 2000). This possibility is raised in *Acropora* because species vary in their ploidy levels (Kenyon 1997). Mendelian inheritance of microsatellites can be confirmed by performing crosses of known parental genotypes, as is routine in the plant and yeast literature (e.g. Jakse et al. 2001; Dobrowolski et al. 2002).

Beyond their application to genetic structure questions in coral populations, microsatellite loci can be used to assess other aspects of their reproductive biology. Although fragmentation is the most well-studied and obvious means of asexual reproduction in reef cnidarians (Highsmith 1982; Coffroth and Lasker 1998; Lirman 2000), genetic analyses have revealed several cases of selfed and/or parthenogenic larvae (Ayre and Resing 1986;

Ayre et al. 1997; Brazeau et al. 1998). Genetically confirmed cases to date involve both brooded and broadcasted larvae, but very few species have been analyzed in this manner.

Here we use a hybridization approach to assess the relative abundance of different microsatellite repeats in the *Acropora palmata* genome. Using this information we then develop microsatellite markers from an enriched library and confirm their Mendelian inheritance by controlled crosses. The goal of this work is to produce a suit of loci suitable for assessing aspects of demography and population genetic structure in *A. palmata* across its Caribbean range.

MATERIALS AND METHODS

DNA Extraction

Because adult *Acropora palmata* Lamarck harbor zooxanthellae (*Symbiodinium spp.*), but their free-spawned gamete bundles do not, we extracted DNA from gametes to build a library. *A. palmata* reproduces sexually once a year (Szmant 1986). Spawn was collected during the August 2001 spawning event at Horseshoe Reef, Key Largo, Florida (N25° 08.392, W80° 17.649) and Green Turtle Cay, Abacos, Bahamas (N26° 42.448, W77° 09.215). DNA from 3-5 gamete bundles was extracted using Phytopure (Amersham Pharmacia), yielding high molecular weight DNA.

Adult DNA for assaying levels of variation in clonal and population structure was extracted from individuals with the QIAmp DNA Mini Kit (Qiagen). 3-10 polyps were scraped off with a sterile razor and ground with a plastic pestle in a 1.5 ml microcentrifuge tube. Extraction was performed overnight at 65° C following the manufacture's instructions.

Larval DNA was extracted by transferring each ethanol- preserved larva into a 0.5 ml strip tube and squashing it with a pipet tip. Any remaining ethanol was evaporated by heating the larvae for 2 min at 100° C. Twenty µl 5% Chelex and 2 µl Proteinase K (Promega, WI) were added to the dried larvae and incubated overnight at 56° C. Proteinase K was inactivated by heating for 15 min at 100° C (D. Williams, pers. Comm.). After spinning at 13000 rpm for 2 min, the supernatant containing the DNA was transferred to a fresh tube and 2 µl of it was added to the multiplex PCR reactions.

For a Southern blot to compare repeat abundances, DNA was isolated from additional species (Roseate Tern, *Sterna dougallii*; Giant Asian Turtle, *Orlitia borneensis*; Bicolor damselfish, *Stegastes partitus*) using a standard proteinase K/SDS digestion (Mullenbach et al. 1989) followed by phenol/chloroform extraction. *Acropora palmata* DNA was extracted using Phytopure (see above).

Southern Blotting

DNA concentrations were measured using a spectrophotometer. Four μg of DNA was digested with 15 U of *DpnII* (New England Biolabs). Digests were divided among 4 lanes of a 1.2% agarose gel, which was then run at 60 V for 16 hours; the 500 bp band in the size marker had run 16.2 cm. The gel was stained with ethidium bromide, photographed (Fig 2.1, left panel, size standard from New England Biolabs), and subjected to a standard Southern blot. The nylon membranes (Nytran N, Schleicher and Schuell Keene N.H.) were probed with 5' ^{32}P labeled oligonucleotides: $(\text{AAT})_{10}$, $(\text{AAC})_{10}$ and $(\text{AAG})_{10}$ and exposed to X-ray film. Relative signal intensity was compared among species and among probes to determine whether particular repeat types were more frequent than others, and therefore which repeat type(s) should be used for enrichment in library construction.

Microsatellite Enriched Genomic Library Development

A genomic library was developed using a repeat enrichment protocol by Hamilton et al. (1999). Genomic DNA was cut with a set of restriction enzymes (*RsaI*, *SnaB*, *HaeIII*; all restriction enzymes used from New England Biolabs) that yielded fragments between 200 bp and 1 kb. These restriction fragments were ligated to a SNX linker (see Hamilton et al. 1999 for sequence). The cut genomic DNA with attached SNX linkers was heat denatured and then hybridized to biotinylated nucleotides ($(\text{AAT})_{10}$ and $(\text{AC})_{10}$ in two separate trials) at 45° C overnight. Streptavidin-coated magnetic beads (Dynabeads, Dynal) were added to this mixture, and incubated at 43° C overnight. Fragments that did not bind to the beads were eliminated (Promega) during a set of 3 washes, each lasting 5 min, with increasing stringency (first wash: 2x at RT with 2xSSC, 0.1% SDS wash buffer, second wash 2x at 33° C with 1xSSC, 0.1 % SDS, third wash 2x at 43° C with 1xSSC, 0.1% SDS). Fragments bound to the magnetic beads (i.e. those which had hybridized to biotinylated oligos) were retained using a magnet that pulled the beads to the side of the tube during the washes. The AAT- and AC-repeat enriched DNA was made double-stranded using a primer that annealed to the SNX linker, then digested with *StuI* to produce cloning ends. The repeat-enriched DNA was ligated into *EcoRV* digested pBluescript SK (Stratagene) vector. Supercompetent cells (Epicurian Coli® XL1-Blue MRF', Stratagene) were transformed following the manufacturer's instructions and plated on Ampicillin/X-gal/IPTG containing plates. Colonies were picked, and DNA was prepared (Promega Wizard Plus SV Miniprep Kit, Promega, WI) and sequenced (using BigDye 3 chemistry (Applied Biosystems) on an ABI 377 automated sequencer in both directions). Sequences were aligned with the program Sequencher (Gene Codes Corporation).

Primer Design and Testing

Primers were designed for sequences that contained 7 or more AC or AAT repeats using the program Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi, Table 2.1). Some sequences were similar to others in the library (Table 2.2); these were excluded from marker development because they are likely to represent multi-copy loci. The non-labeled (reverse) primer of marker 201 has an addition (GTTCTT) on the 5' end to ensure a high percentage of adenylation (Brownstein et al. 1996). Three populations of *A. palmata* from the Key Largo area of Florida were screened for polymorphism. Eight primers were polymorphic (most common allele frequency <90%) and amplified consistently (Table 2.1, 2.3) in the adults. For these, two sets of multiplex PCRs were developed for efficient scoring on an ABI 3730.

Microsatellite Scoring

Two 10 µl multiplex PCR reactions (M-I and M-II, Table 2.1) were performed per sample. M-I consisted of 2 µl each of primer pairs 166-PET (5 µM), 201-VIC (3 µM), 192-6FAM (5 µM) and 181-NED (3 µM), 1 µl 10x PCR Reaction Buffer (Promega), 0.8 µl of MgCl₂ (25 mM), 0.2 µl of dNTP (10 mM) and 0.2 µl of *Taq*-Polymerase (5 U µl⁻¹, Storage Buffer B, Promega). M-II consisted of 2 µl each of primer pairs 207-PET (5 µM), 180-VIC (5 µM), 182-6FAM (5 µM) and 187-NED (4 µM), 1 µl Promega 10x PCR Reaction Buffer, 1.2 µl of MgCl₂ (25 mM), 0.2 µl of dNTP (10 mM) and 0.2 µl of *Taq*-Polymerase (5 U µl⁻¹). 100-200 ng adult DNA was added to each reaction (1 µl). Adults and larvae were amplified using the same recipes except that twice the amount of *Taq* was used per reaction for the larvae. Thermal cycling was carried out with MJ Research PT200 or PT100 cyclers with an initial denaturation step at 95° C for 5 min followed by 35 cycles of 95° C for 20 sec, 50° C for 20 sec, 72° C for 30 sec. A final extension of 30 min at 72° C ensured that the majority of amplicons were +A. PCR products were visualized using an ABI 3730. An internal size standard (Gene Scan 500-Liz, Applied Biosystems CA) ensured accurate sizing. Electropherograms were analyzed with GeneMapper Software 3.0 (Applied Biosystems, Ca).

*Sampling of *Acropora palmata**

We sampled *Acropora palmata* stands at Horseshoe Reef (see DNA section above), Little Grecian Reef (N25° 07.106, W80° 19.029) and Sand Island (N25° 01.074, W80° 22.117), located off of Key Largo, Florida. These sites are separated by distances ranging from 3.3 km (Horseshoe - Little Grecian) to 15.4 km (Sand Island - Horseshoe). Since no information was available on the clonal structure of *A. palmata* in these populations, we

sampled at three spatial scales within the Little Grecian and Horseshoe populations to detect both common and rare genets. Random numbers were generated for each of three nested circles with radii of 15m, 10m and 5m. The random numbers generated were precise to 5° of arc and 50 cm along strike. At each reef, a stake was placed in the center of the patch. Using a measuring tape attached to the center stake and a compass, the coordinates were located. The colony underneath each of the coordinates was sampled; this was repeated 8 times per circle. If there was no colony at a particular random coordinate, that coordinate was crossed out and the next random number was sampled. No colony (defined here as a continuous, upright entity of skeleton with a stalk that attaches it to the bottom) was sampled twice. At Sand Island reef, > 90% of the colonies (ramets) were sampled, making a random sampling approach unnecessary. One 1 cm long tip per identified colony was snipped off using a bolt cutter and placed in a labeled zip lock bag. Coral samples were transferred into 70% ethanol upon returning to shore and stored at -80° C until genotyping. Only samples where amplicons could be scored at all eight loci were included in the analyses.

Crossing Experiments

We identified five colonies representing four genets at Sand Island Reef, Florida as potential gamete donors (Fig 2.2). Gametes were collected on 17 August 2003 using nets (ca. 80 µm). Egg/sperm bundles were transferred into 15 ml centrifuge tubes where they remained until the bundles broke apart (60-90 min). Eggs were carefully siphoned off the surface and washed at least three times in filtered sea water until no sperm remained. Crosses were set up by adding eggs and sperm to a culture dish and left to fertilize for one hour. Sperm concentrations (10^6 - 10^8 sperm ml⁻¹) used here were within in the range reported to give good fertilization success in Pacific Acroporids (Willis et al. 1997). The fertilized eggs were then transferred to Petri dishes and raised at 25° C until they were 80 hrs old. Larvae were pipetted to dishes with fresh filtered sea water when unfertilized eggs and sperm began to foul the dish. Larvae were observed under a dissecting microscope to check for normal development. The fertilization solution was preserved with Lugol's Solution (10 parts of potassium iodide, 5 parts of iodine, 85 parts H₂O). Sperm concentration was determined by counting sperm in a hemocytometer in duplicate. Eighty hr larvae were preserved in 95% ethanol and stored at -80° C until analysis. In some cases (mean = 3 per cross, Table 2.5), fewer than four loci could be scored for a larva, resulting in the exclusion of that sample.

Larvae were classified into one of three types based upon their genotypes. (1) Outcrossed sexual larvae had one maternal and one paternal allele at all loci and were used for tests of co-dominant inheritance of the eight microsatellite loci. We compared expected and observed ratios of offspring classes using Chi-square tests. Chi-square tests

are sensitive to small sample sizes; the expected number of observations should not be < 5 per class. For a cross between two heterozygous individuals, four classes of gametes are possible and the expected ratio is 1:1:1:1. Hence, we only analyzed crosses for which > 25 larvae were scored. (2) Larvae were scored as triploid if three alleles were found at least at one locus and the alleles scored at the other loci did not exclude the possibility that the larva could be triploid (i.e. one of the parents was homozygous at that locus). All triploid larvae possessed both maternal and paternal alleles. (3) Some larvae had maternal genotypes only at all loci. Because genotypes were not always identical to the maternal genotype at all heterozygous loci, these were considered to be the products of self-fertilization.

RESULTS

Southern Blot

Fig. 1 shows that the sequence motif AAC is nearly absent from the *A. palmata* genome, while the sequence AAT is comparatively abundant. Indeed, despite the underloading of *A. palmata* DNA (see left panel), the coral lane is as dark or darker than all the other species when probed with AAT. Note also the appearance of dark bands in the AAC lanes of all species indicative of some repetitive elements that contains an AAC microsatellite. No species showed an abundance of AAG repeats (data not shown).

Microsatellite Marker Development

Of 40 bacterial clones sequenced from the AAT-enriched library, 15 (38%) contained AAT-repeats (Table 2.2). The number of repeat units in these positive clones ranged from 4 to 28 [mean (SD) = 13 ($\pm$ 7.9)]. Primers were designed and tested for the ten sequences that contained AAT repeats with more than eight repeat units (Table 2.1). Eight of these ten primer pairs amplified and were polymorphic in the Florida populations (see next section).

Screening of an earlier library constructed using AC as the enrichment motif produced a large number (9 out of 25) of repeat-containing bacterial colonies that aligned with a sequence from the histone gene cluster of *Acropora formosa* (Miller et al. 1990). Specifically, this *A. palmata* repeat [(GT)₃TT(GT)₂GA(GT)₄] aligned with the non-coding region between the H2a and H4 coding regions of the histone box (alignment available from IB upon request). The prevalence of the multi-copy histone-box repeat limited the usefulness of the AC- library for Mendelian marker development. No primers to AC-repeat loci were included in the study.

Spawning and Crosses

Acropora palmata was observed spawning during August of 2003 at Sand Island Reef, Key Largo, Florida. On 17 Aug 2003, the 6th night after the August full moon, bundles formed between 21:37 and 21:55 EDT. Egg/sperm bundles were released from 22:00 until 22:25. Of the 60 colonies present at Sand Island reef, eight colonies representing four different genets spawned.

A total of 21 crosses were attempted (Table 2.4A), resulting in 2826 larvae. Some crosses could not be performed due to limited amounts of sperm or eggs (empty cells in Table 2.4A). *Acropora palmata* larvae followed the development pattern described by Hayashibara et al. (1997) and Ball et al. (2002). Larvae had obtained an elongated shape with an indentation around the center at ca. 14 hrs after fertilization indicating gastrulation. By 31 hrs, larvae were round again. First signs of cilia were observed after 41 hrs along with a change in shape from round to pear-like. Larvae began swimming at 78 hrs.

The number of larvae remaining after 80 hrs varied widely among crosses (Table 2.4A). Sperm concentration ranged from 10^6 to 10^8 ml sperm ml⁻¹ (Table 2.4B). In self-fertilization trials, fewer than 10 larvae survived for 80 hrs (Table 2.4A). This also holds true for the cross between clonemates G and D (Table 2.4A). Most eggs in the self-fertilization trials had broken apart 14 hrs after the fertilization attempt was made.

Inheritance Patterns

Larval genotypes were compared to known parental genotypes (Table 2.7). Primers for presumptive loci 187 and 201 commonly exhibited more than two peaks per larva; 187 amplified between two and five peaks per individual, while 201 amplified up to eight haplotypes per individual. Thus, these two primer pairs likely amplify multiple loci. In addition, primers for presumptive locus 180 commonly failed to amplify one of the parental alleles in larvae, creating apparent homozygote excess; this probably indicates the presence of null alleles. Expectations for offspring classes were thus impossible to formulate for these three primer sets and chi-square tests were not performed.

Tests of co-dominant inheritance were conducted for the five remaining presumptive loci (166, 181, 182, 192, 207). In each of the four reciprocal crosses that produced over 25 scorable larvae, chi-square tests for departure from expected Mendelian ratios were non-significant for these five markers (Table 2.7).

In 5 (24%) of the 21 attempted crosses, at least some larvae were scored as triploids (Table 2.5). The proportion of triploid larvae ranged from 0.07 – 0.42 in those crosses that showed triploids (Table 2.5). Selfed larvae occurred in 6 of the 17 (35%) outcrosses (Table 2.5). The proportion of selfed larvae in those crosses that produced them ranged from 0.04

– 1.00 (Table 2.5). The proportion of both triploid and selfed larvae varied among egg donors. At least some triploid larvae developed from eggs in 3 out of 4 out-crosses in which colony R was the egg-donor (Table 2.5). Additionally, triploid larvae were observed in cross D x R (36%) and cross G x Y (2 out of 29). Selfed larvae were mainly found in crosses where colony O was the egg donor. Also noteworthy is the low number of larvae surviving when colony O was the sperm donor (Table 2.4B), even though sperm concentrations were similar between out-crosses with O as a sperm donor and other sperm donors fertilizing eggs from the same colony (compare cross O x G and O x Y, Table 2.4B). Note that colonies G and D are clonemates as previously identified through genotyping. Both G and D were crossed with Y, producing the same classes of larval genotypes (Table 2.7).

Marker Testing in Adult Populations

Three *A. palmata* populations from Key Largo, Florida were genotyped using the five scoreable loci. The number of alleles sampled per locus ranged from 5 to 11 (Table 2.3) in a sample of 94. These 94 colonies represented 20 unique genotypes (Table 2.6). Observed heterozygosity per locus was high, ranging from 0.76 to 0.95 (Table 2.3). All loci showed heterozygote excess (mean $Het_{obs} = 0.88$, mean $Het_{exp} = 0.79$, $p < 0.001$, Table 2.3). When assessing all five markers, the probability of identity by chance was very low (Table 2.3). The three reefs had markedly different amounts of genotypic diversity. At the 15 m scale, Horseshoe Reef ($n=20$, Fig 2.3A) and Little Grecian Reef ($n=20$, Fig 2.3B) were dominated by one genet each, whereas Sand Island Reef ($n=32$) had 10 genets (Fig 2.2). Colonies subsequently selected to supply gametes for crossing experiments are indicated on the clonal map for Sand Island Reef (Fig 2.2).

DISCUSSION

Microsatellite Markers

We report the development of five highly polymorphic microsatellite markers that demonstrate Mendelian inheritance patterns for the threatened Caribbean coral *Acropora palmata*. The Acroporidae have proven to be difficult subjects for microsatellite development (Marquez et al. 2000). Three labs had used five methodological approaches to screen Pacific acroporid corals for 15 different di-, tri-, and tetra-nucleotide repeats (ACA, AG, AGTG, ATT, CA, CAAT, CAC, CGA, CT, GATT, GT, TA, TAG, TC, TG) (Marquez et al. 2000). In the face of the apparent paucity of microsatellite loci in the *Acropora* genome, we first determined which repeat type was comparatively abundant using Southern blots.

The observed differences in the abundance of probed sequences (AAT, AAC and AAG) between *A. palmata* and other organisms may explain why earlier attempts at microsatellite development failed. Furthermore, the AAC probe revealed a repetitive element that contains an AAC microsatellite (Fig 2-1). Developing such loci would raise technical difficulties in subsequent DNA amplification: primers might amplify several distinct loci, or sequences may be found that are partly unique (in the flanking region of the repetitive element) and partly shared with other microsatellite loci (the repetitive element itself). Such problems arose twice in this project; first, two primer pairs designed for an AAT locus amplified more than one locus. Second, we found that an intergenic spacer region between two highly repetitive histone genes, H2a and H4 contained an AC-rich motif. Due to the PCR based enrichment method used in the protocol (Hamilton et al. 1999), these sequences swamped the genomic library and rendered it useless for isolating single locus markers. Instead, directed by the Southern blots, AAT was targeted as the motif for microsatellite development.

The AAT-microsatellite markers had 8.5 alleles on average ranging from 5 to 11 in the three Florida populations examined ($n = 20$ genets, Table 2.3). Five CA microsatellites developed for *Seriatopora hystrix* had fewer alleles per locus (3 - 9, mean = 5, $n = 31 - 98$, Maier et al. 2001). Observed heterozygosity for *A. palmata* was high ($H_o = 0.76 - 0.95$, Table 2.3).

Initial screening of *Acropora cervicornis* ($n = 3$) and the *A. palmata* / *A. cervicornis* hybrid (van Oppen et al. 2000a) *A. prolifera* ($n = 3$) showed that the markers developed here for *A. palmata* also amplify the DNA of these congeneric species. The one exception is locus 192, which did not amplify the *A. cervicornis* individuals tested. Microsatellite primers commonly amplify in closely related species (Primmer et al. 1996; Primmer and Merila 2002), enhancing the cost- and time-effectiveness of their development, although such borrowed loci tend to show lower heterozygosity in populations and species other than those for which they were developed (Hutter et al. 1998).

Microsatellite motifs are not evenly distributed among genomes and have different characteristics within genomes (Primmer et al. 1997). Several groups have been identified as depauperate in microsatellites (e.g. birds, Lagercrantz et al. 1993; Primmer and Ellegren 1998). Dinucleotides are commonly more polymorphic than tri- and tetranucleotide repeats. Of dinucleotides, CA is the most abundant (and variable, Bachtrog et al. 2000) repeat type in *Drosophila* and mammals. However, in *Arabidopsis thaliana* and yeast, AT is most abundant. Even among *Drosophila* species differences in microsatellite abundance have been found (Warner and Noor 2000). Thus, the discovery of polymorphic microsatellite repeats in Caribbean acroporids does not necessarily contradict the apparent

lack of microsatellites in Pacific acroporids. Interestingly, Chenuil et al (1997) suggest that an under-representation of dinucleotides in barbel compared to other fish might result from selection pressure to eliminate DNA from polyploidized species via preferential loss of repeat units during slip-strand repair. Comparisons of motif abundance, cell size and DNA content between diploid and polyploid *Acropora* species (Kenyon 1997) might be enlightening.

Mendelian Inheritance of Markers

Co-dominant inheritance cannot necessarily be assumed for microsatellite markers (Dobrowolski et al. 2002). In addition, zooxanthellate DNA might have contaminated the genomic library we screened for our coral markers, even though we had taken the precaution of using gamete-derived coral DNA for library construction. Our controlled crosses, however, indicate that five of the eight loci are host-specific and conform to Mendelian expectations. These five markers should prove useful for both inferring population connectivity and evaluating the contribution of asexual reproduction in this clonal organism. This information is crucial to the evaluation of the extinction threat faced by *Acropora palmata*.

Controlled crosses addressing the inheritance of a molecular marker have been performed once previously within *Acropora*. Hatta et al. (1999) demonstrated Mendelian segregation for the mini-collagen marker for a limited number of larvae ($n = 11$) in Pacific acroporids. A number of authors confirmed Mendelian inheritance of allozyme markers with one (Stoddart 1983a; Ayre and Resing 1986) or both (Stoddart et al. 1988; Hellberg and Taylor 2002) parental genotypes known for both hermatypic and ahermatypic corals. In the plant literature, such controlled crosses are a standard part of marker development (Jakse et al. 2001). Many broadcast spawners reproduce only once or a few times per year, at night and somewhat unpredictably. Thus, it is understandable that controlled crosses have been rare in the coral literature.

Selfed and Triploid Larvae

The controlled crosses yielded some larval genotypes that were unexpected if larvae in *Acropora palmata* result solely from sexual outcrossing. Acroporids are known to be poor self-fertilizers (Fukami et al. 2003), and indeed this was the case here: crosses between sperm and egg from the same colony yielded far fewer larvae than were obtained from out-crosses (Table 2.4A). However, in crosses between different genets, high levels of selfed larvae were sometimes observed (Table 2.5). The number of selfed larvae produced appears to vary among egg donors (Table 2.5). Incomplete washing of eggs once egg-

sperm bundles had broken apart may have left some self-sperm in the egg-suspension, resulting in self-fertilized eggs.

Triploid larvae were frequently observed in the offspring of egg donor R (Table 2.5). Three alternatives can be proposed for their origin. (1) Polyspermy occurred: haploid eggs were first self fertilized, and then fertilized by foreign sperm. (2) Asexual (mitotic - parthenogenic) eggs could have been fertilized by a foreign sperm, resulting in a triploid organisms. However, only 4 of 287 *diploid* larvae show a genotype consistent with a mitotic - parthenogenic origin, making this explanation unlikely. (3) The retention of a polar body could explain the presence of both maternal alleles (meiotic parthenogenesis). Triploid larvae never contained both paternal alleles, but always had both maternal alleles and only one paternal allele. Several coral species are reported to release polar bodies within 30 min of spawning (Szmant-Froelich et al. 1980; Harrison and Wallace 1990). We are not able to discriminate between retention of a polar body, self-fertilization or mitotic parthenogenesis as the origin of the second maternal allele.

The high proportion of triploid larvae may be artifactual but is worth noting because the triploid larvae survived 80 hrs. The occurrence of self-fertilized and triploid larvae, even at low percentages, underlines the necessity for genetic confirmation of larval origin when interpreting the fertilization success of acroporid crosses. Triploid larvae may be one route to developing variation in ploidy levels among *Acropora* species. Observation of triploid adults would lend further support to this hypothesis.

Application of Markers in three adult Acropora palmata Populations

The high heterozygosities of the five microsatellite markers we have developed result in a low probability that non-clonemates will share identical genotypes (Table 2.3), thus we can distinguish genets. We applied the five Mendelian microsatellite markers to three *A. palmata* populations in the Florida Keys. Clonal maps were constructed from the physical location of the colonies recorded at time of collection and overlaid with the genotypes (Fig 2.2, 2.3). A clear picture of clonal structure emerges. While Little Grecian and Horseshoe reef have very little (or no) clonal variation, Sand Island harbors many genets. There is a wide range in the number of ramets per genet. Clonemates are clumped as expected since clonal propagation occurs through breakage of branches and their subsequent re-attachment (Highsmith 1982; Coffroth and Lasker 1998).

The dominance of a single genet at Horseshoe Reef helps to explain previous, failed fertilization studies. In 1999 and 2001, gamete bundles were collected at Horseshoe Reef during a vigorous spawning event and crossed. Survival rate was so low that no larvae

could be settled (Miller and Szmant, unpubl. data), and this may be due to the absence of clonal variation at Horseshoe Reef.

The differences in levels of clonal diversity between reefs within a 15 km area raise management concerns. The total number of colonies ($n = 94$) represents only 20 genets. At Horseshoe Reef, ramets of the dominant genet were found over a maximum distance of 21.3 m (Table 2.6, Fig 2.3A). The Little Grecian genet spread over 10.5 m (Table 2.6, Fig 2.3B). Edmunds (1994) suggest genets might differ in their susceptibility to bleaching. Thus, the dominance of a single genet at Horseshoe Reef and Little Grecian Reef could make populations at these localities highly vulnerable to disturbance, even though they coped with white band disease, bleaching, hurricanes and predators over the last five years (Baums et al. 2003a; Baums et al. 2003b, Miller, Williams and Baums, unpubl. data).

Conclusion

The targeted enrichment protocol might help to circumvent problems encountered when developing microsatellites for scleractinian corals and other problematical taxa. The development of highly polymorphic, Mendelian markers for the threatened coral *Acropora palmata* will prove useful for the investigation of both clonal structure and population connectivity. Preliminary trials indicate that the markers developed here may also be useful in *A. cervicornis* and in the hybrid *A. prolifera*. Information on the genetic population structure of Caribbean acroporids is urgently needed because these corals are currently being evaluated as candidates for listing under the U.S. Endangered Species Act (Diaz-Soltero 1999).

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Table 2.1 Microsatellite primers for *Acropora palmata*. All primers amplify regions with AAT repeats. The size of the amplicon (in bp) and the number of AAT repeats in the bacterial clones to which the primers were first designed are given.

Primer	Primer Pair	Multiplex	Product Length (bp)	Num of AAT Repeats	Mendelian
166	TCTACCCGCAATTTTCATCA PET-CGCTCTCCTATGTTTCGATTG	I	168	28	Yes
180	VIC-TTTCTCAGTGGGTTCCATCA CCTTTCGTTGCTGCAATTTT	II	134	19	No
181	NED-TTCTCCACATGCAAACAAACA GCCAGGATAGCGGATAATGA	I	152	10	Yes
182	6FAM-TCCCACAACCTCACACTCTGC ACGCGGAAATAGTGATGCTC	II	165	18	No
187	NED-CGGATCTCACACTGATGCAA CATATAGATATCTGCGCAATAAG	II	112	11	Yes
192	6FAM-TTTGAGCATTTAAGGAGCAACA CAGCAGACTCAACAGCAGGA	I	180	28	Yes
201	VIC-CCAAAACCTCAGAAACCCATT GTTTCTTCGCAGAATCCATGTTGATAGC	I	134	12	No
207	ATCCACGCCCAAACAATGTA	II	183	20	Yes

Table 2.2 Genomic libraries of *Acropora palmata* enriched for AAT and AC microsatellite repeats. The number of bacterial clones sequenced is given as well as the number and proportion of those that contained a microsatellite repeat. In some cases bacterial clones aligned with others from the library and were excluded from primer development since that sequence might be part of a multi-gene locus. NA = not applicable.

	AAT library	AC library
Bacterial colonies sequenced	40	144
Bacterial colonies with repeat	15	25
Num of repeat units (range) in positive bacterial colonies	4 - 28	5 - 17
Average repeat units (SD)	13.0 (7.9)	12.3 (4.5)
Primers designed	10	12
Polymorphic loci	8	0
Bacterial colonies aligning to others in library	8	48
Bacterial colonies aligning with <i>A. formosa</i> histone	NA	21

Table 2.3 Genetic variation of *Acropora palmata* microsatellite markers. Data based on 20 genets pooled from Horseshoe, Little Grecian and Sand Island Reefs, Florida. Probability of identity (PI) after sequentially multiplying PI values over all loci. Biased: based on Paetkau and Strobeck (1994); unbiased: based on Kendall and Stewart (1977) as calculated by Gimlet (Valiere 2002).

Locus	Number alleles sampled	Gene diversity/ locus + population	Het _{Obs}	Het _{Exp}	Prob. of identity - biased	Prob. of identity - unbiased
166	10	0.885	0.90	0.86	0.033	0.016
181	5	0.667	0.81	0.65	0.184	0.158
187	10	0.876	0.95	0.86	0.037	0.020
192	8	0.804	0.76	0.78	0.071	0.043
207	11	0.868	0.95	0.85	0.038	0.018

Table 2.4 Crosses between *Acropora palmata* colonies. Letters identify the colonies gametes were collected from (N = 6). Because either the amount of sperm or the amount of eggs was limiting, not all crosses were attempted (empty cells in the matrix). **A)** Number of larvae that survived until day 4 when the experiment was terminated. **B)** Sperm concentration (# of sperm ml⁻¹). Bold numbers indicate self-crosses. Some samples were lost (dashes). Colonies D and G are clonemates (i.e. share the same alleles at all 8 loci). NA = not applicable

A

Sperm Donor	Egg Donor					
	R	O	G	D	Y	H
R	1.77x10⁸	1.82x10 ⁸	3.69x10 ⁸	2.48x10 ⁸	1.29x10 ⁸	3.05x10 ⁷
O	1.03x10 ⁷	Na	8.78x10 ⁷	-	2.37x10 ⁷	Na
G	1.16x10 ⁷	1.36x10 ⁷	Na	5.27x10 ⁶	1.35x10 ⁶	Na
D	2.32x10 ⁷	Na	Na	-	-	Na
Y	1.62x10 ⁸	2.41x10 ⁸	Na	-	-	Na
H	4.00x10 ⁸	Na	Na	Na	Na	Na

B

Sperm Donor	Egg Donor						Total
	R	O	G	D	Y	H	
R	2	259		224	868	19	1372
O	6		3	4	30		43
G	386	54		1	119		560
D	94			2	175		271
Y	96	210		265	9		580
H	0						0
Total	584	523	3	496	1201	19	2826

Table 2.5 *Acropora palmata* larval genotypes. See methods for explanation of larval classes. Prop = proportion, N = sample size, SD = standard deviation. Note that colonies D and G are clonemates.

Cross (Male x Female)	Total Larvae	<4 Loci genotyped	≥4 Loci genotyped	Outcrossed Sexual	Maternal genotypes only	Triploid pattern
		N	N (Prop)	Prop (N)	Prop (N)	Prop (N)
RxD	31	5	26 (0.84)	0.88 (23)		0.12 (3)
GxR	31	11	20 (0.65)	0.80 (16)		0.20 (4)
DxR	31	4	28 (0.90)	0.61 (17)		0.36 (10)
YxR	31	0	31 (1.00)	0.58 (18)		0.42 (13)
DxD	2	2	1 (0.50)	0.00 (0)		
DxY	31	4	28 (0.90)	0.93 (26)	0.04 (1)	
GxO	31	4	27 (0.87)	0.37 (10)	0.63 (17)	
GxY	30	1	29 (0.97)	0.93 (27)		0.07 (2)
OxD	4	3	1 (0.25)	1.00 (1)		
OxG	3	0	3 (1.00)	0.67 (2)	0.33 (1)	
OxR	6	1	5 (0.83)	1.00 (5)		
OxY	8	3	5 (0.63)	0.40 (2)	0.60 (3)	
RxH	1	1	0 (0.00)			
RxO	31	12	20 (0.65)	0.30 (6)	0.65 (13)	
RxR	2	0	2 (1.00)		1.00 (2)	
RxY	31	2	29 (0.94)	1.00 (29)		
YxD	31	3	28 (0.90)	1.00 (28)		
YxO	31	4	27 (0.87)	0.41 (11)	0.59 (16)	
YxY	9	1	8 (0.89)	0.00 (0)	1.00 (8)	
DxG	1	0	1 (1.00)	0.00 (0)	1.00 (1)	
Total	376	61	319 (0.85)	0.69 (221)	0.19 (62)	0.10 (32)
Mean ± SD	19 ± 14	3 ± 3	16 ± 13 0.78 ± 0.27	0.73 ± 0.26 15 ± 10	0.65 ± 0.33 7 ± 7	0.23 ± 0.15 6 ± 5

Table 2.6 Genotypic diversity in three Floridian *A. palmata* populations as detected by five microsatellite markers. For Sand Island, the number of colonies sampled and the number of genets found within a 15 m range are listed in parentheses. Average (Aver) and standard deviation (SD), maximum and minimum distances (Dist) between ramets per reef are given. All distances in meters (m).

Population	# of Colonies genotyped	# of Genets	Aver. dist ramets (m) (SD)	Max dist ramets (m)	Min dist ramets (m)
Horseshoe	20	1	8.2 (4.3)	21.3	0.3
Little Grecian	20	1	5.2 (2.5)	10.5	0.3
Sand Island	53 (32)	18 (10)	7.5 (2.1)	25.1	0.4

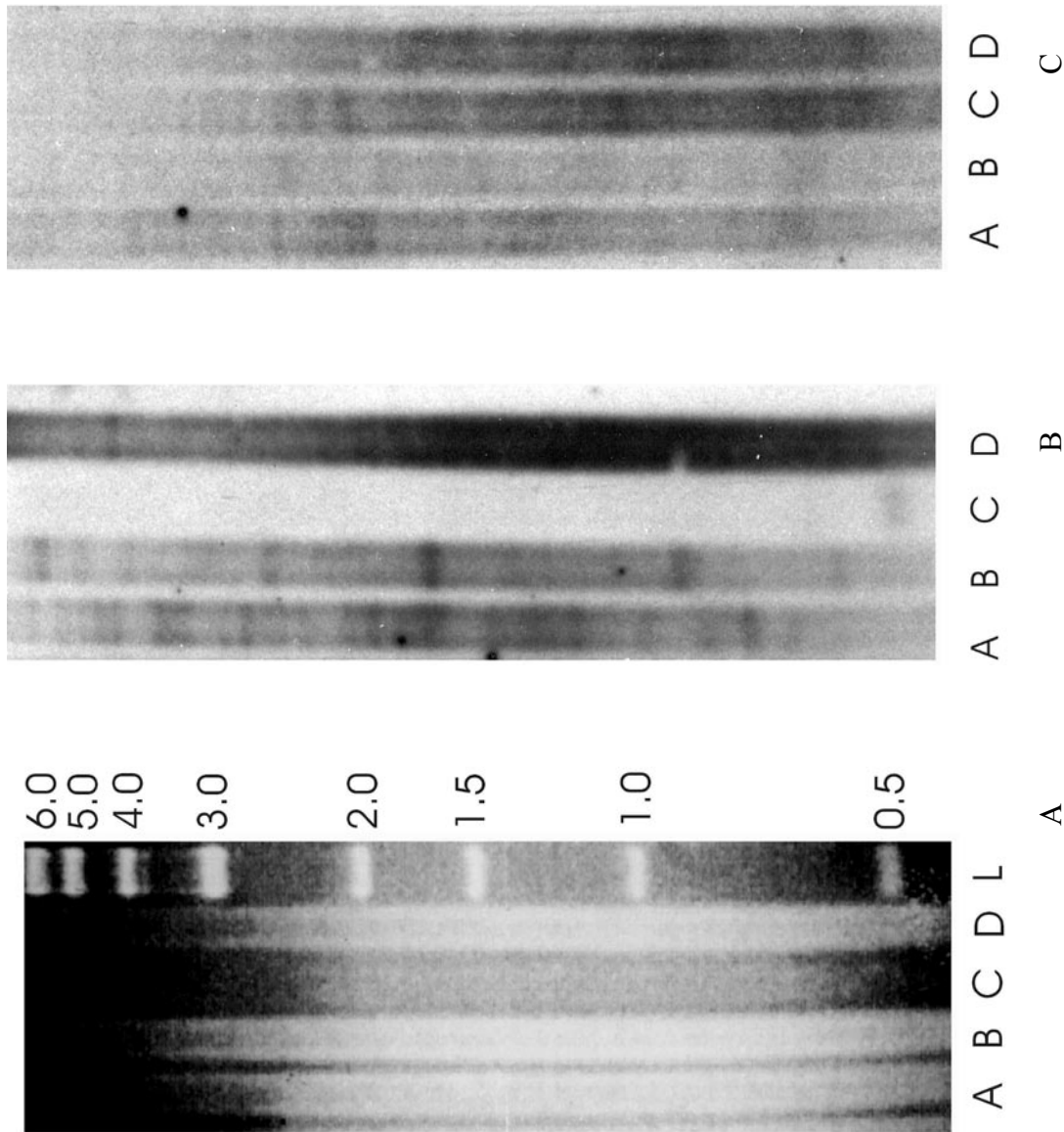
Table 2.7 Chi-squared analysis of larval genotypes observed from five *Acropora palmata* crosses at 5 loci. Genotypes are represented as six digit numbers. The first three digits refer to the paternal allele (in basepairs, bp) and the second three digits refer to the maternal allele. Cross G x D is between clonemates. Chi-square tests were carried out without applying a continuity correction. A simple Bonferroni correction would be dividing the cut off significance level ($p < 0.05$) by the number of tests carried out ($n = 50$). The new significance level then equals $p < 0.001$. Thus, none of the chi-square tests are significant ($p = 0.006$ for locus 181 and D x Y, Y x D).

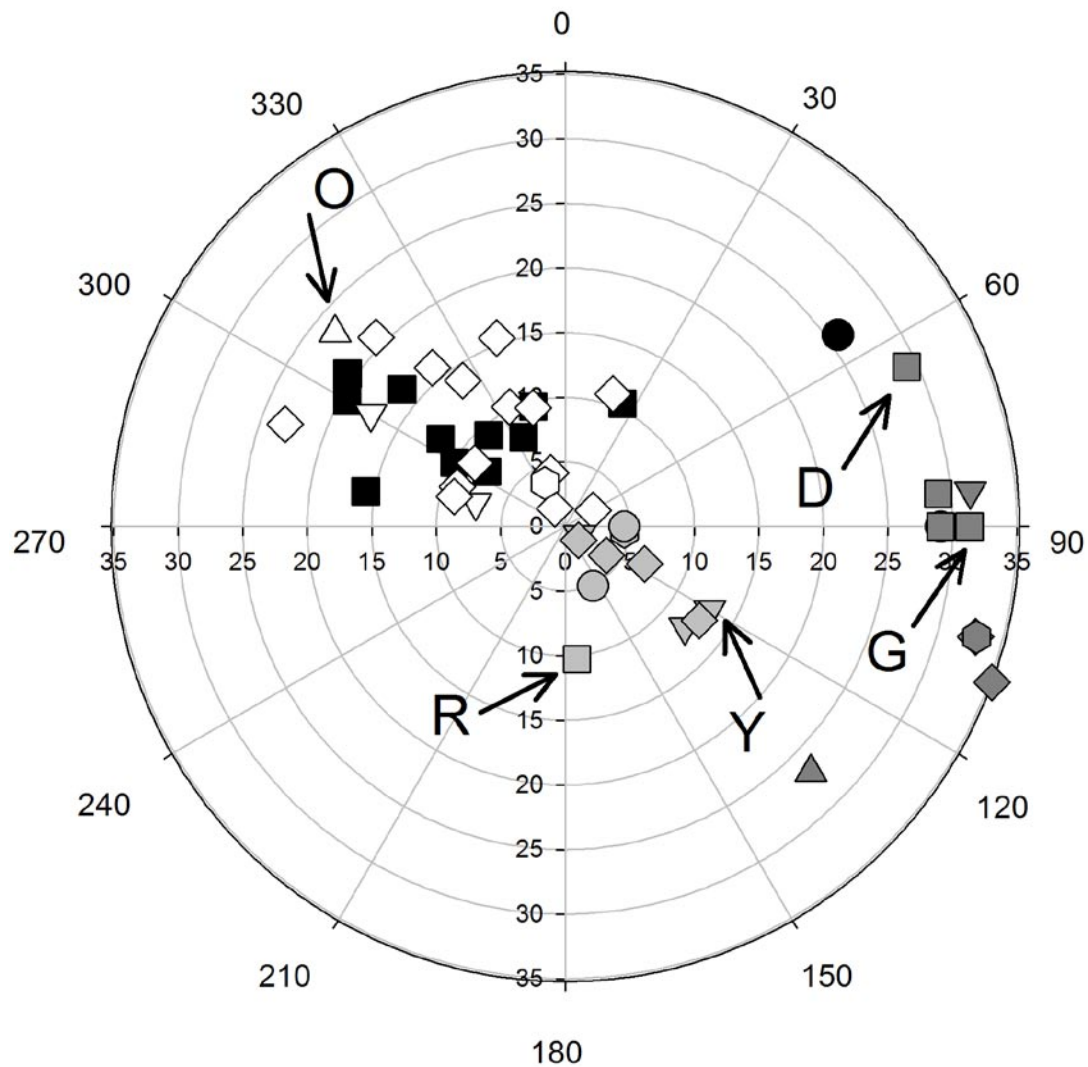
Locus	Cross	Parental Genotypes		No. of Progeny	Distribution of Larval Genotypes Observed						Expected Ratio	X ² – p value
		Father	Mother									
166	RxD	164173	155161	22	8 (155164)	2 (155173)	8 (161164)	4 (161173)			1:1:1:1	0.18
	DxR	155161	164173	16	3 (155164)	4 (155173)	4 (161164)	5 (161173)			1:1:1:1	0.92
	RxD, DxR			38	11 (155164)	6 (155173)	12 (161164)	9 (161173)			1:1:1:1	0.54
	YxR	170170	164173	17	11 (164170)	6 (170173)					1:1	0.23
	RxY	164173	170170	28	10 (164170)	18 (170173)					1:1	0.13
	YxR, RxY			45	21 (164170)	24 (170173)					1:1	0.65
	YxD	170170	155161	25	11 (155170)	14 (161170)					1:1	0.55
	DxY	155161	170170	26	13 (155170)	13 (161170)					1:1	1
	DxY, YxD			51	24 (155170)	27 (161170)					1:1	0.98
	GxY	155161	170170	24	13 (155170)	11 (161170)					1:1	0.68
181	RxD	156156	159162	22	12 (156159)	10 (156162)					1:1	0.67
	DxR	159162	156156	16	8 (156159)	8 (156162)					1:1	0.32
	RxD, DxR			38	18 (156159)	20 (156162)						0.75
	YxR	156159	156156	17	15 (156156)	2 (156159)					3:1	0.66
	RxY	156156	156159	28	22 (156156)	6 (156159)					3:1	0.21
	YxR, RxY			45	37 (156156)	8 (156159)						0.26
	YxD	156159	159162	25	11 (156159)	8 (156162)	3 (159159)	3 (159162)			1:1:1:1	0.06
	DxY	159162	156159	26	9 (156159)	10 (156162)	3 (159159)	4 (159162)			1:1:1:1	0.13
	DxY, YxD			51	20 (156159)	18 (156162)	6 (159159)	7 (159162)				0.00
	GxY	159162	156159	25	8 (156159)	10 (156162)	5 (159159)	2 (159162)			1:1:1:1	0.08

Locus	Cross	Parental Genotypes		No. of Progeny	Distribution of Larval Genotypes Observed						Expected Ratio	X ² – p value
		Father	Mother									
182	RxD	142176	142173	22	7 (142142)	5 (142173)	4 (142176)	6 (173176)			1:1:1:1	0.67
	DxR	142173	142176	17	2 (142142)	8 (142173)	5 (142176)	2 (173176)			1:1:1:1	0.12
	RxD, DxR			39	9 (142142)	13 (142173)	9 (142176)	8 (173176)				0.68
	YxR	158191	142176	18	2 (142158)	4 (142191)	6 (158176)	6 (176191)			1:1:1:1	0.49
	RxY	142176	158191	28	9 (142158)	7 (142191)	4 (158176)	8 (176191)			1:1:1:1	0.57
	YxR, RxY			46	11 (142158)	11 (142191)	10 (158176)	14 (176191)				0.85
	YxD	158191	142173	28	5 (142158)	8 (142191)	4 (158173)	11 (173191)			1:1:1:1	0.23
	DxY	142173	158191	26	9 (142158)	6 (142191)	8 (158173)	3 (173191)			1:1:1:1	0.38
	DxY, YxD			54	14 (142158)	14 (142191)	12 (158173)	14 (173191)				0.97
	GxY	158191	158191	27	9 (142158)	5 (142191)	9 (158173)	4 (173191)			1:1:1:1	0.17
192	RxD	163166	151160	23	8 (151163)	5 (151166)	7 (160163)	3 (160166)			1:1:1:1	0.46
	DxR	151160	163166	17	6 (151163)	0 (151166)	4 (160163)	7 (160166)			1:1:1:1	0.08
	RxD, DxR			40	14 (151163)	5 (151166)	11 (160163)	10 (160166)				0.24
	YxR	144172	163166	18	6 (144163)	2 (144166)	5 (163172)	5 (166172)			1:1:1:1	0.57
	RxY	163166	144172	28	9 (144163)	5 (144166)	8 (163172)	6 (166172)			1:1:1:1	0.70
	YxR, RxY			46	15 (144163)	7 (144166)	13 (163172)	11 (166172)				0.38
	YxD	144172	151160	28	11 (144151)	3 (144160)	7 (151172)	7 (160172)			1:1:1:1	0.20
	DxY	151160	144172	26	6 (144151)	6 (144160)	9 (151172)	5 (160172)			1:1:1:1	0.70
	DxY, YxD			54	17 (144151)	9 (144160)	16 (151172)	12 (160172)				0.38
	GxY	151160	144172	25	5 (144151)	5 (144160)	5 (151172)	10 (160172)			1:1:1:1	0.11

Locus	Cross	Parental Genotypes		No. of Progeny	Distribution of Larval Genotypes Observed						Expected Ratio	X ² – p value
		Father	Mother									
207	RxD	185188	182197	12	6 (182185)	1 (182188)	4 (185197)	1 (188197)			1:1:1:1	0.11
	DxR	182197	185188	17	5 (182185)	6 (182188)	2 (185197)	4 (188197)			1:1:1:1	0.56
	RxD, DxR			29	11 (173185)	7 (173188)	6 (176185)	5 (176188)				0.41
	YxR	173176	185188	17	5 (173185)	3 (173188)	4 (176185)	5 (176188)			1:1:1:1	0.89
	RxY	185188	173176	28	5 (173185)	13 (173188)	5 (176185)	5 (176188)			1:1:1:1	0.08
	YxR, RxY			145	10 (173182)	16 (173197)	9 (176182)	10 (176197)				0.43
	YxD	173176	182197	28	8 (173182)	8 (173197)	6 (176182)	6 (176197)			1:1:1:1	0.90
	DxY	182197	173176	25	3 (173182)	8 (173197)	8 (176182)	6 (176197)			1:1:1:1	0.44
	DxY, YxD			53	11 (173182)	16 (173197)	14 (176182)	12 (176197)				0.77
	GxY	182197	173176	26	6 (173182)	8 (173197)	7 (176182)	5 (176197)			1:1:1:1	0.54

Fig 2.1 Abundance of microsatellite motifs in *Acropora palmata* and three other species. The left panel shows the gel stained with ethidium bromide and UV illuminated. It shows the relative amounts of DNA in each lane. This gel was Southern blotted; the membrane was probed and exposed to x-ray film (middle and right panels). The middle panel shows the autoradiograph produced by the (AAC)₁₀ probe. The arrow indicates a repetitive element in the coral genome. The right panel shows the autoradiograph produced by the (AAT)₁₀ probe. A=Roseate Tern, *Sterna dougallii*; B=Giant Asian Turtle, *Orlitia borneensis*; C=Elkhorn Coral, *A. palmata*; D=Bicolor Damselfish, *Stegastes partitus*, L=Ladder (kilobases).





N=50

Fig 2.2 *Acropora palmata*. Polar plot of population at Sand Island Reef. Each mark represents a colony. Ramets of the same genet indicated by common dot shape and color. Arrows indicate colonies that served as gamete donors during the August 2003 spawning event. Letters correspond to parental designation in Table 2.4. Radial axis: distance in (m), angular axis: angle in degrees. N = number of colonies.

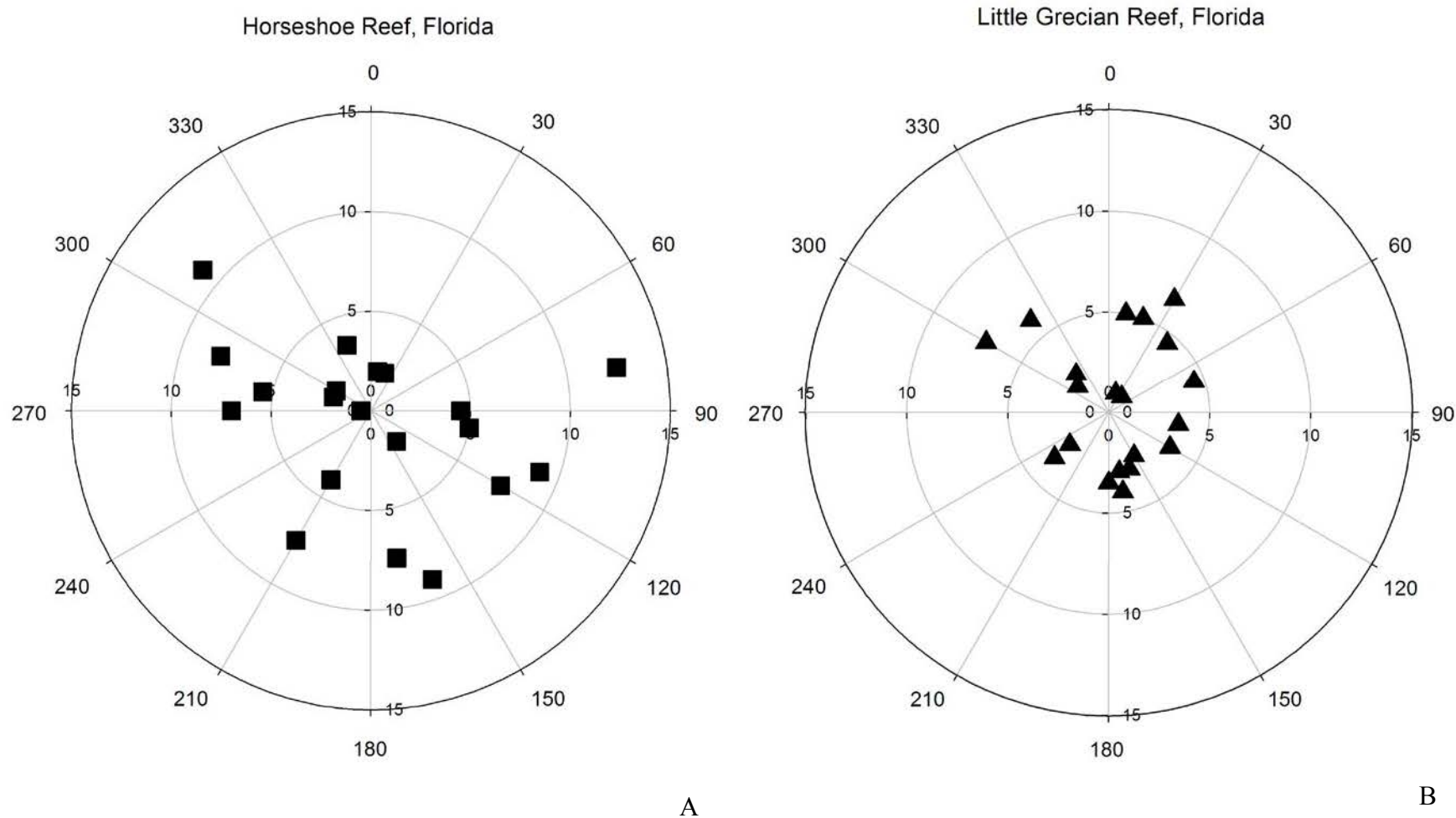


Fig 2.3 *Acropora palmata*. Polar plots of populations at Horseshoe (**A**) and Little Grecian (**B**) Reef. Only one genet was found on each of these reefs. Radial axis: distance in (m), angular axis: angle in degrees. N = number of colonies.

CHAPTER 3: MICROSATELLITES DEFINE TWO POPULATIONS OF *ACROPORA PALMATA* IN THE CARIBBEAN

ABSTRACT

The extent to which pelagic larvae connect marine populations has been the subject of ongoing controversy. The debate centers on both the degree of correlation between surface currents and larval dispersal routes and the time scale over which larval exchange between populations takes place. Theoretically, genetic similarity between populations can be maintained by low levels of migration. Such a small number of migrants, however, would be insufficient to sustain populations in demographic decline. In the face of the depressed state of many tropical benthic organisms, such as reef-building corals, this question is now of concern to conservation biologists. We employ microsatellite markers to estimate the population structure and migration rates of the ecologically important, Caribbean reef-building coral *Acropora palmata*, a candidate for the U.S. Endangered Species List. We used a Bayesian clustering approach to estimate the number of populations present in our samples. This analysis identified two major clusters, a western (W) cluster comprised of samples from Panama, Mexico, Florida, the Bahamas and Navassa and an eastern (E) cluster containing samples from the U.S. Virgin Islands and the Lesser Antilles (St. Vincent and the Grenadines, Bonaire and Curacao). Both F_{st} (0.056) and R_{st} (0.327) estimates were significant and indicate moderate differentiation between the two clusters. Bayesian estimates of migration (implemented by the Program BayesAss) suggest little to no recent exchange between these clusters. Within each cluster, asymmetric migration patterns were observed. In the east, 30% of *A. palmata* genets in the US Virgin Islands as well as the Netherlands Antilles are immigrants from St. Vincent and the Grenadines (SVG). SVG was self-recruiting. Panama does not experience measurable amounts of immigration from the other localities in the W (or the E). The cluster's split between Navassa and the US Virgin Islands in the North, and between Panama and Curacao in the South, is congruent with previously suggested biogeographical breaks at the Mona Passage (between Puerto Rico and Hispanola) and the Peninsula de Guajira (eastern Colombia). The detection of two distinct populations of *A. palmata* challenges the assumption of panmixia among Caribbean populations of benthic broadcast spawning corals and suggests that these populations need to be managed separately.

INTRODUCTION

Marine populations have been thought to be well connected via long-distance dispersal of planktonic larval stages (Heck and McCoy 1978; Veron 1995; Scheltema et al. 1996). High connectivity between populations would have important implications for the management of marine resources. Compared to highly structured populations, a smaller number of marine reserves would theoretically be required to achieve adequate protection of larval supplies. The extent of interconnectedness of coral-reef associated organisms is the subject of ongoing controversy (e.g. Roberts 1997, and references herein; Cowen et al. 2000). Roberts (1997) proposed a high correlation between ocean surface currents and larval dispersal routes, such that species with planktonic larvae inhabiting regions connected by strong currents should show high genetic similarity. In contrast, Cowen et al. (2000) suggested that high diffusion and mortality rates, aided by behavioral adaptations, should result in local larval retention and closed populations over ecologically relevant time scales. Indeed, an increasing number of studies report far more limited dispersal than previously predicted (reviewed in Hellberg et al. 2002; Swearer et al. 2002; Thorrold et al. 2002).

Marine fishes reveal a gradient of genetic similarity in Caribbean populations that is weakly correlated with the larval life span (Shulman and Bermingham 1995). Recently, Taylor and Hellberg (2003) presented mtDNA evidence for strong differentiation among Caribbean populations of cleaner gobies (*Elacatinus evelynae*). Some populations within color forms were even reciprocally monophyletic, suggesting thousands of generations of isolation despite modest geographic separation and larvae with a three-week pelagic duration. These gobies may be regarded as poor representatives of benthic reef inhabitants, however, for several reasons. First, they belong to an especially species-rich lineage, so their strong subdivision could be considered a propensity to differentiate. Second, their planktonic distributions suggest possible adaptation for nearshore development (Smith et al. 1987), perhaps providing them with the potential to restrict their dispersal by ocean currents. Third, their eggs are attached to the substrate prior to fertilization, so that embryos do not spend the first six days of their ontogeny as plankton (Valenti 1972). Here we examine population structure in a Caribbean broadcast spawning coral to which these conditions do not apply: the elkhorn coral *Acropora palmata*.

Acropora palmata is one of the major reef building corals in the Caribbean, providing essential habitat for a multitude of reef organisms (Gladfelter et al. 1978; Lirman 1999) and thus can be regarded as a foundation species (Vermeij 2001). Historically, it dominated the reef crest and the shallowest depth of the fore reef (0-5m) (Bruckner 2002). It forms thickets of juxtaposed colonies, thus complicating the identification of individuals. Asexual

reproduction by breakage and reattachment of branches can be the predominant mode of recruitment for *A. palmata* (Highsmith 1982), making this species potentially very long-lived. This is facilitated by its capacity for lesion repair (Gladfelter et al. 1978; Bak 1979; Highsmith 1982). In contrast to asexual reproduction, sexual recruitment appears relatively limited (Dustan 1977; Bak and Engel 1979; Hughes and Jackson 1980; Rylaarsdam 1983; Rosesmyth 1984; Hughes and Jackson 1985) at least in large populations that produce numerous fragments. *A. palmata* generally releases sexually produced gametes into the water column once a year after the August full moon in a synchronized spawning effort (Szmant 1986). Each hermaphroditic colony simultaneously releases eggs and sperm in bundles that float to the surface where they break apart and mix with gametes from other colonies (acroporids are poor self-fertilizers, Willis et al. 1997; Fukami et al. 2003, Chapter 2). Pelagic larvae become competent to settle within 5 days, but can remain planktonic for up to two weeks (Szmant pers. comm., Baums pers. observ.).

Populations of both *Acropora palmata*, and the congener *A. cervicornis* decreased across the Caribbean in the 1980s and remain in a depressed or possibly still declining state (Aronson and Precht 2001b; Bruckner 2002). White Band Disease, which is specific to acroporids (Antonius 1981; Gladfelter 1982; Peters 1993) may be the primary cause of the recent mortality (Rogers 1985; Wells 1988; Sheppard 1993; Aronson and Precht 2001a). Additionally, acroporids are particularly susceptible to hurricane breakage (Stoddart 1963; Glynn 1964; Shinn 1976; Woodley et al. 1981; Shinn et al. 1989; Curran et al. 1994; Steneck 1994) and have experienced severe bleaching in the last decade (Porter and Meier 1992; Winter et al. 1998). The depressed state of acroporids in the Caribbean has led to their listing as candidate species under the U.S. Endangered Species Act (Diaz-Soltero 1999) and formal review of their status is ongoing (Bruckner 2002).

Knowledge of the population structure of *Acropora palmata* is both potentially informative as to the generality of proposed phylogeographic breaks in the Caribbean and essential to assessing potential conservation measures. In this study, we test the null hypothesis of Caribbean panmixia in *A. palmata*. The expectation of no population structure is based on the presumed long larval life spans of this coral and the strong and consistent surface currents running through its range during the spawning season. We applied microsatellite markers to understand the population structure of *A. palmata*. We present evidence for the existence of two distinct population clusters in the Caribbean that show little recent migration between them, and a high degree of self-recruitment. This information will prove useful for formulating effective conservation strategies.

MATERIAL AND METHODS

Sampling

Acropora palmata Lamarck colonies (n=1051) were sampled and genotyped from eight localities spanning much of the Caribbean and Bahamas using SCUBA (Table 3.1, Fig 3.1). At some reefs (marked with a * in Table 3.1), colonies were sampled using a random sampling procedure (described in Chapter 2) to estimate the contribution of asexual reproduction to the population. Sample sizes in Table 3.1 indicate the number of unique genets identified rather than the total number of colonies sampled. A description of clonal structure in *A. palmata* will be provided in a separate report (Chapter 4). Large variation in clonal structure led to sometimes-drastic reductions of genet sample sizes (in two extreme cases from n = 27 colonies (= ramets) sampled to n = 1 genotype). The depressed state of *A. palmata* populations at some localities led to further departures from ideal sample sizes. At other reefs, all or most colonies present were sampled. The maximum distance between colonies on a given reef did not exceed 100 m. In St. Vincent and the Grenadines (SVG), samples were taken at haphazardly chosen intervals until a minimum of 24 colonies per reef was sampled or no more colonies could be located within swimming distance (usually 600m but up to 2000m). One cm-long tip per identified colony was snipped off using a bolt cutter and placed in a labeled bag. Coral samples were immersed in 70% ethanol upon returning to shore and stored at -80° C until genotyping.

Genotyping

Estimation of genetic population structure in corals poses a number of particular difficulties. Most reef corals harbor intracellular algal symbionts, complicating coral-specific marker development (Shearer et al. in press). Mitochondrial DNA is generally invariant within coral species, making it useless for population genetic studies (Shearer et al. 2002). Due to frequent asexual reproduction, the genetic markers employed must be sufficiently polymorphic to identify individual genets. This allows for the exclusion of multilocus genotypes that are identical by descent and thus prevents inflation of interpopulation differences due to asexual reproduction. Microsatellite markers have become available for study of corals only recently (Maier et al. 2001; Severance et al. in press; Shearer in press). Five newly developed microsatellite loci for *A. palmata* (Chapter 2) are polymorphic enough to distinguish genets. Through controlled crosses conducted previously (Chapter 2), it was demonstrated that all five of these loci were Mendelian and unlinked. We used these to genotype colonies sampled in this study.

Tissue samples were extracted and genotyped as described in Baums et al. (subm). Briefly, two multiplex Polymerase Chain Reactions (PCR) were performed per sample using fluorescently labeled primers to assay five loci containing AAT repeats. PCR products were visualized with an automated sequencer (ABI 3730). An internal size standard (Gene Scan 500-Liz, Applied Biosystems CA) ensured accurate sizing. Electropherograms were analyzed with GeneMapper Software 3.0 (Applied Biosystems, Ca). Alleles were scored as PCR product size and converted to repeat number by subtracting the size of the flanking region when necessary (e.g. for R_{st} estimates, see below). A total of 1050 colonies were genotyped at all five loci. Of these, 578 multilocus genotypes were unique (= genets) and thus included in the following analyses.

Bayesian Analysis of Population Structure

No *a priori* information on the population structure of *Acropora palmata* was available. Instead, the number of populations K was estimated using a Bayesian approach implemented by the program STRUCTURE (Vers. 2, Pritchard et al. 2000). STRUCTURE uses a clustering method to assign individuals (in our case genets) with similar multilocus genotypes to probable common populations. Admixed individuals (those with genotypes that do not readily cluster with others) are assigned jointly to probable parental populations. This approach assumes that (posterior) populations are in Hardy -Weinberg equilibrium (HWE), but is robust against slight linkage disequilibrium between markers (Falush et al. 2003). Five independent runs of $K = 1 - 8$ were performed at 10^6 Markov Chain Monte Carlo (MCMC) repetitions and a 100000 generation burn in period. The “admixture ancestry model” (since the planktonic larval stage of *A. palmata* might lead to highly interconnected populations) was run under the assumption of “correlated allele frequencies”. The posterior probability ($\log \Pr (K|X)$), was calculated for each K . X denotes the genotypes of the sampled individuals. Similar likelihood values for several K were obtained. The smallest K is the most likely (informal pointers in the documentation of STRUCTURE, Cegelski et al. 2003) and thus we chose $K = 2$ (see results). Based on the highest average proportion of membership (q), the eight sampled localities of the Caribbean (Fig 3.3) were divided into two clusters. Proportion of membership q and number of genets was estimated in each of the clusters by taking the average of q of all those genets having their maximum probability of membership in the respective cluster.

In a second round of analyses, we evaluated the individual membership q_i for each genet to one of the two clusters. Genets were coded for the cluster in which they were sampled (their assumed population of origin). Using option USEPOPINFO, STRUCTURE

then calculated the probability that the genet's ancestry lies exclusively within the cluster that it was sampled from or in the other cluster.

Testing for Conformity to Expectations

Sampled genotypes were tested for conformity with Hardy Weinberg expectations in FSTAT, Vers. 2.9.3 (Goudet 1995). Tests were based on either the eight sampled localities or the two clusters suggested by STRUCTURE. A sequential Bonferroni correction (Holm 1979) was applied when multiple tests were carried out on the same dataset. Markers were shown to be unlinked in a previous crossing study (Chapter 2). However, tests for linkage disequilibria between all loci were performed within and overall clusters to check for hidden population structure with FSTAT.

Some loci can skew estimates of population structure when SMM (Stepwise Mutation Model) based statistics such as R_{st} or $\delta\mu^2$ are employed. Landry et al. (2002) developed a straightforward test of the variances in size frequency distributions of alleles between loci. Only loci with similar variance in allele size differences should be included in one study. The statistic F_{ctr} was calculated using the program ANIMAL FARM (Landry et al. 2002).

Estimates of Population Differentiation using F- and R-Statistics

Population structure is routinely analyzed with an analysis of variance framework ("F-statistics", see Balloux and Goudet 2002; Balloux and Lugon-Moulin 2002 for recent reviews). The amount of genetic variation distributed within and among groups is estimated and taken as a reflection of the degree of differentiation. The measure of variance can be based either on the number of different alleles (F_{st} , F-statistics) or the sum of squared size differences between alleles (R_{st} , R-statistics), which accounts for the probability that similar sized microsatellites are closely related. F- and R-statistics were estimated for the two clusters suggested by STRUCTURE. The program FSTAT was run with 1000 repetitions to return Weir and Cockerham's (Weir 1984) estimator of F_{st} , θ , and Slatkin's estimator of R_{st} , ρ (Slatkin 1995). Bootstrapping over samples and loci, and jackknifing over samples was performed when programs permitted (> 4 loci in FSTAT). A G-test (Balloux and Goudet 2002) was performed to estimate population differentiation with and without locus 166 (locus 166 had the largest effect on F- and R-statistics, see Results). Heterogeneity in F-statistics among loci and localities was estimated by calculating F_{st} values based on 4 out of 5 loci at a time (1000 replicas, calculated by MSA). All analyses mentioned above were conducted for all five loci and in a second round, excluding locus 166.

Multilocus genotypes were also subjected to an analysis of molecular variance (Michalakis and Excoffier 1996) as implemented by ARLEQUIN (Vers. 2.0 Schneider et al. 2000). The two clusters were used as “Groups”, and the eight sampling localities were designated as the “populations. Differentiation among groups and within populations was estimated based on both the number of different alleles (F_{st}) and the sum of squared size differences (R_{st}).

Detection of Migration Rates

Various algorithms designed for assigning and excluding individuals to previously defined populations are implemented in the program GENECLASS (Piry et al. subm). We used GENECLASS to exclude populations (clusters) as places of origin for genets following Cornuet et al. (1999). The underlying thought is that not all potential source populations might have been sampled in the study. The criteria used were those of Rannala and Mountain (Bayesian model, 1997) and of Paetkau et al (frequency model, 1995). 10000 individuals were simulated and the exclusion threshold was set at 0.01. If the genet’s multilocus likelihood of belonging to a population is smaller than 0.01, that population is excluded as the population of origin for this genet.

In a second approach we used a Bayesian model to estimate the direction of recent migration rates, m , among clusters and sampling localities (implemented in the program BayesAss, Wilson and Rannala 2003). This model relaxes assumptions about Hardy-Weinberg equilibria. It upholds assumptions about unlinked loci and non-overlapping generations, both of which the dataset may violate: in the W some populations show linkage disequilibrium at several loci and *Acropora palmata* genets are potentially long-lived due to frequent asexual reproduction. The model was run under the following (default) parameters: 3,000,000 iterations of which 999,999 are burn in; sampling frequency was 2,000. BayesAss performs a likelihood ratio test to determine if the migration rates posterior probabilities are significantly different than the prior. For both clusters, χ^2 values were significant, indicating that enough information is present to detect migration rates.

RESULTS

Identification of Subpopulations using STRUCTURE

A significant drop in $\log P(K|X)$ is observed from $K = 1$ to $K = 2$ predefined clusters (Table 3.2). $\log P(K|X)$ continues to decline until $K = 4$ and then rises again when $K = 5$. The posterior probabilities of K indicate that $K = 4$ is the most likely clustering.

Given a range of K values with nearly equal likelihoods, the smallest identified clustering has been considered the most probable (Pritchard et al. 2000). This is corroborated by the high values of membership q seen in the assignment analysis (0.91 and 0.91, Table 3.2, Fig 3.2). Any further subdivision into $K > 2$ results in significant reduction of q . Examining the membership of genets in the 4 clusters identified by STRUCTURE indicates that several of those clusters overlap geographically whereas the membership of genets when split into two clusters is congruent with a split into a W and a E subregion (Fig 3.3, Table 3.2). Thus $K = 2$ was chosen as the most likely clustering. The two clusters are henceforth referred to as the W cluster with members Panama, Mexico, Florida, Bahamas and Navassa and the E cluster with members US Virgin Islands, St. Vincent and the Grenadines, Bonaire and Curacao. Within each of these clusters, genotypes conform to Hardy-Weinberg expectations, indicating that a Wahlund (Wahlund 1928) effect is absent and no undetected population structure within each cluster is present. However, while there was no evidence of linkage disequilibrium in the E cluster, 6 out of 10 pairwise tests in the W cluster were significant after Bonferroni correction. The test for linkage disequilibrium was repeated based on the eight sampling localities. In fact, samples from Panama (5 out of 10 pairwise locus comparisons were significant at $p < 0.0056$), Florida (2 out of 10 tests) and the Bahamas (6 out of 10 tests) were responsible for the detection of linkage between loci within the E cluster. All other tests were not significant. Since loci were shown to be unlinked in crossing studies (Chapter 2), this is taken as an indication of subtle population structure that remains undetected by the other statistics.

Population Structure as estimated by F-Statistics

There was significant differentiation between clusters as estimated by F_{st} (0.056, Table 3.3). AMOVA gave similar results with 5% of the variation distributed between clusters (Table 3.4A). When based on the sum of squared size differences, higher estimates of population differentiation are obtained ($R_{st} = 0.327$ or 32% of the variation based on AMOVA, Table 3.4A). Only between 1.4% (F_{st} and R_{st}) of the variance was attributed to variation among localities within the clusters (AMOVA, Table 3.4). For comparison, F_{st} and R_{st} estimates are given when grouping is based on the eight sampling localities instead of the two clusters identified by STRUCTURE. This results in reduced F_{st} and R_{st} values as expected since populations are now artificially divided into subpopulations (Table 3.3).

Locus 166 contributed strongly to estimates of population differentiation, as is apparent from the reduction in F_{st} and R_{st} values calculated with just the remaining four loci (Table 3.3). Inspection of the allele frequency distribution for locus 166 indicated discrete modes in allele frequencies in the two clusters (Fig 3.2). Some microsatellite loci

can disproportionately influence population distance measures based on size differences between alleles such as R_{st} and $(\delta\mu)^2$ (Landry et al. 2002) and so might lead to erroneous estimates of population differentiation. Thus, we tested for equal variance of mean allele size among clusters for each locus. These tests indicated that the amount of variance between loci is comparable (Table 3.5). A G-test of population differentiation between the two clusters remained strongly significant ($p < 0.001$) even without locus 166. Heterogeneity of F-statistics was calculated by bootstrapping over loci (Table 3.3). The bootstrapped F_{st} estimates are slightly lower than the global value but remain different from zero with or without locus 166.

Assessment of Migration Rates

STRUCTURE identified a small number of individuals with likely immigrant ancestry in the W ($n=1$) and the E ($n=4$) clusters (Table 3.6). All other genets were assigned to the cluster from which they were sampled. Using the program GENECLASS, for only four genets, both the W and the E cluster could be excluded as the population of origin when applying the frequency based criterion of Paetkau et al. (1995). All four genets were sampled in the W (one in Mexico and in Navassa and two in the Bahamas). Overall 87 % of genets were correctly assigned using this approach (i.e. genets were assigned back to the population they were sampled from). The Bayesian model in GENECLASS (Rannala and Mountain 1997) identified 7 genets in addition to the four identified by the frequency approach that it could not assign to either cluster ($n=9$ total: 4 sampled in the Bahamas, 1 sampled in Florida, 1 sampled in Mexico, 2 sampled in Navassa, and 1 sampled in the USVI). Again, 87 % of all genets were correctly assigned. Only one of the samples that could not be assigned to any of the clusters by GENECLASS was also flagged by STRUCTURE (the sample from the USVI).

Immigration between the two *Acropora palmata* clusters was essentially absent as estimated with the Bayesian approach implemented in BayesAss (Wilson and Rannala 2003). The absence of migration is not attributable to lack of information as indicated by significant χ^2 values when comparing the posterior probabilities of migration rates to their prior (log-likelihood ratio test, Wilson and Rannala 2003). The same result of no exchange between the W and E localities is obtained when immigration is assessed for all eight sampling localities. Again, log likelihood ratio tests were significant. Within each cluster some interesting patterns emerge. Panama does not appear to receive propagules from any of the potential sources sampled in this study. Some of the larvae, however, recruit into the localities along the Florida current. Florida, the Bahamas and Navassa are all estimated to derive >10% of their population from Panama. Note, that the standard deviation associated

with the estimates for Panama as a source population are high. The Bahamas do not receive propagules from Florida as estimated by this approach. Within the E cluster, standard deviations around immigration proportions are lower than within the W cluster. St. Vincent and the Grenadines act as larvae donors for both the USVI and the Netherlands Antilles, a result that is congruent with prevailing current patterns in the region.

DISCUSSION

*Two populations of *Acropora palmata**

Patterns of microsatellite differentiation reported here reject the hypothesis that the elkhorn coral *Acropora palmata* constitutes a single, interbreeding population throughout its broad geographic range. Instead, our results reveal a significant genetic discontinuity that indicates populations from the eastern Caribbean (roughly the U.S. Virgin Islands and the Lesser Antilles) and from the western Caribbean (Panama, Mexico, Florida, the Bahamas and Navassa) have experienced little if any gene flow between them in the recent past. Subdivision within these two clusters is subtler. It is evident, however, that immigration patterns between localities within clusters are asymmetrical and follow general current patterns.

Support for population differentiation of *Acropora palmata* in the Caribbean is twofold. Two distinct population clusters were identified as the most plausible scenario by the Bayesian modeling approach of STRUCTURE (Pritchard et al. 2000) without providing prior geographical information (Fig 3.3). Based on the high mean membership q in each cluster (Table 3.2), the eight sampling localities were unambiguously identified as belonging to one of the two clusters. STRUCTURE identified only five individuals as not originating from the cluster where they were sampled (Table 3.6). When asking the question for which genets **both** clusters could be **excluded** as the population of origin, between 4 and 9 genets were identified by exclusion tests (carried out by GENECLASS). The Type I error rate was set to 0.01; we would thus expect about 6 genets to be excluded by chance alone. The small number of a) recent migrants between the two clusters and b) genets from some theoretical third, not sampled population are in good agreement with a split of *A. palmata* samples into two clusters. F- and R-statistics (Table 3.3, 3.4) substantiate this grouping as well. Based both on F_{st} (0.056) and R_{st} (0.327), differentiation between the two clusters was highly significant and more so than between the eight original sampling localities.

Differentiation estimates based on R_{st} (Slatkin 1995) are expected to, and often do, (Balloux and Goudet 2002) result in higher values than when the test is based on F_{st} . Because R_{st} takes differences in allele sizes into account (under the assumption of a Stepwise Mutation Model, SMM), it can be strongly influenced by loci that show discrete allele size frequency distributions between populations. Locus 166 shows such a distribution (Fig 3.2). When excluding locus 166, R_{st} values become smaller than estimates of population differentiation based on F_{st} . This raises the question whether locus 166 estimates alone create the significant R_{st} values we observed. We tested for homogeneity of variance (Landry et al. 2002) in allele size between populations and could reject this hypothesis. The observed modality of allele frequency distributions between clusters is intriguing, however. All loci in this study are trinucleotide (AAT) repeats. If these repeats are subject to selection or are closely linked to a region that is, the assumption of neutrality underlying F-statistics is violated. Still, a disruption of gene flow between the western and eastern clusters is evident, regardless of the evolutionary mechanism that produces this result.

Results of the Bayesian assignment model developed recently by Wilson and Rannala (2003) strongly support the split of *Acropora palmata* into a W and an E cluster (Table 3.7A). This model specifically estimates migration over the past few generations. Within the clusters, > 98% of genets originated from local sources. This absence of recent migrant exchange holds when the data are analyzed on the basis of the eight sampling localities (Table 3.7B). Statistical confidence in patterns obtained for the E populations is relatively strong, perhaps due to close adherence of population parameters to model assumptions (large sample sizes, linkage equilibrium). St. Vincent and the Grenadines are clearly identified as a major source of *A. palmata* larvae for both the US Virgin Islands and the Netherlands Antilles (Bonaire and Curacao). Local current patterns are congruent with this result. Flow direction on the west side of the Windward Islands is influenced by the development of rings from the North Brazil current (Cowen et al. 2003b). Rings may either travel northward along the western shores of the Windward Islands or “squeeze” through one of the passages (e.g. St. Vincent Passage) to continue along a westward path toward the Central American coast (Fig 3.4). This is evident in the variable dispersal patterns of simulated fish larvae released from Barbados (compare releases for July and October in Fig 3 of Cowen et al. 2003a).

Within the W subregion, two interesting results stand out. First, Panamanian *A. palmata* stands are entirely self-recruiting and second, Bahamian populations do not seem to receive larvae from Florida. Both of these results are again congruent with current patterns (Fig 3.4, Centurioni and Niler 2003). 

Gene Flow Patterns in Highly Clonal Acroporids

Geographic variation has been described in a number of corals in temperate (Hoffman 1986; Hellberg 1994; Hellberg 1995; Hellberg 1996; McFadden 1997) and tropical studies (Ayre and Dufty 1994; Ayre et al. 1997; Adjeroud and Tsuchiya 1999; Ayre and Hughes 2000; Takabayashi et al. 2003; Mackenzie et al. 2004) using nuclear markers. However, we know of none using multi-locus microsatellite genotypes nor of any performed in the Caribbean. Comparison of our results to other coral studies is complicated by two factors. First, geographic structure estimates from allozymes using F_{st} statistics are likely to yield higher values than if the same pattern would have been ascertained with microsatellites. This is attributable to the higher mutation rates of microsatellites that result in a high degree of polymorphism, thereby deflating F_{st} values (Neigel 1997; Balloux and Lugon-Moulin 2002). Secondly, while studies of tropical corals routinely measure the contribution of asexual reproduction to population structure (using the statistic N^* , Johnson and Threlfall 1987; Uthicke et al. 1998), clonemates are rarely removed from the dataset before F_{st} values are calculated. This would likely lead to upward bias in population differentiation measures. Here, the great polymorphism of the markers used led to high confidence in the identification of clonemates and thus each unique genotype was included only once. Hence, only qualitative comparisons to other studies tropical reef corals with a broadcast spawning mode are warranted.

Ayre and Hughes (2000) used allozymes and found significant subdivision in two broadcasting acroporids sampled from populations spanning 500-1200 km along the Great Barrier Reef. Mackenzie et al. (2004) found similar differentiation in *A. nasuta* over a similar spatial scale using a combination of two intron sequences and one microsatellite locus. These three acroporids showing subdivision had little clonal structure. However, two other broadcast spawners, (*Acropora millepora* and *A. valida*) surveyed by Ayre and Hughes (2000) were moderately to highly clonal, as is *Acropora palmata* (Chapter 4. These two clonal species showed F_{st} values that did not differ from 0 over the 500-1200 km range, even with the inclusion of clonemates in the dataset. In the present study, sampling localities were separated by a maximum distance of about 1800 km within the W cluster (Bahamas – Panama) and about 840 km within the E cluster (Bonaire/Curacao – St. Vincent and the Grenadines, estimated midpoints of each locality, Fig 3.1). Comparison of spatial scale is not straightforward however, since the Great Barrier Reef is essentially linear in contrast to the oval shaped Caribbean basin.

Gene flow in the Caribbean

Studies of Caribbean metazoans other than corals have until recently found little genetic differentiation even over evolutionary time scales. Of eight species of reef fish surveyed by Shulman and Bermingham (1995), using mtDNA RFLPs, only three showed significant population structure. No relation between geographical and genetic distance was observed for these three subdivided species nor could geographic structure be related to larval characteristics. Queen conch (*Strombus gigas*), a species with a pelagic larval duration of 12 to 39 days, also showed no subdivision throughout the Caribbean (Mitton et al. 1989; Campton et al. 1992). Spiny lobster (*Panulirus argus*), with an even longer pelagic larval period (6-12 mo), are similarly highly connected throughout their range (Silberman et al. 1994).

Coral biologists frequently view the Caribbean as one province, with too few geographic barriers to allow for further distinctions (Veron 1995). Starck and Colin (1978), however, noted a break at the Mona Channel, where swift currents pass between Puerto Rico and Hispaniola. This area is coincident with the geographic division between genetically distinct white and blue color forms of the goby *Elacatinus evelynae* (Colin 1975; Taylor and Hellberg 2003). This is congruent with the finding of population subdivision between the Bahamas/Navassa and U.S. Virgin Island *Acropora palmata* samples. Most Caribbean endemics described to date are from the Western Caribbean (Collette 1974; Johnson and Brothers 1989; Colin 2002), where gyres could facilitate local retention (Fig 3.4). A second division was found between Panama and the Netherlands Antilles. Unsuitable habitat of the Venezuelan coast seems to cause disruptions in species' distribution for other organisms. For example, Pta. Guajira in the southern Caribbean divides coral reef dwelling species from those tolerant of productive upwelling waters (Colin 1975). A study of a Caribbean coral-reef fish using a trace-element marker revealed higher recruitment of locally retained larvae opposed to larvae originating from distant islands (Sweater et al. 1999), emphasizing the importance of behavioral and physical dispersal mechanisms. Simulation studies of larval fish dispersal came to similar conclusions, providing evidence for local retention of larvae as a result of larval behavior (Cowen et al. 2000; Cowen et al. 2003a). Studies of other coral reef species with a variety of life history characteristics should be investigated to ascertain the generality of these suggested biogeographical breaks in the Caribbean.

Conservation and Reservation Design

Marine reserves are the current tool of choice to manage coral reef organisms. Optimal design strategies, i.e. their placement, number and size, are the subject of much debate

(Palumbi 2003). Depending on the desired effect of the reserve (spill-over of larvae to neighboring sites, source for long-distance dispersing larvae to unprotected areas, habitat preservation), the assumption of highly dispersing larvae is more or less important. The existence of two populations of *Acropora palmata* in the Caribbean, possibly separated by a biogeographical barrier, suggests that marine reserves designed to protect this foundation species will have effects within their respective populations only if the goal is to provide a source of larvae for other imperiled reefs. This leads to an increase in the number of marine reserves necessary (Cowen et al. 2000).

There are immediate conservation applications of our findings. Ship groundings often impact the shallow *Acropora palmata* populations. Discussions are underway to reseed the impacted reef with colonies and/or larvae from other locations (A. Szmant pers com.). Based on this study, any such reseeded efforts would need to ascertain that transplanted colonies/larvae belong to the same population cluster than colonies from the impacted areas.

Summary

Bayesian analysis of population structure, along with F-statistics and genotype assignment tests, suggest that *Acropora palmata* in the Caribbean form two distinct populations with limited gene flow between them. The most likely locations for biogeographical barriers between the two clusters are the Mona Passage between Mona and Puerto Rico and the stretch of unsuitable habitat along the Venezuelan coast. St. Vincent is identified as an important source for *A. palmata* larvae within the eastern cluster. Congruent with current patterns, Panamanian stands experience low immigration. There is no evidence of larval export from the sampled Florida stands to the Bahamas. Taken together, our results indicate that 1) biogeographical barriers may exist across species boundaries in the Caribbean and 2) larval exchange between *A. palmata* stands is asymmetrical between most sites.

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Table 3.1 *Acropora palmata* genets identified in eight localities of the Caribbean. * samples were taken using a random sampling approach described in Baums et al. (subm); + colonies were sampled haphazardly and their location was mapped ; - colonies were sampled haphazardly and no information on colony location was recorded . NA not available.

Clusters	Locality	Reef Name	Latitude (°)	Longitude (°)	# of Genets	Per Locality		
W Cluster	Bahamas	Great Iguana*	26.7075	-77.1536	27	98		
		Halls Pond+	24.3539	-76.5699	13			
		Rocky Dundas+	24.2788	-76.5387	4			
		Little Darby+	23.8474	-76.2088	10			
		Bock Cay+	23.8075	-76.1601	10			
		Black Bouy+	23.8022	-76.1460	11			
		Charlies Beach*	23.7808	-76.1039	14			
		Perry Shallow*	23.7733	-76.0954	5			
		Childrens Bay+	23.8473	-76.2081	4			
		Dry Tortugas-	24.6209	-82.8675	2			
	Florida	Rock Key+	24.4560	-81.8596	4	30		
		Western Sambo	24.4799	-81.7187	9			
		Horseshoe*	25.1395	-80.2944	1			
		Little Grecian Snail Patch-	NA	NA	1			
		Little Grecian*	25.1184	-80.3172	1			
		Sand Island*	25.0179	-80.3686	12			
		Mexico	Banco Chinchorro+	18.3833	-87.4500		7	7
			North Shelf+	18.4135	-75.0229		18	
		Navassa	NW Point+	18.4136	-75.0294		35	68
			Lulu Bay+	18.3958	-75.0199		15	
	Panama	Bocas Del Drago+	9.4162	-82.3309	9	38		
		Cayo Wild Cayne+	9.3459	-82.1718	6			
		Bastimentos*	9.2650	-82.1201	17			
		Tobobe West-	9.12268	-81.8183	6			
Total		23				241		
E Cluster	Bonaire	Taylor's Made*	12.2238	-68.4051	32			
		Blue Bay*	12.1352	-68.9898	32			
	Curacao	Sea Aquarium*	12.0838	-68.8958	32	132		
		Awa Blanca*	12.0407	-68.7830	28			
		Boka Patrick-	12.2873	-69.0427	15			
		Blue Lagoon-	13.1285	-61.1993	9			
		Petit Byahaut W-	NA	NA	7			
		Petit Byahaut E-	NA	NA	17			
		Mustique-	12.8915	-61.1863	24			
	SVG	Bequia-	13.0150	-61.2491	16	164		
		Canouan-	12.6943	-61.3364	21			
		Tobago Cays-	12.6253	-61.3499	32			
		Union Island-	12.5916	-61.4160	24			
		Mayreau Gardens-	12.6322	-61.3816	14			
		Johnsons Reef*	18.3617	-64.7743	16			
		USVI	Grounding VI-				11	41
	Hawksnest Bay*		18.3472	-64.7807	14			
	Total		17				337	
	Total	8	42				578	

Table 3.2 Average and variance of average of Log P (K|X) from 5 runs of STRUCTURE with 10^6 repetitions each for *Acropora palmata* multilocus genotypes. Proportion of membership q and number of genets (in parentheses) was estimated in each of the clusters by taking the average of q of all those genets having their maximum probability of membership in the respective subpopulation for one of the 5 runs. The number of genets assigned to each cluster is given in parentheses.

K	Average Log P (K X)	Variance Log P (K X)	Cluster 1	Cluster 2	Cluster 3	Cluster 4
1	-11456	0.0				
2	-10927	1.4	0.91 (253)	0.91 (325)		
3	-10922	35.2				
4	-10918	529.1	0.71 (124)	0.48 (162)	0.49 (148)	0.68 (141)
5	-11225	80211.5				
6	-11279	1222.6				
7	-11330	48932.5				
8	-11408	16622.5				

Table 3.3 Estimates of F_{st} , F_{it} , F_{is} (Weir and Cockerham's theta, F , f) and R_{st} (Rousset 1996 and Goodman 1997) based on grouping the data into 2 clusters identified by STRUCTURE and into the eight sampling localities. Heterogeneity of the F_{st} estimate was evaluated using MSA (Dieringer and Schlotterer 2003) by calculating F_{st} with 4 out of 5 loci (bootstrapping). F_{st} and R_{st} were obtained with FSTAT (Goudet 1995). All estimates are reported based on either all loci or with locus 166 excluded. In the latter case FSTAT cannot compute confidence intervals due to the limited number of loci. CI = 95% confidence interval.

Grouping	Statistic	Heterogeneity of F_{st}	F_{st}	F_{it}	F_{is}	R_{st}
2 Clusters						
All Loci	Mean	0.051	0.056	0.056	0.000	0.327
	Lower CI	0.018	0.018	0.020	-0.008	
	Upper CI	0.114	0.107	0.104	0.010	
Without 166	Mean	0.026	0.026	0.028	0.002	0.002
	Lower CI	0.016	NA			
	Upper CI	0.038	NA			
8 Localities						
All Loci	Mean	0.042	0.045	0.035	-0.010	0.235
	Lower CI	0.019	0.020	0.014	-0.021	
	Upper CI	0.080	0.075	0.065	0.001	
Without 166	Mean	0.026	0.028	0.020	-0.008	0.021
	Lower CI	0.016	NA			
	Upper CI	0.038	NA			

Table 3.4 AMOVA on **A)** the number of different alleles (F_{st}) and **B)** the sum of squared size differences (R_{st}) as implemented by ARLEQUIN ver. 2.0 (Schneider et al. 2000). DF = degrees of freedom, SS = sum of squares, Var = Components of variance, Sign = Significance based on 10100 permutations, % Var = Percentage variation. Groups: W and E cluster, populations: 8 sampling localities.

A)

Source of variation	DF	SS	Var	Sign	% Var
Among Groups	1	63	0.10	0.02+-0.00	5.0
Among populations within groups	6	32	0.03	0	1.4
Within populations	1148	2128	1.85	0	93.6
Total	1155	2222	1.98		

B)

Source of variation	DF	SS	Var	Sign	% Var
Among Groups	1	8210	14.33	0.02+-0.00	31.5
Among populations within groups	6	672	0.66	0	1.4
Within populations	1148	34989	30.48	0	67.0
Total	1155	43871	45.46		

Table 3.5 Test of the inequality of microsatellite loci contribution to SMM-based distance coefficients (ANIMAL FARM, ver1.0). Results of the permutation test (1000). Given are the original value of the F_{ctr} statistic for each locus, the probability that the null hypothesis is true and the adjusted p-values following Sidak (1967). Adjusted significance threshold (Bonferroni correction) = 0.010000.

Locus	Original value F_{ctr}	Probability of H_0	Sidak adj. p-value
166	719.87	0.03	0.13
181	0.01	0.39	0.91
187	0.01	0.51	0.97
192	0.00	0.88	1.00
207	0.00	0.98	1.00

Table 3.6 Assessment of ancestry for genets with $q < 0.5$ of belonging to the cluster they were sampled from (Sample Cluster). Given are the probabilities of these genets not having an immigrant ancestry (P), the probabilities of being a first generation immigrant (G_0), of having one immigrant parent (G_1) and of having one immigrant grandparent (G_2). As calculated by STRUCTURE.

Sample Cluster	Locality	Reef Name	Genet ID	P	$P(G_0)$	$P(G_1)$	$P(G_2)$
E	Curacao	Boka Patrick	490	0.11	0.69	0.10	0.10
E	Curacao	Sea Aquarium	538	0.25	0.57	0.08	0.11
E	Curacao	Sea Aquarium	297	0.40	0.01	0.36	0.23
E	USVI	Johnsons Reef	1	0.31	0.65	0.02	0.02
W	Navassa	Nshelf	65	0.11	0.76	0.08	0.05

Table 3.7 Migration rates among *Acropora palmata* **A)** clusters and **B)** sampling localities. Means and standard deviations of the posterior distributions of m , the migration rate into each population, are given (%). The clusters into which genets are migrating are given in columns while the origins of the genets are given in rows. The values along the diagonal (in bold) indicate the proportion of individuals that are retained in each cluster. Values >10% in black. Bon/Cur = samples from Bonaire and Curacao.

A)

	W Cluster n = 241		E Cluster n = 337	
	Mean	SD	Mean	SD
W cluster	98.5	0.9	1.5	0.9
E cluster	0.8	0.6	99.2	0.6

B)

	West Cluster										East Cluster					
	Panama n = 38		Mexico* n = 7		Florida n = 30		Bahamas n = 98		Navassa n = 68		USVI n = 41		SVG n = 164		Bon/Cur n = 132	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Panama	98.6	1.4	3.6	4.2	18.0	6.2	15.6	7.3	10.2	5.9	0.4	0.6	0.1	0.2	0.3	0.4
Mexico	0.1	0.3	71.5	4.2	0.7	0.9	0.3	0.4	0.2	0.4	0.3	0.5	0.1	0.1	0.1	0.2
Florida	0.2	0.4	2.1	2.8	67.9	1.2	0.4	0.4	0.3	0.5	0.3	0.5	0.1	0.1	0.1	0.2
Bahamas	0.2	0.6	2.8	3.4	4.4	3.9	73.2	5.8	8.4	9.0	0.4	0.6	0.2	0.3	0.3	0.4
Navassa	0.2	0.4	5.1	4.5	4.3	5.6	8.8	10.8	78.7	10.4	0.4	0.6	0.1	0.1	0.4	0.5
USVI	0.2	0.3	2.6	3.3	0.7	1.1	0.3	0.5	0.5	0.7	67.9	1.1	0.1	0.1	0.2	0.4
SVG	0.3	0.6	10.2	6.1	2.6	2.6	0.3	0.5	1.2	0.9	29.2	2.1	99.4	0.5	30.9	1.1
Bon/Cur	0.2	0.4	2.1	2.6	1.4	1.9	1.1	1.0	0.5	0.7	1.0	1.5	0.1	0.2	67.7	0.7

* Low Sample Size

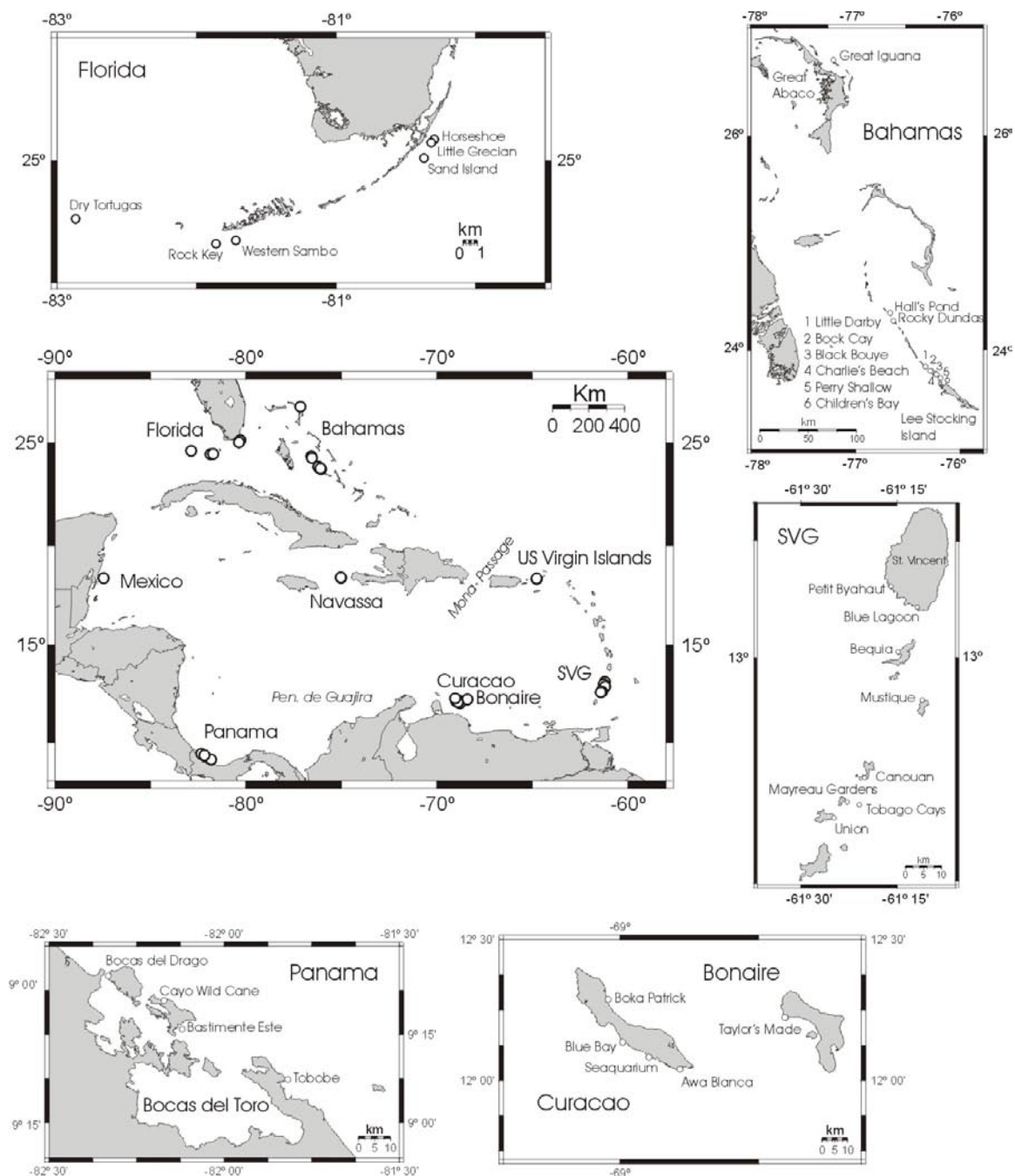


Fig 3.1 *Acropora palmata* stands sampled in the Caribbean. Maps created with OMC (<http://www.aquarius.geomar.de/omc>).

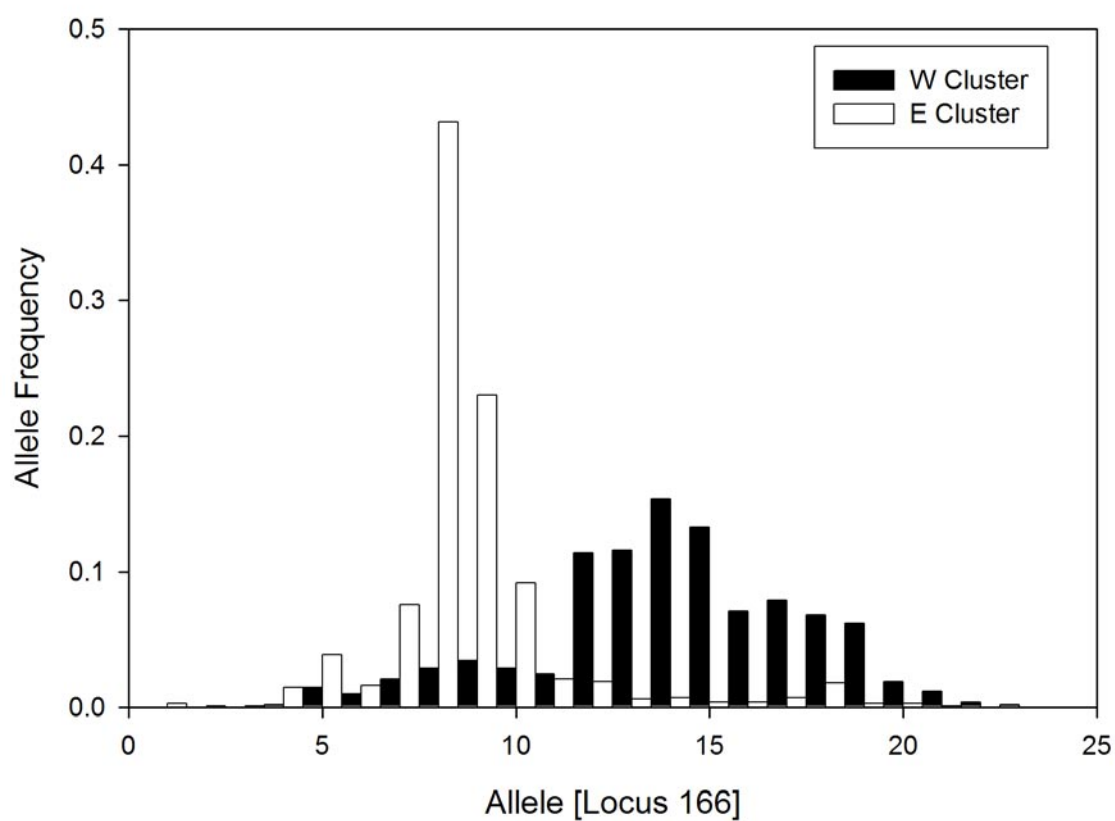


Fig 3.2 *Acropora palmata* allele frequency distributions of locus 166 in the W cluster (black bars) and the E cluster (white bars).

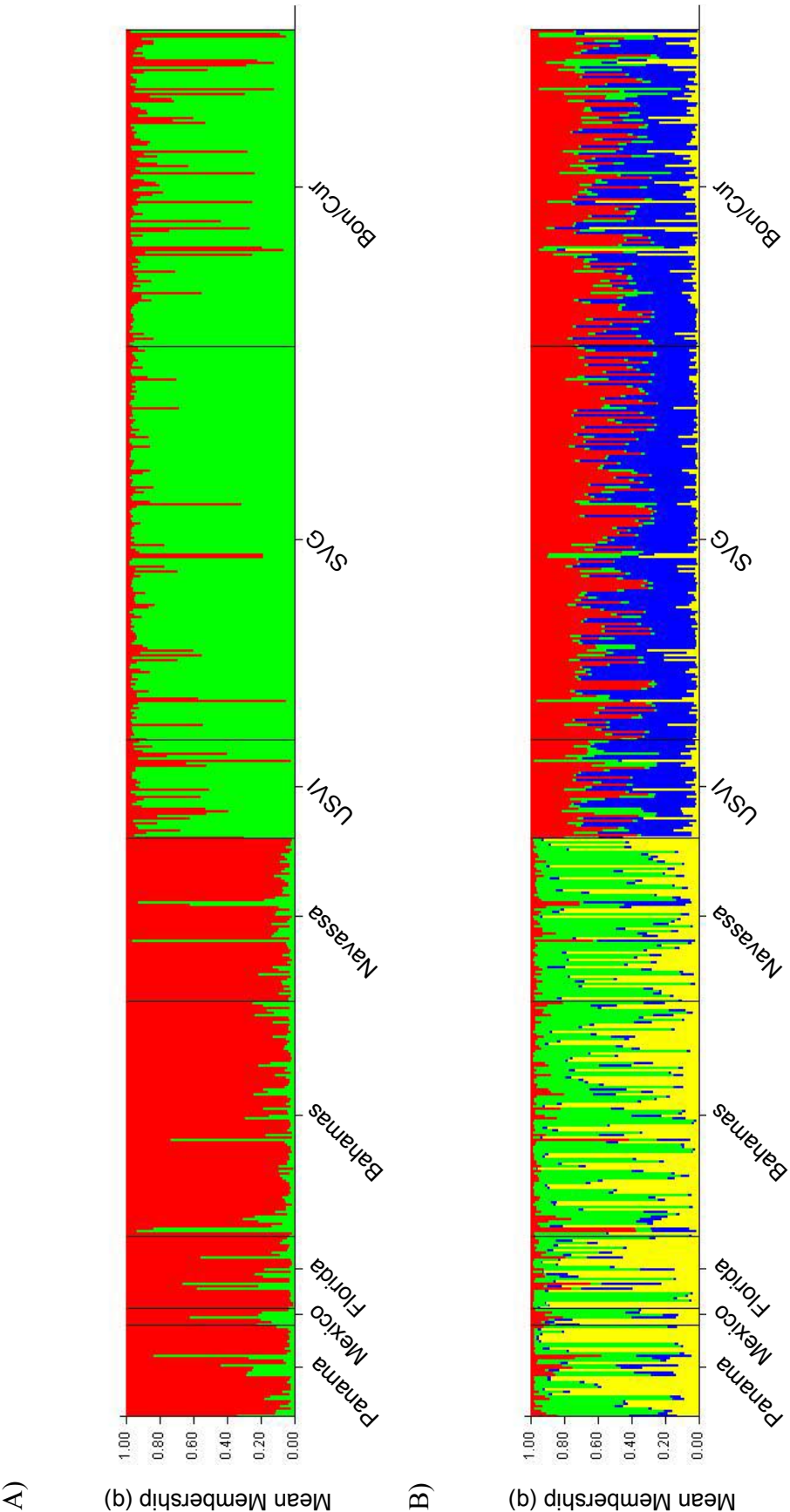


Fig 3.3 Estimates of individual membership q_i to **A**) each of two clusters **B**) each of four clusters plotted by locality. Bon/Curacao = Bonaire and Curacao. The mean membership of each individual to a given cluster ranges from 0 (very unlikely) to 1 (very likely). Same color indicates membership in the same cluster. However color codes are not the same in the two panels. Sampling localities are bracketed with vertical black lines. As estimated by STRUSTRUCTURE (Pritchard et al. 2000).

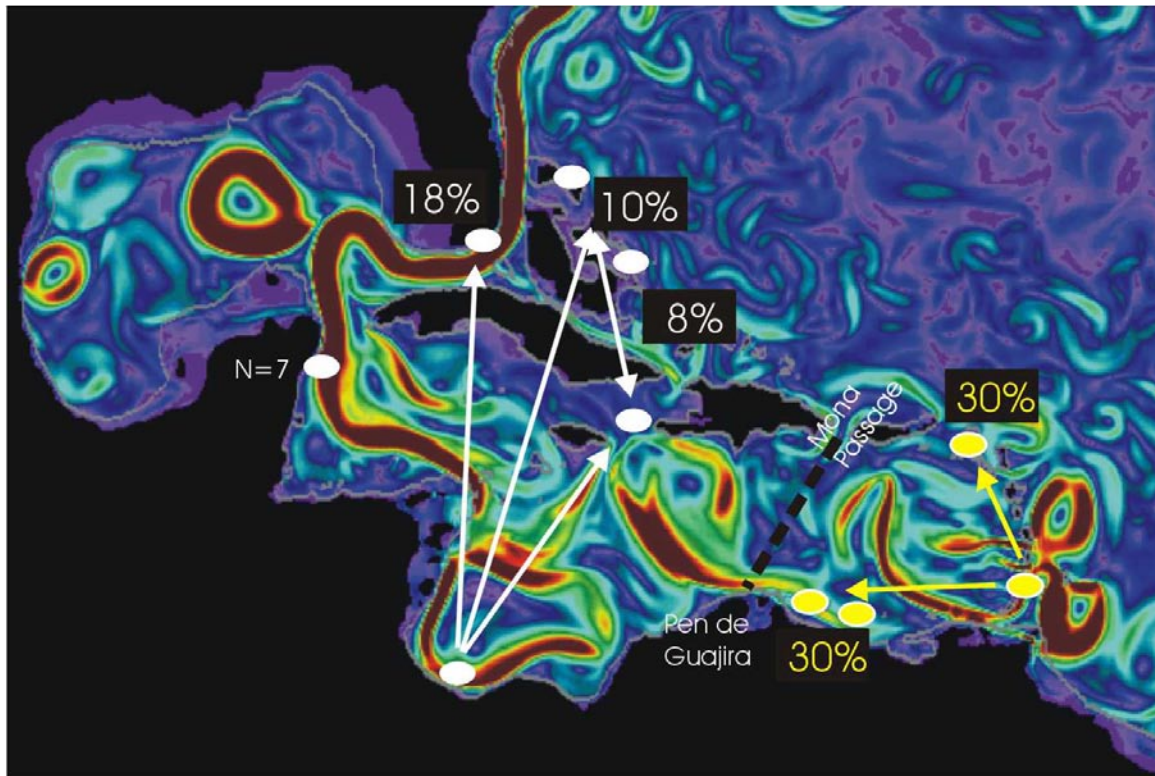


Fig 3.4 Immigration patterns of *Acropora palmata* in the Caribbean. The underlying map shows the average daily surface velocity in the Caribbean on Aug 1 (shown is the 10-year mean kinetic energy). Dark red indicates highest energy, blue lowest. August is the month in which *Acropora palmata* spawns most often. Map courtesy of Chassignet and Garaffo (MICOM highresolution model. <http://panomramix.rsmas.miami.edu>). The black dashed line indicates the most likely location of the phylogeographic break between the East and the West based on Goby studies (see text). Sampling localities are indicated by ovals. Arrows point towards the localities into which larvae are migrating. The percentage of immigration along those dispersal routes is shown, considering only values above 10% from Table 3.7B. 

CHAPTER 4: CLONAL STRUCTURE VARIES GEOGRAPHICALLY IN THE CARIBBEAN ELKHORN CORAL, *ACROPORA PALMATA*

ABSTRACT

A hierarchical survey of *Acropora palmata* clonal population structure was undertaken at 25 reefs in 8 localities in the Caribbean and western North Atlantic. Clonal structure, assayed using highly polymorphic microsatellite markers, was unpredictable at scales of meters to kilometers. Populations sampled 30-70 m apart on the same reef sometimes varied from being pre dominantly asexual to sexual, emphasizing the influence of micro-scale processes on clonal structure. Most populations showed intermediate patterns of genotypic diversity. Similar variation was evident between reefs within localities. In Florida i.e., only one genet each was found on two reefs, while a reef just 15 km away showed considerable genotypic variation. Clonal structure became predictable on a Caribbean-wide scale. A genetic break divides the 8 localities into a western and an eastern subregion. Highly asexual, genotypically depauperate populations with low colony densities characterized the western subregion, while sexual processes led to genotypically diverse, dense stands in the eastern subregion populations. Clonal evenness, spread of clones, and colony size were similar in both subregions. The contribution of sexual reproduction to population structure and genotypic diversity were negatively related to colony density indicating that within subregions, denser populations rely more on asexual reproduction and have lower diversity than less dense populations. The input of sexual recruits might be the main difference between these subregions causing the observed large-scale patterns. Additionally, local rates of fragmentation, geographical location, exposure and other factors may determine the degree of asexuality within subregions, localities and reefs.

INTRODUCTION

Recent declines of coral reefs (Gardner et al. 2003; Hughes et al. 2003; Pandolfi et al. 2003) have intensified interest in the ability of reef-building corals to adapt to changing environments (e.g. the adaptive bleaching hypothesis, Baker 2001; Douglas 2003). Sexual reproduction generates diversity among genotypes and this variation is sorted non-randomly according to selection pressures thus allowing adaptation to changing conditions. However, in corals, asexual reproduction through fragmentation can be the dominant mode of proliferation. This is especially true for fragile, branching coral species,

including members of the families Acroporidae, Agariciidae, Pocilloporidae and Poritidae (Tunncliffe 1981; Highsmith 1982; Jokiel et al. 1983; Willis 1985; Hunter 1993; Ayre et al. 1997; Adjeroud and Tsuchiya 1999; Lirman 2000). Asexuality *per se* has no effect on allelic or genotypic frequencies in populations, but it limits segregation and recombination and might slow adaptation to changing environmental conditions.

The consequences of asexual reproduction on genotypic diversity depend largely on how frequently sexual recruits replenish genetic diversity and how long genets live. Both empirical (Ayre 1985; Hunter 1993) and theoretical studies (Sebens and Thorne 1985) have suggested that genotypic diversity at a local scale might decrease over time through elimination of genets by intraspecific competition or stochastic effects. In contrast, genotypic diversity might remain high if sexual recruits, however rare, have a long life span after successful establishment (Burnett et al. 1995; McFadden 1997). Regardless of the consequences on genotypic diversity, asexual proliferation of clones might enhance persistence when colony densities become so low that dilution of gametes is too great for fertilization to occur (Allee effect, Knowlton 1992; Kojis and Quinn 1994; Levitan 1995; Levitan and Petersen 1995).

Physical disturbance is the most commonly invoked mechanism responsible for fragmentation leading to clonal proliferation (Hunter 1993; McFadden 1997; Coffroth and Lasker 1998). Once fragments are produced, their survival and successful establishment in the population depends on how suitable the environment is for retention and survival of fragments. Over time, different disturbance regimes might lead to characteristic patterns of population density, genotypic diversity, size structure, and clonal spread of genets as was demonstrated for *Porites compressa* (Hunter 1993). For example, large genets were associated with the five densest populations in *Pavona cactus* (Ayre and Willis 1988). Coffroth and Lasker (1998), studying clonal population structure in the gorgonian *Plexaura kuna*, formalized their observations of disturbance effects in a conceptual model. In contrast to the results obtained for *Porites compressa* (Hunter 1993), their model suggests that intermediate disturbance should lead to the highest genotypic diversity.

The Caribbean elkhorn coral, *Acropora palmata* has both sexual and asexual modes of reproduction. *A. palmata* reproduces sexually once annually by releasing bundles of egg and sperm into the water (broadcast spawning, Szmant 1986). Eggs are fertilized in the water column, and larvae settle after about 1-2 weeks in the plankton (Szmant 1986). Asexual reproduction via wave-induced branch breakage (fragmentation) can be the dominant mode of spreading (Highsmith 1982). The high acroporid cover of Caribbean reefs prior to the 1980s resulted from the combined effects of fragmentation and high growth rates (Shinn 1966; Gilmore and Hall 1976; Tunncliffe 1981; Highsmith 1982). Acroporid populations

have since declined due to a combination of factors such as an acroporid-specific disease (White Band Disease, Ritchie and Smith 1998; Aronson and Precht 2001b), bleaching and hurricanes. Bak (1983) hypothesized that high asexual reproduction rates might have led to low genotypic diversity and, hence, high disease susceptibility in acroporids. Whatever the direct cause of the decline of *A. palmata*, it was severe enough to justify its listing as a candidate for the U.S. Endangered Species List (Diaz-Soltero 1999). A formal review of their status is ongoing (Bruckner 2002).

Clonal structure has not been studied in *Acropora palmata*, but some information is available for the Caribbean congener and sister species, *A. cervicornis*. Self-recognition analyses in *A. cervicornis* revealed that clones do not extend further than 20 m (Neigel and Avise 1983). A single clone may dominate areas of 10 m² and these clones are generally spatially discrete with tight boundaries. The genetic basis of tissue compatibility assays underlying these analyses has since been questioned by studies showing fusion of electrophoretically distinct colonies (Heyward and Stoddart 1985; Willis 1985) and so indicating that tissue compatibility assays are unreliable means of detecting clonal structure.

Genetic studies of clonal structure depend on the ability to distinguish genotypes that are identical as a result of clonal replication (ramets) from genotypes that are identical by chance. Previous workers used surveys of allozyme variation to investigate clonal structure (Stoddart 1984; Hunter 1993; McFadden 1997; Ayre and Hughes 2000). Because allozyme loci have a relatively small number of alleles, the resolution of this marker system is limited unless a large number of loci are assayed. Coffroth and Lasker (Coffroth et al. 1992; Coffroth and Lasker 1998) used a DNA fingerprinting technique that allowed for the unambiguous identification of genets. However, due to the presence of intracellular symbionts (zooxanthellae of the genus *Symbiodinium*), anonymous fingerprinting techniques are technically challenging. The recent development of highly polymorphic, coral-specific microsatellite markers (Maier et al. 2001; Miller and Howard in press; Severance et al. in press; Shearer in press; Shearer et al. in press) solves this problem. Here we employ microsatellite markers (Chapter 2) to describe fine- and broad-scale variation in clonal structure of *Acropora palmata*.

The spatial scale on which clonal structure varies in *Acropora palmata* was not known. We thus employed a hierarchical sampling design. A previous study (Chapter 3) had identified eastern and western phylogeographic subregions for *Acropora palmata* in the Caribbean, with little or no recent gene exchange between them. The split occurs along a north-south running boundary between the Mona Passage to the Guajira Peninsula,

Colombia. Clonal structure was assessed within reefs (30-70m), between reefs (10-100 km), within localities (1000 km) and between subregions in the Caribbean.

MATERIAL AND METHODS

Sampling

A total of 717 *Acropora palmata* Lamarck colonies was sampled and genotyped from 29 stands representing 25 reefs in 8 localities of the Caribbean and western North Atlantic (Fig 4.1, Table 4.1, 4.2). The eight localities are split into 2 phylogeographic subregions: populations in Florida, Bahamas, Navassa, Panama and Mexico belong to the western subregion; the U.S. Virgin Islands, St. Vincent and the Grenadines, Bonaire and Curacao belong to the eastern subregion. The term reef is used when referring to duplicate sets of samples (= stands) from the same site. Colonies were sampled using a random sampling procedure (described in Chapter 2) to estimate the contribution of asexual reproduction to population structure. We sampled on three spatial scales to detect both common and rare genets. Random numbers were generated for each of three nested circles with radii of 15 m, 10 m and 5 m. The random numbers generated were precise to 5° of arc and 50 cm along strike. At each reef, a stake was placed in the center of the patch. Coordinates were located using a compass and a measuring tape attached to the center stake. The colony underneath each coordinate was sampled until 8 samples per circle were obtained. If there was no colony at a particular random coordinate, that coordinate was crossed out and the next random number was sampled. No colony was sampled twice. By design, this approach results in higher sampling effort on the 5 m scale compared to the 10 and 15 m scales. All colonies within the 15 m radius circle were counted so that colony density (colonies m⁻²) at each site could be estimated. A colony (ramet) is defined as a continuous, upright entity of skeleton with a stalk that attaches it to the bottom. Where the size of the *Acropora palmata* patch allowed, two non-overlapping, duplicate sets of circles were sampled (noted by the subscripts a and b as part of the reef code in Table 4.1). Distances between center points of duplicate sets of circles ranged from 35 m-70 m.

This random approach was not always feasible, especially when colony density was low. In these cases, either all or a large proportion of the colonies present were sampled haphazardly (Table 4.2). When feasible, the number of colonies present at the site was estimated (Table 4.2). The sampling scale for haphazardly collected sites was estimated by calculating the maximum pair-wise distance between samples. In the following discussion, we define a population as all samples originating from one set of circles or from one

haphazardly sampled reef (see below). For randomly and haphazardly collected sites, maps were prepared identifying the location of each colony in relation to a center point (Fig 4.3). Colony size was measured as two diameters and the height to the nearest 10 cm. One 1 cm long tip was snipped off from each identified colony using a bolt cutter and placed in a labeled zip bag. Coral samples were transferred into 70% ethanol upon returning to shore and stored at -80°C until genotyping.

Genotyping

We refer to an assemblage of genetically identical colonies (clones) that are descendants of a single zygote as “genets” (Harper 1977; Hughes 1989; Carvalho 1994). Physiologically distinct colonies that can function and survive on their own but belong to the same genet are termed “ramets” (Kays and Harper 1974) or clonemates.

Most reef corals harbor intracellular algal symbionts, complicating coral-specific marker development (Shearer et al. in press). The genetic markers employed must also be sufficiently polymorphic to identify individual genets. Five newly developed microsatellite loci for *Acropora palmata* (Chapter 2) are sufficiently polymorphic (mean observed heterozygosity = 0.88) to distinguish genets. The unbiased probability of identity (PI) of two non-clonemates is (ca. 10^{-7}). PI is an estimator of the probability that two individuals drawn at random from a population share the same genotype at all assayed loci and is calculated based on allele frequencies (Waits et al. 2001). Microsatellites were shown to be coral-specific by controlled crosses (Chapter 2).

Tissue samples were extracted and genotyped as described in Chapter 2. Briefly, two multiplex Polymerase Chain Reactions (PCR) were performed per sample using fluorescently labeled primers to assay five microsatellite loci containing AAT repeats. PCR products were visualized with an automated sequencer (ABI 3730). An internal size standard (Gene Scan 500-Liz, Applied Biosystems CA) ensured accurate sizing. Electropherograms were analyzed with GeneMapper Software 3.0 (Applied Biosystems, Ca). Alleles were scored based on amplicon size. Samples with identical alleles at all five loci were regarded as ramets belonging to the same genet.

Statistics

Rarefaction Analysis

The number of genets detected will invariably increase with sample size and sampling effort (i.e. proportion of the population sampled) unless one genet completely dominates a population. To standardize datasets from different populations, rarefaction curves

were calculated (Gotelli and Colwell 2001). By repeatedly re-sampling the pool of N colonies, the average number of genets represented by 1 to N individuals was obtained and plotted. Biases are likely to differ between randomly and haphazardly sampled reefs (see “Sampling” above) and only samples collected using similar protocols could be compared in this manner (Gotelli and Colwell 2001). Sample sizes were relatively even for randomly sampled populations ($N = 20-24$), however sampling effort was not (the proportion of the population sampled ranged from 0.001 to 0.4 in random samples, Table 4.1). The number of individuals sampled and the area searched differed substantially for haphazardly sampled reefs. Rarefaction analysis was carried out using Analytic Rarefaction (vers 1.3, <http://www.uga.edu/strata/software/Software.html>), which was based on the formulation of Tipper (1979).

Clonal Structure Analysis

Clonal population structure was expressed as clonal richness, genotypic richness, or genotypic evenness using the indices N_g/N , G_o/G_e , G_o/N_g (see below). These are the most commonly used matrices in the benthic marine invertebrate literature (e.g. Stoddart and Taylor 1988; Ayre et al. 1997; Coffroth and Lasker 1998; Ayre and Hughes 2000). Clonal richness is the ratio of the number of unique genotypes (= genets, N_g) identified over the total number of colonies sampled (N), N_g/N (e.g. Coffroth and Lasker 1998). This also provides a measure of the contribution of sexual reproduction to the population.

Observed genotypic diversity, G_o , as defined by Stoddart and Taylor (1988) was calculated as

$$G_o = 1/[\sum_i^k g_i^2]$$

where g_i is the relative frequency of the i th of k genotypes. This is conceptually similar to species diversity in a habitat as calculated by the Shannon index (Magurran 1988). Unlike the case for species diversity, an expected level of genotypic diversity equal to the number of samples obtained can be posed for an outcrossing population. Here, the expected genotypic diversity equals the number of samples obtained. The multilocus genotypes produced by the five microsatellite markers in this study were unique to each genet, so that the genotypic diversity expected in solely sexually reproducing population (G_e) equals the number of colonies genotyped. Previous studies using less polymorphic allozyme markers had to estimate G_e (Stoddart and Taylor 1988). The ratio of G_o/G_e gives an indication of the richness of genotypic diversity in a population. G_o/G_e has a maximum of 1 in a solely sexual population and approaches 0 in a population dominated by a single genet.

The ratio of G_o/N_g gives a measure of clonal evenness. In a population dominated by one clone, the evenness approaches 0 whereas in a population where each genet is

represented by equal numbers of ramets, the ratio approaches one. A peculiarity of this statistic is that populations with only one genotype also have an evenness of 1 though it could be argued that evenness has no meaning in a population with a single genet. Based on the combination of clonal richness (G_o/G_e) and evenness (G_o/N_g), populations were classified into four groups (sexual, mostly sexual, asexual and mostly asexual).

For comparison with the plant literature (Ellstrand and Roose 1987), two additional measures of dominance and evenness are presented. The complement of the Simpson index corrected for finite samples, D (Pielou 1969) was calculated as

$$D = 1 - \{[\sum n_i(n_i - 1)]/[N(N - 1)]\},$$

where n_i is the number of colonies of genotype i and N is the total sample size. D is 0 in a population with only one genet and 1 in a population comprised of genets with only one member. The evenness measure of Fager (1972), was calculated as

$$E = (D_{\text{obs}} - D_{\text{min}})/(D_{\text{max}} - D_{\text{min}}),$$

where $D_{\text{min}} = [(N_g - 1)(2N - N_g)]/[N(N - 1)]$ and $D_{\text{max}} = [N(N_g - 1)]/[N_g(N - 1)]$. E is 0 in a population where all colonies represent different genets or where all colonies belong to the same genet (note that this measure of evenness does not have the same peculiarity as G_o/N_g). E is 1 when all genets of a population have the same number of ramets.

Ecological parameters

Colony density was expressed as the number of colonies counted within the sample area (see “Sampling”). Data on colony density was available for 18 populations (Table 4.1).

Size histograms based on three dimensional colony size (calculated from two orthogonal diameters and one height) measured at the time of collection were prepared for each stand. The distribution of size classes was skewed toward small colonies. Hence, size estimates were log transformed (see appendix) and then mean size of colonies was compared among groups. In addition, the coefficient of variation (CV) of log transformed size estimates for each reef was calculated. The CV provided estimates of the skewness of size frequency distributions in the different populations.

Spread of ramets was expressed as the average distance between clonemates (m). First, radial sampling coordinates were converted to XY distances. Then, pair wise comparisons of distance (in m) were made between all ramets of a genet and the average of these was taken. Note that this average can be based on very different numbers of N . The maximum extent of genets on a reef was measured as the maximum distance (in m) between any two ramets of a genet in that population.

Spatial Analysis

The above ecological parameters and genetic descriptors of clonal structure were calculated for each population (n=31). Populations were assigned to one of four groupings with differing clonal structures ranging from asexual to sexual (see Results). We then tested for associations between the degree of clonality of a population and colony density, size structure, and clonal spread using one-way ANOVA. Variances were homogenous for all comparisons (Levene's test, $p > 0.05$). Tukey's *post hoc* tests were used to compare groups. Population values of genetic and ecological parameters were averaged a) within the 8 sampling localities and b) within the two provinces (with 4 localities each) to assess the spatial scale at which clonal structure varies predictably. Regression analysis was performed to test for significant correlation between ecological parameters and clonal structure within each subregion. We tested for significant differences in clonal structure and ecological parameters between provinces using an independent t-test after homogeneity of variances was ascertained (Levene's test).

RESULTS

Rarefaction Analysis

A total of 717 *Acropora palmata* colonies were sampled from 25 reefs throughout the Caribbean. Among these were 373 unique genotypes. Most genets were represented by a single ramet (Fig 4.2). Fourteen of the 25 reefs were sampled using a random nested sampling design to facilitate inter-reef comparisons (Table 4.1), but population densities differed substantially (mean proportion of population genotyped [± 1 SD]: 0.18 ± 0.11 , Table 4.1) among these sites. At the remaining reefs (haphazardly sampled, $n = 11$), both the area searched and the proportion of colonies sampled varied and were larger than for higher density stands (average proportion of colonies genotyped for populations where data are available = 0.81 ± 0.15 , Table 4.2). The entire dataset was standardized by calculating individual rarefaction curves for each population (Gotelli and Colwell 2001). The number of genets present clearly differed between populations even when comparing them at the level of the smallest sample size ($N=17$ from Rocky Dundas for haphazardly sampled reefs and $N=15$ from Lulu Bay for randomly sampled reefs, Table 4.1 and 4.2, Fig 4.6). The ranking of populations in terms of the number of genets present remained the same for these reduced sample sizes as when comparing them at their full sample size (Fig 4.6). Thus, in the following, raw, non-standardized data is used to describe clonal structure

patterns to prevent the loss of information associated with rarefaction curves (Gotelli and Colwell 2001).

Variation in Population Structure within and among Reefs

Clonal Population Structure

Clonal diversity (N_g/N) ranged from 0 (only one clone present) on two Florida reefs to 1 (each genotype unique) on all three Navassa reefs. The Caribbean-wide average of N_g/N was 0.54 ± 0.27 . At the 14 reefs randomly sampled using the nested circle approach, the sexual contribution to the population averaged 0.52 ± 0.26 . The average contribution of sexual reproduction (0.56 ± 0.29) was virtually identical for haphazardly sampled reefs despite the potential bias towards detecting a larger number of genets when sampling a larger proportion of the population.

Genotypic diversity of populations is described by both their genotypic richness and evenness. Again, sampled *A. palmata* populations show a continuum between 0 and 1 for both measures (Table 4.1, 4.2).

Inspection of the relation between genotypic richness and evenness values distinguishes four groupings of population structures (Fig 4.4, Table 4.1, 4.2). Four populations (all three Navassa reefs and one population from Blue Bay, Curacao) were classified as sexual with a genotypic richness of 0.98 ± 0.05 (1 SD) indicating that asexual reproduction is nearly absent. This results in high genotypic evenness values (0.99 ± 0.02). The “mostly sexual” group was characterized by moderate genotypic richness (0.45 ± 0.14) and evenness (0.70 ± 0.12) values. While some asexual reproduction occurs, no one genet dominates these populations. This group consisted of 14 populations. “Mostly asexual” populations ($N = 14$) had low genotypic richness (0.13 ± 0.05) and evenness (0.39 ± 0.10). Here, one or a few clones dominated the population. In two populations, Horseshoe and Little Grecian reefs in Florida, only one genet was identified (see Fig 2.3 in Chapter 2.). Both stands are linear in shape and additional samples ($n = 4$ and 5 respectively, Fig 4.3B) collected from the edges of the stands ($27.5\text{m} \pm 11.8\text{m}$ and $58.2\text{m} \pm 8.4\text{m}$ average $\pm$ SD distance to center point, respectively) confirmed that each was monotypic. At Little Grecian reef, the maximum extent of the clone was 75.3 m , at Horseshoe reef the maximum distance was 69.7 m . The relationship between G_o/N_g and G_o/G_e is a power function ($f=x^{0.45}$, $r^2=0.92$, $p>0.0001$, excluding the asexual group, see Methods). The addition of just one genet to a mostly asexual population results in a relatively higher gain in evenness than the addition of one genet to a mostly sexual population.

Similar patterns of clonal richness and evenness were obtained when using Simpson's diversity index and Fager's evenness measure (Table 4.3). The increase in clonal diversity from the asexual to the mostly-asexual group (0 to 0.72) was much higher, however, than the increase of genotypic diversity between the two groups (0.05 to 0.13, Table 4.3). Genotypic and clonal evenness showed similar increases from asexual to sexual groups (Table 4.3).

In three cases, duplicate sets of circles sampled on the same reef within 50 – 75 m of one another resulted in such different estimates of clonal richness and evenness that duplicates were placed in different population structure groups (Table 4.1, 4.3, Fig 4.4). One duplicate set from Sea Aquarium, Curacao, was placed into the mostly asexual group, whereas the other was placed into the mostly sexual group. The same was true for the duplicate sets from Bastimentos, Panama. Duplicate sets from Blue Bay, Curacao, showed such different patterns of clonal structure as to warrant classification for one as mostly asexual whereas the other was sexual (Table 4.1, 4.3, Fig 4.4).

Despite these differences in population structure between duplicate sets collected from one reef, comparison of the number of genets identified when sampling on smaller spatial scales (5 m, 10 m, and 15 m radii) showed that most of the clonal richness of a population was captured by the smallest radius (Fig 4.5). Widening the radius from 5 m to 10 m and then again to 15 m resulted in small, non-significant gains in the number of genets identified.

Ecological Parameters

Colony density varied considerably among the sampled stands, from 0.09 to 0.79 colonies m⁻², however there was no relationship between colony density and the degree of clonality of a population (one-way ANOVA, $df = 3$, $F = 1.19$, $p = 0.34$, Fig 4.7A). Similarly, neither mean colony size (nor the variation in size structure, data not shown) was predictably distributed among the different groups (One-way ANOVA, $df = 3$, $F = 2.33$, $p = 0.096$, Fig 4.7B). Note that the sexual group does have less variation in size structure than the mostly-sexual group, probably due to the smaller n .

As is apparent from radial plots (Fig 4.3), clones tend to be clumped. Nevertheless, some ramet-rich clones were spread over a wide area. Populations dominated by one or a few clones (the mostly asexual group) showed wider average clonal spread than populations with fewer ramets per genet (the mostly sexual group) (Fig 4.7C, one-way ANOVA, $df = 3$, $F = 10.40$, $p < 0.001$). Average clonal spread for the four groups was 6.8 ± 2.2 m (asexual), 4.7 ± 1.6 m (mostly asexual), 3.2 ± 1.8 m (mostly sexual), and 2.8 ± 4.8 m (sexual, not zero due to one genet with two ramets in the Blue Bay II population). Maximum distance between ramets decreased with increasing sexuality (data not shown).

Variation in Clonal Structure within and among Localities and Provinces

Within the western subregion (populations in Florida, Bahamas, Navassa, Panama and Mexico), both purely sexual ($n=3$ in Navassa) and purely asexual ($n=2$ in Florida) populations were observed (Table 4.1, 4.2). Overall, sexual reproduction contributed $44 \pm 35\%$ to population structure (Table 4.4). The large variation of this estimate is due to the entirely sexual populations in Navassa, the only such locality that we sampled. When excluding Navassa, due to its outlier status, mean $N_g/N = 0.30 \pm 0.17$ (Table 4.4). The sexual contribution of population structure was greater and more homogeneous (mean $N_g/N = 0.65 \pm 0.12$) in the eastern (U.S. Virgin Islands, St. Vincent and the Grenadines, Bonaire and Curacao, Table 4.4) than western provinces. Excluding Navassa, the western populations have a lower contribution of sexual reproduction to clonal structure ($N_g/N = 0.30 \pm 0.17$ and 0.65 ± 0.12 , $p < 0.001$) and lower genotypic diversity ($G_o/G_e = 0.20 \pm 0.16$ and 0.44 ± 0.16 , $p < 0.001$) than populations in the eastern Caribbean (Table 4.4). This pattern holds when using the complement of Simpson's diversity index instead of G_o/G_e ($p < 0.05$, Table 4.4) *Acropora palmata* populations in the western Caribbean are also less dense within the sampled localities (0.13 ± 0.08 and 0.30 ± 0.21 colonies m^{-2} , $p < 0.05$, including Navassa) than in the eastern Caribbean (Table 4.4). Genotypic evenness, spread of clones and colony size were similar in both provinces (Table 4.4).

Within the provinces, the contribution of sexual reproduction to population structure (N_g/N , East: $r^2 = 0.93$, West: $r^2 = 0.89$, $p < 0.001$, Fig 4.8A) and genotypic diversity (G_o/G_e , East: $r^2 = 0.59$, West: $r^2 = 0.50$, $p < 0.05$, Fig 4.8B) were negatively related with colony density, indicating that within provinces, denser populations rely more on asexual reproduction and have lower diversity than less dense populations. Clonal spread and mean colony size did not vary, however, with the degree of sexuality (N_g/N) or the genotypic diversity (G_o/G_e) of populations within provinces (Table 4.4).

DISCUSSION

The combination of highly polymorphic microsatellite markers that ensured the unambiguous distinction of genets, with a hierarchical sampling design on spatial scales from within reef to Caribbean-wide, revealed that clonal structure in *Acropora palmata* becomes predictable at large scales that coincide with previously identified, phylogeographic provinces in the Caribbean. Highly asexual, genotypically depauperate populations with low colony densities characterized the western subregion, while sexual processes led to genotypically diverse, dense stands in the eastern subregion. The input of sexual recruits might be the main difference between these provinces causing the observed patterns.

Additionally, local rates of fragmentation, geographical location, exposure and other factors may determine the degree of asexuality within provinces, localities and reefs.

Patterns of Clonal Structure in Acropora palmata

Clonal population structure of *Acropora palmata* ranges from entirely asexual to entirely sexual over spatial scales from 10s of meters (within reef variation) to 10s of kilometers (variation among adjacent reefs). Asexual reproduction can be the sole apparent mode of reproduction. On two reefs in Florida, only one genet was found with maximum linear extensions of 69.7 and 75.3 m, respectively. Another 12 populations were not monotypic, but were still dominated by one or a few ramet-rich genets (Table 4.1, 4.2, Fig 4.4). Ramets tended to be spatially clumped, as is apparent from the physical maps (Fig 4.3). However, asexual reproduction is not important everywhere. Populations in Navassa showed no evidence of clonal replication (Table 4.1, 4.2, Fig 4.3A). An additional 13 populations consisted mostly of singleton genets, with some genets having 2 or 3 members. Across the Caribbean, sexual contribution of population structure was around 54% ($\pm 27\%$) and genotypic diversity as measured by G_o/G_e was 0.38 (± 0.30 , Table 4.1, 4.2). Simpson's index (1-D) gave larger average values of genotypic diversity (0.79 ± 0.25 , Table 1, 2) which is higher compared to the mean value of 1-D (0.62) for clonal plants (Ellstrand and Roose 1987).

The scale at which the contribution of sexual reproduction to the population varies can be quite small. High patchiness within a reef was observed in three instances: Bastimentos, Blue Bay, Sea Aquarium (Table 4.1, Fig 4.3C, D). At these reefs, high clonal replication occurred only in one of the two sets of circles sampled at 30-70 m distances. At the scale of reefs, similar variation was observed. Horseshoe and Little Grecian Reefs are only separated from Sand Island by 15 km, and have similar depth and exposure but contrasting clonal structure. Johnson's Reef and Hawksnest Bay Reef in the USVI, separated by just 2 km, also had markedly different degrees of asexual reproduction (Table 4.1). On the within and between reef scale, clonal structure patterns are unpredictable.

Storm and wave impact alone are unlikely to vary on a scale of meters. Abiotic disturbance is clearly required for the generation of fragments; a necessary but not sufficient condition as discussed by Coffroth and Lasker (1998) for *Plexaura kuna*. Large colonies must be present in the population to provide the source of fragments, and, depending on the severity of the disturbance, a structure to retain the fragments within that population. Hence, the density of the *A. palmata* stands, other corals, and/or bottom topography may determine the relative contribution of asexual recruits to a given population. For example, the entirely sexual populations at Navassa grow on a narrow shelf that offers poor retention

potential for *Acropora* fragments, which are likely generated by the strong currents and high wave assault characteristic of this island.

Patterns emerge when considering estimates of the sexual contribution and genotypic diversity on the scale of the two provinces. *Acropora palmata* populations in the western Caribbean appear to rely more heavily on asexual reproduction (around 65%), which led to lower genotypic diversity than in the east (Table 4.4). The notable exception is Navassa with solely sexual populations and associated high genotypic diversities. Despite the prevalence of asexual reproduction in the western Caribbean, colony density within surveyed areas was actually lower than in the eastern Caribbean (Table 4.4). This is surprising since *A. palmata* is thought to be a poor sexual recruiter (Dustan 1977; Bak and Engel 1979; Hughes and Jackson 1980; Rylaarsdam 1983; Rosesmyth 1984; Hughes and Jackson 1985) and thus achieving higher population densities in the west with less asexual reproduction is unexpected.

Hurricanes have large impacts on some reefs in both localities, while other localities like Curacao, Bonaire and Panama rarely lie in the path of destruction (Goldenberg et al. 2001). If hurricane frequency were the main cause underlying observed population structure, one would expect localities with low hurricane frequency like Panama ($N_g/N = 0.45 \pm 0.00$), Curacao, ($N_g/N = 0.74 \pm 0.11$) and Bonaire ($N_g/N = 0.67 \pm 0.15$) populations to be similar with respect to the amount of asexual reproduction, which is not the case. Thus, differences in the rate of asexual reproduction are not a simple function of hurricane occurrence. In addition, hurricanes do not always lead to increased population sizes through fragment generation. Lirman's (2003) stage model of *Acropora palmata* predicted that when storm frequency increases over a certain threshold (once every two years), fragmentation may come at considerable cost to the colonies, leading to overall population decline especially when sexual input is limited. Apart from having a negative effect on growth rates and survivorship potential, sexual reproduction is depressed in both the surviving fragments and the source colony (Lirman 2000).

The prevalence of sexual reproduction in the eastern Caribbean might be attributable to higher rates of larval exchange between reefs in that subregion (Chapter 3). *Acropora palmata* stands from St. Vincent and the Grenadines were found to be a source for larvae for both the USVI and the Netherlands. In the west, most immigrants have their ancestry in Panama; however, overall immigration rates are lower in the west than the east. So, superimposed on the small-scale, complex and unpredictable fragmentation processes discussed above, are those that determine the relative influx of sexual recruits in a given population and may be responsible for the large scale patterns seen in the Caribbean.

Clonal structure patterns have been analyzed on a species-wide scale in a number of Scleractinia. Analyses of protein (allozyme) markers show a wide variety of asexual reproduction patterns within the Acroporidae. Of four broadcast-spawning Pacific acroporids studied on the Great Barrier Reef, two, *Acropora valida* and *A. millepora*, were mainly asexual while *Acropora hyacinthus* and *A. cytherea*, were mainly sexual (Ayre 2000). Two acroporid brooders, however, (*A. cuneata* and *A. palifera*) had high genotypic diversities (Benzie et al. 1995, Ayre and Hughes 2000). Other scleractinian genera are equally diverse in their clonal structures. *Pavona cactus* on the Great Barrier Reef (Ayre and Willis 1988) and *Pocillopora damicornis* in Japan (Adjerdoud and Tsuchiya 1999) are highly clonal. In contrast, *P. damicornis* populations in Australia seem to be mainly sexually derived and randomly mating (Ayre et al. 1997, Benzie et al. 1995). Contrasting reproductive patterns over large geographical scales within the same species are not exceptional in the Scleractinia (reviewed in Harrison and Wallace 1990).

Studies of variation in clonal population structure over several spatial scales within a species were conducted for *Acropora valida* and *A. millepora* along the Great Barrier Reef (Ayre and Hughes 2000). Similar variation for values of N_g/N and G_o/G_e within a reef (100s of m) was observed than for *A. palmata* in the Caribbean (this study). Again, the amount of sexual larval input might be the structuring force. Connell et al. (1997) showed that over 30 years recruitment rates varied >7.5 fold among sites within one of Ayre and Hughes's study reefs. In addition, a regional pattern in clonal structure emerged for *A. valida* that was attributed to gradients in larval recruitment along the GBR (Hughes et al. 2000). Interestingly, variation in acroporid larval recruitment along the GBR was largely determined by differences in fecundity of adults, not their abundance.

Small-scale patterns of clonal variation within species have usually been attributed to disturbance. Highly asexual populations of *Porites compressa* in Hawaii had experienced recent, severe disturbance while populations dominated by sexual reproduction had been undisturbed for longer periods (Hunter 1993). A similar correlation between clonal structure and disturbance was found for the gorgonian *Plexaura kuna* (Coffroth and Lasker 1998). However, in this case intermediate disturbance caused the highest clonal diversity. In contrast, habitat preference was found to determine the location and spread of several clones of *Pavona cactus* in Australia (Ayre and Willis 1988).

Colony density, average colony size and variation in colony size were poor predictors of clonal structure for *Acropora palmata* on the within and between reef scale (Fig 4.7). However, when comparing populations among localities within provinces, higher colony densities were associated with a higher degree of asexual reproduction and, consequently,

lower genotypic diversity (Fig 4.8). Similar observations were made in *Porites compressa* and *Pavona cactus* (Ayre and Willis 1988; Hunter 1993).

Consequences of Variation in Clonal Structure

The effective population size for sexually reproducing species may be less than the total census count of organisms if all individuals do not successfully breed, generations are overlapping, the sex ratio deviates from one-to-one, and population sizes fluctuate (reviewed in Ellstrand and Elam 1993). For clonal species, the number of breeding genets (not ramets) will determine the upper limit of effective population size. The current study has determined that approximately half of the individual colonies sampled represent distinct genets ($N_g/N = 0.54 \pm 0.27$ on average across the Caribbean). However, the relative contribution of these genets to successful sexual reproduction is still unknown. Broadcast spawning corals like *Acropora palmata* are expected to have a small effective population size (i.e. few genets contributing successfully to sexual reproduction): both fertilization success of spawned gametes (pers observ) and the recruitment of larvae (Dustan 1977; Bak and Engel 1979; Hughes and Jackson 1980; Rylaarsdam 1983; Rosesmyth 1984; Hughes and Jackson 1985) are highly stochastic. Also, large clones might contribute disproportionately (assuming that sufficient gametes are available nearby for outcrossing). Once local genet diversity becomes low, the distance between different genets might limit fertilization success (Allee effect, Knowlton 1992; Kojis and Quinn 1994; Levitan 1995; Levitan and Petersen 1995) even further. Populations with small effective population sizes are far more prone to extinction due to demographic stochasticity, reduction in gene diversity, or accumulation of deleterious mutations (Ellstrand and Elam 1993; Grosberg and Cunningham 2000). However, asexual proliferation of clones ensures persistence at two Florida reefs with only one genet each. In these cases, dilution of reproductive cells traveling between reefs might be too great for fertilization to occur.

An important factor that led to the decline of *Acropora palmata* populations in the Caribbean was the outbreak of the acroporid-specific White Band Disease. Different genets may show differential susceptibility for the disease. Genets have been shown to be differentially susceptible to bleaching (Edmunds et al. 2003). Populations with low genet diversity, such as stands in the western Caribbean, are thus likely to be at higher risk of critical decline than populations with high diversity.

The depressed status of acroporid populations in the Caribbean has led to their listing as candidates for the endangered species list. Conservation measures such as marine protected areas might be useful means to persevere this species. Only genotypically diverse stands can serve as effective larval sources for surrounding populations and harbor potentially

resistant genotypes against future disease outbreaks. Because no easily measurable population characteristic correlates with clonal diversity, molecular surveys of *Acropora* stands will need to be included as part of any protection measures.

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Table 4.1 *Acropora palmata* randomly sampled for clonal structure analysis. At some reef duplicate sets of circles were collected (indicated by the 'a' or 'b' ending of the reef code). Total area sampled was always 707 m². Num Col: number of colonies within each set of circles. Number of colonies (N) and proportion of colonies (Prop sampled) of those present within each set that were sampled. Col Dens = colony density m⁻². Genet dens = Genet density m⁻². N_g: number of genets, G_o: observed genotypic diversity, G_e: expected genotypic diversity, G_o/G_e: assesses the relative importance of sexual reproduction in a population. G_o/N_g: genotypic evenness (number of ramets per genet), 1-D: complement of Simpson's diversity index, E: Fager's Evenness. Populations were classified into groups based on their combination of N_g/N and G_o/G_e as asexual (1), mostly asexual (2), mostly sexual (3), and sexual (4) (Fig 4.4).

Locality	Reef Code	Reef Name	Num Col	Col Dens	Genet Dens	Prop Sampled	N	N _g	N _g /N	G _o	G _o /G _e	G _o /N _g	1-D	E	Group
Bahamas	Ba1	Great Iguana	61	0.09	0.010	0.4	23	7	0.30	3.33	0.14	0.48	0.73	0.62	2
	Ba5	Bock Cay	105	0.15	0.014	0.2	23	10	0.43	6.37	0.28	0.64	0.90	0.76	3
	Ba7	Middle Beach	62	0.09	0.014	0.4	23	10	0.43	4.85	0.21	0.49	0.83	0.63	2
Bonaire	Bo1a	Taylors Made I	92	0.13	0.020	0.2	21	14	0.67	10.26	0.49	0.73	0.95	0.75	3
	Bo1b	Taylors Made II	52	0.07	0.025	0.4	22	18	0.82	13.44	0.64	0.75	0.97	0.4	3
	Cu1a	Blue Bay I	131	0.19	0.018	0.2	21	13	0.62	4.74	0.23	0.36	0.83	-0	2
Curacao	Cu1b	Blue Bay II	78	0.11	0.027	0.3	20	19	0.95	18.18	0.91	0.96	0.99	0	4
	Cu2a	Sea Aquarium I	560	0.79	0.018	0.0	22	13	0.59	8.07	0.37	0.62	0.92	0.79	3
	Cu2b	Sea Aquarium II	370	0.52	0.017	0.1	24	12	0.50	4.06	0.17	0.34	0.79	0.42	2
	Cu3a	Awa Blanca I	160	0.23	0.023	0.1	21	16	0.76	11.92	0.57	0.74	0.96	0.6	3
	Cu3b	Awa Blanca II	200	0.28	0.017	0.1	22	12	0.55	8.64	0.39	0.72	0.93	0.83	3
Florida	Fl4	Horseshoe	175	0.25	0.001	0.1	21	1	0.05	1.00	0.05	1.00	0	0	1
	Fl5	Little Grecian	131	0.19	0.001	0.2	20	1	0.05	1.00	0.05	1.00	0	0	1
	Pa1	Bocas Del Drago	156	0.22	0.013	0.1	22	9	0.41	2.69	0.12	0.30	0.66	0.16	2
Panama	Pa3a	Bastimentos I	102	0.14	0.007	0.2	21	5	0.24	1.68	0.08	0.34	0.42	0.15	2
	Pa3b	Bastimentos II	107	0.15	0.018	0.2	20	13	0.65	10.00	0.50	0.77	0.95	0.80	3
	Vi1	Johnson's Reef	204	0.29	0.023	0.1	23	16	0.70	11.76	0.51	0.73	0.96	0.74	3
USVI	Vi2a	Hawksnest Bay I	264	0.37	0.011	0.1	22	8	0.36	3.32	0.15	0.41	0.73	0.5	2
	Vi2b	Hawksnest Bay II	212	0.30	0.013	0.1	24	9	0.38	3.06	0.13	0.34	0.70	0.38	2
Navassa	Na3	Lulu Bay	NA				15	15	1.00	15	1	1	1.00	1.00	4
Total		14 Reefs (20 Populations)	3222				430	221							
Average			169.6	0.24	0.02	0.18	21.5	11.1	0.52	7.17	0.35	0.64	0.79	0.57	
SD			123.1	0.17	0.01	0.11	2.0	5.0	0.26	5.03	0.28	0.24	0.25	0.31	

Table 4.2 *Acropora palmata* stands sampled haphazardly for clonal structure analysis. When available, the total number of colonies present is given. The maximum distance (max dist sampled in m) between samples (not genets!) collected indicates the sampling scale. The number (N) and proportion (when applicable, prop sampled) are given. The average distance between samples is given (average dist samples in m). See Table caption 1 for explanation of other categories. Colony densities were 0.012 and 0.005 colonies m⁻² for N Shelf and NW Point Reef, respectively.

Locality	Reef Code	Reef Name	Colonies present	Max Dist sampled	N	Prop sampled	N _g	N _g /N	G _o	G _o /G _e	G _o /N _g	1-D	E	Group
Bahamas	Ba6	Black Buoy	NA	14.4	22		11	0.50	6.91	0.31	0.63	0.96	0.84	3
	Ba2	Halls Pond	23	67.3	19	0.83	13	0.68	10.94	0.58	0.84	0.63	0.63	3
	Ba4	Little Darby	NA	20.3	25		10	0.40	3.79	0.15	0.38	0.77	0.49	2
	Ba3	Rocky Dundas Boka	NA	55.2	17		4	0.24	2.43	0.14	0.61	0.88	0.8	2
Curacao	Cu4	Patrick	NA	76.0	21		15	0.71	12.60	0.60	0.84	0.97	0.83	3
Florida	Fl3	Sand Island	75	58.3	53	0.71	12	0.23	2.99	0.06	0.25	0.68	0.54	3
Mexico	Me1	Chinchorro	NA		35		7	0.20	2.61	0.07	0.37	0.64	0.56	2
Navassa	Na1	N Shelf	19	44.1	18	0.95	18	1.00	18.00	1.00	1.00	1.00	1.00	4
	Na2	NW Point	37	96.2	35	0.95	35	1.00	35.00	1.00	1.00	1.00	1.00	4
Panama	Pa2	Wild Cayne	21	34.2	13	0.62	6	0.46	3.60	0.28	0.60	0.78	0.54	4
SVG	Sv4	Canouan	NA	323.1	29		21	0.72	15.87	0.55	0.76	0.97	0.79	3
Total	11 Reefs				287		152							
Average				78.9	26.1		13.82	0.56	10.43	0.43	0.66	0.84	0.73	
SD				89.4	11.4		8.66	0.29	9.90	0.34	0.25	0.14	0.18	

Table 4.3 Grouping of randomly sampled *Acropora palmata* patches based on their population structure. Given are means (1SD). See caption of Table 4.1 for abbreviations.

Group	Reefs	Col dens		N _g /N		G _o /G _e		G _o /N _g		1-D		E	
		N	Aver	SD	Aver	SD	Aver	SD	Aver	SD	Aver	SD	
Asexual	2	0.22	0.04	0.05	0.00	0.05	0.00	1.00	0.00	0.00	0.00	0.00	0.00
Mostly Asexual	11	0.25	0.16	0.34	0.10	0.13	0.05	0.39	0.10	0.72	0.11	0.46	0.21
Mostly Sexual	14	0.25	0.21	0.63	0.11	0.45	0.14	0.70	0.12	0.93	0.05	0.73	0.13
Sexual	4	0.04	0.06	0.99	0.02	0.98	0.05	0.99	0.02	1.00	0.00	1.00	0.00

Table 4.4 Clonal structure of *Acropora palmata* based on locality and subregion. Given are means (1 SD). See caption of Table 4.1 for abbreviations. Ave (without Nav): average of values for the western subregion excluding Navassa. See text for explanation. T-test significance: * p<0.05, *** p<0.001, ** p<0.01, *p<0.05, #p=0.058, ^p<0.05 (comparing the west including Navassa to the east).

Subregion	Locality	Reefs	Col Dens		N _g /N		G _o /G _e		G _o /N _g		1-D		E	
			Aver	SD	Aver	SD	Aver	SD	Aver	SD	Aver	SD	Aver	SD
West	Bahamas	7	0.11	0.04	0.43	0.14	0.26	0.15	0.58	0.15	0.81	0.11	0.68	0.12
	Florida	3	0.22	0.04	0.11	0.10	0.05	0.00	0.75	0.43	0.26	0.26	0.14	0.14
	Mexico	1			0.20		0.07		0.37		0.68	0.68	0.54	0.54
	Panama	3	0.15	0.01	0.45	0.00	0.29	0.00	0.57	0.00	0.80	1.00	0.50	1.00
	Navassa	3	0.01	0.04	1.00	0.17	1	0.19	1	0.22	1	0.76	1.00	0.51
East	Bonaire	1	0.10	0.04	0.74	0.11	0.56	0.11	0.74	0.01	0.96	0.02	0.57	0.25
	Curacao	4	0.35	0.26	0.67	0.15	0.46	0.25	0.66	0.23	0.90	0.10	0.49	0.37
	SVG	1			0.72		0.55		0.76		0.97		0.79	
	USVI	2	0.32	0.05	0.48	0.19	0.26	0.22	0.50	0.21	0.70	0.27	0.46	0.30
West	Ave		0.12 [^]	0.09	0.44	0.35	0.33	0.39	0.65	0.23	0.71	0.64	0.57	0.47
West	Ave (without Nav)		0.16 [#]	0.06	0.30 ^{***}	0.17	0.17 ^{**}	0.12	0.57	0.15	0.64 [*]	0.26	0.47	0.23
East	Ave		0.26 ^{#^}	0.14	0.65 ^{***}	0.12	0.46 ^{**}	0.14	0.66	0.12	0.88 [*]	0.12	0.58	0.15

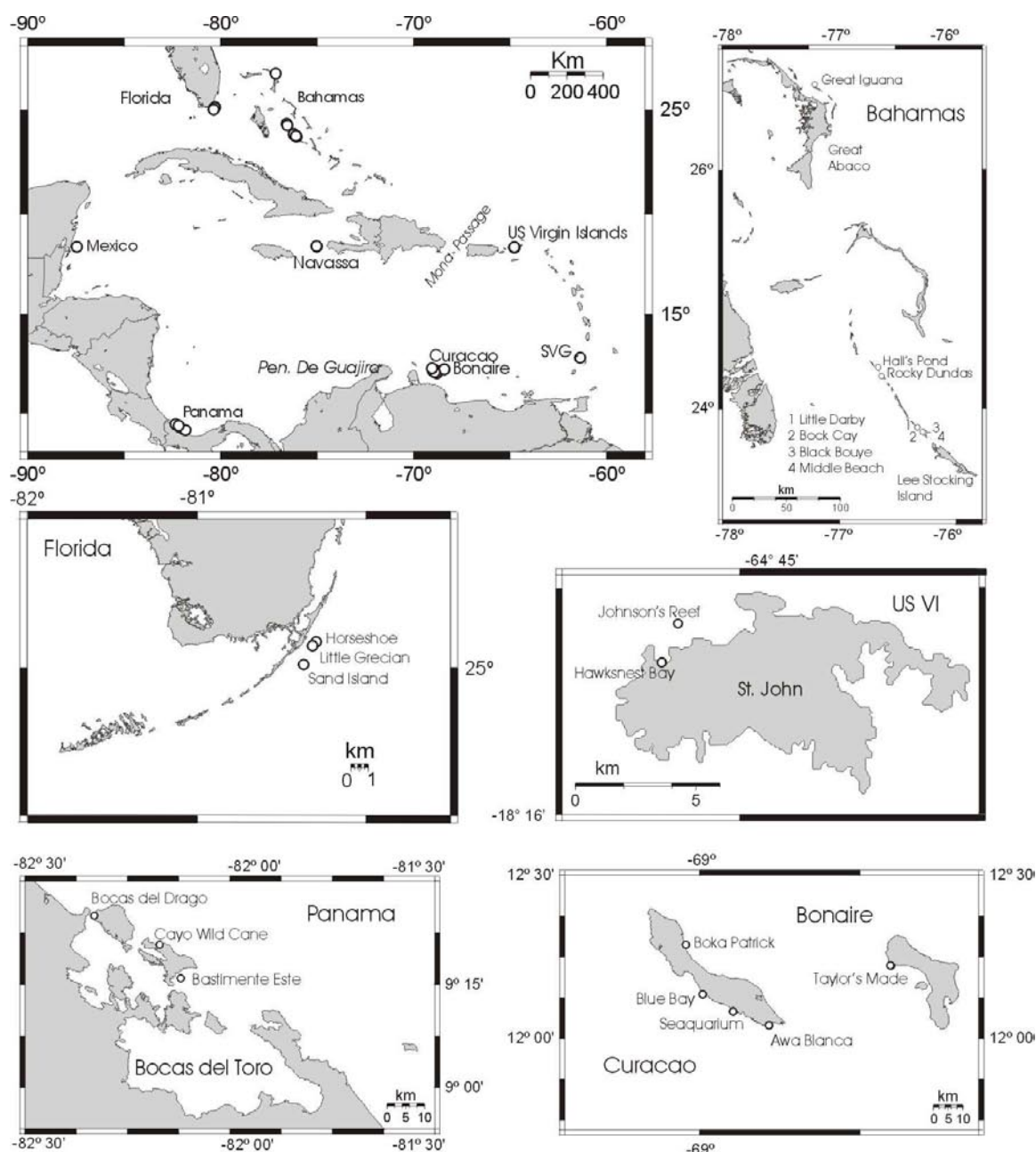


Fig 4.1 Sampling locations of *Acropora palmata* in the Caribbean and western north Atlantic. Details are given for some of the 8 localities to indicate inshore/offshore locations of sampled reefs. Navassa is a small island with narrow shelf and all 3 sampled sites were on reefs with equally exposed locations (details not shown). Information on clonal structure was obtained at only one reef in St. Vincent and the Grenadines (Canouan). This reef is located within a semi-protected bay (details not shown) at the southern end of the island chain. of sampled reefs. Navassa is a small island with narrow shelf and all 3 sampled sites were on reefs with equally

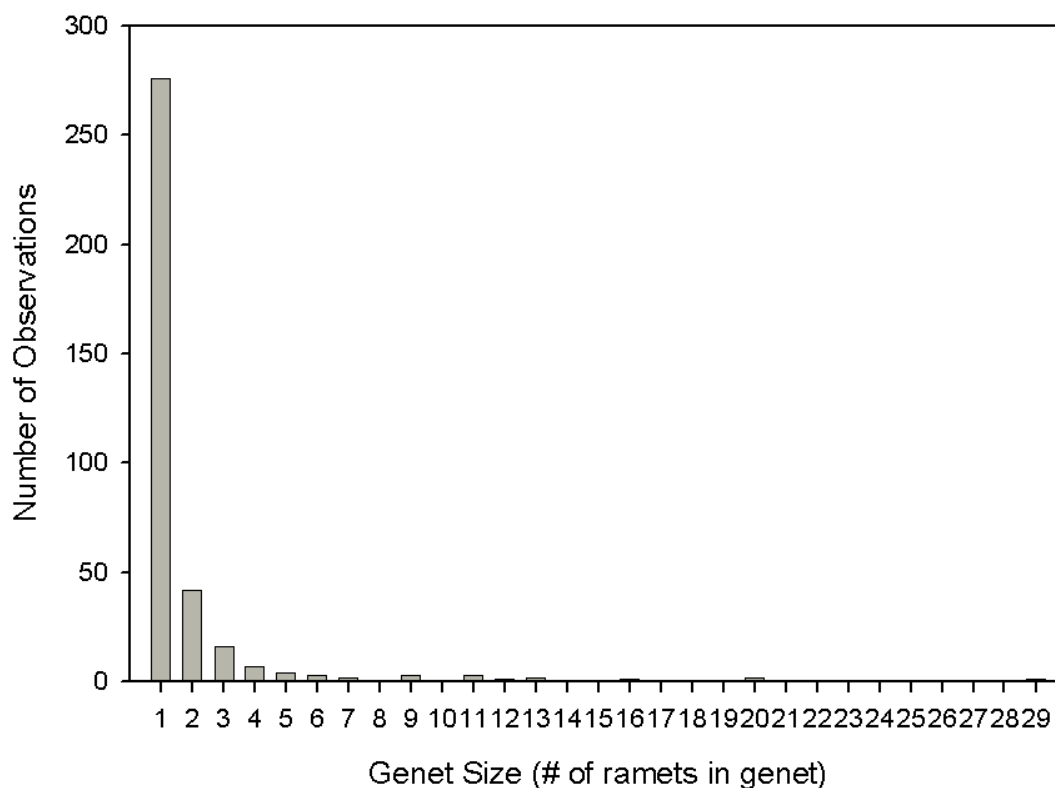
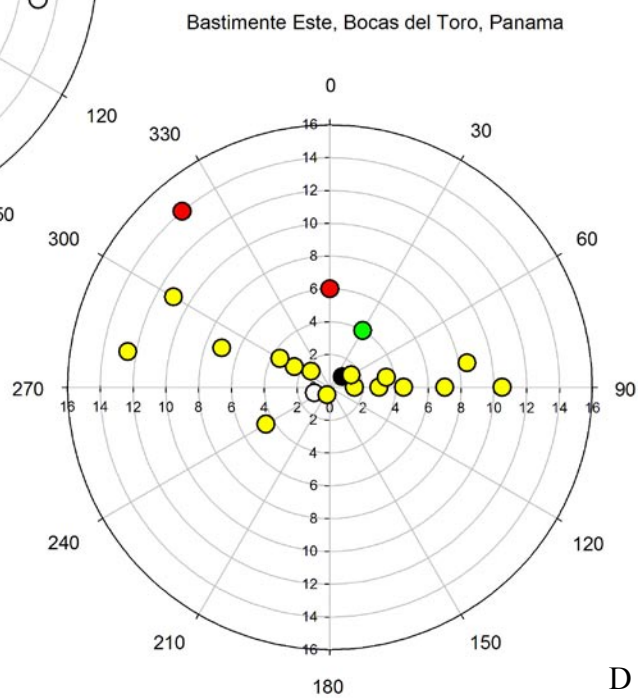
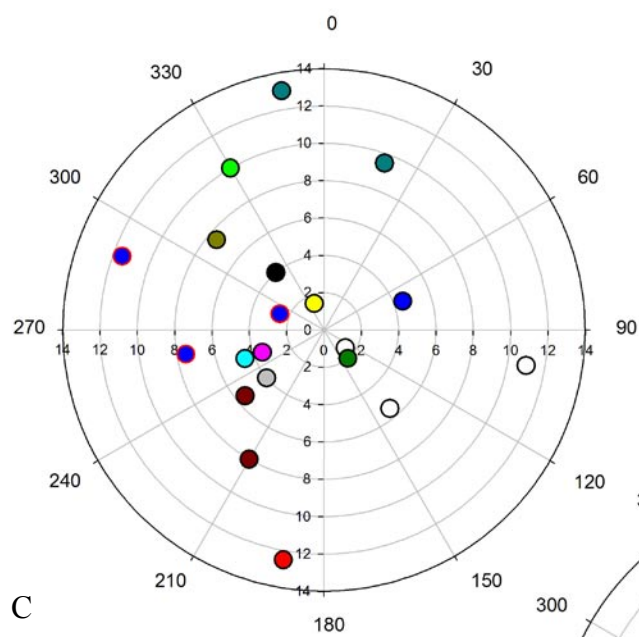
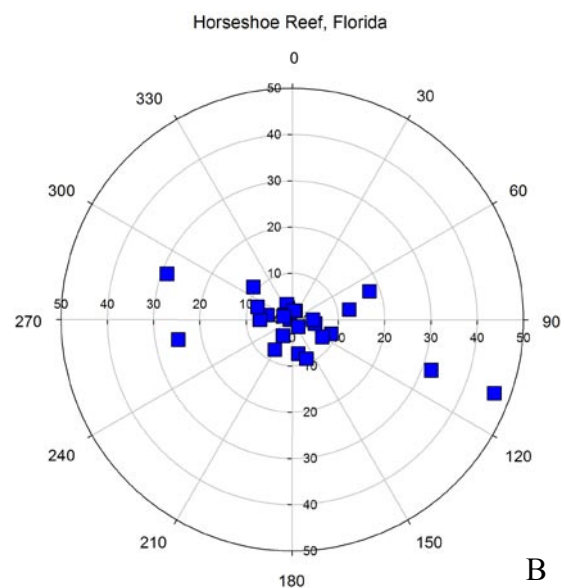
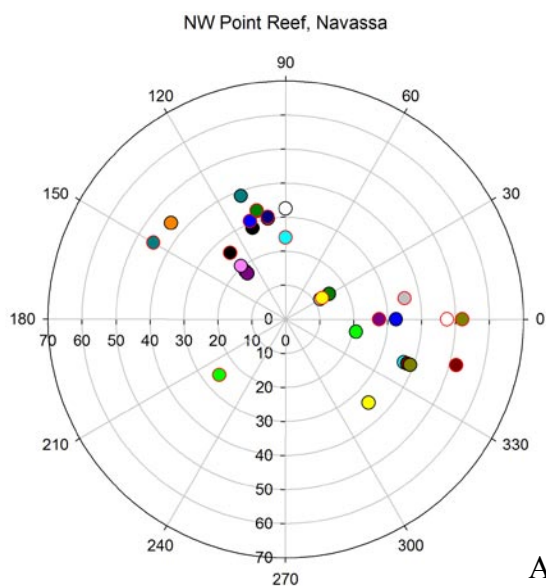


Fig 4.2 Frequency of genet size in *Acropora palmata* in the Caribbean. Genet size is given as the number of ramets in a genet. A total of N=373 genets were observed containing a total of N= 717 ramets.

Fig 4.3 Polar plots of *Acropora palmata* populations. **A)** At NW Point reef, Navassa, every point represents a unique genet. **B)** At Horseshoe Reef, Florida each point represents a ramet of the same genet. **C)** And **D)** Duplicate sets of circles sampled at Bastimentos Reef, Panama. One genet (red with black outline) occurred in both sets of circles. The plots are arranged graphically as they were laid out on the reef (i.e. 310° and 31.5m between center points). The plot depicted in D) was sampled first (Bastimentos I). Each mark represents a colony. Ramets of the same genet indicated by common symbol and color. Radial axis: distance in (m), angular axis: angle in degrees. See Tables 1 and 2 for sample sizes.



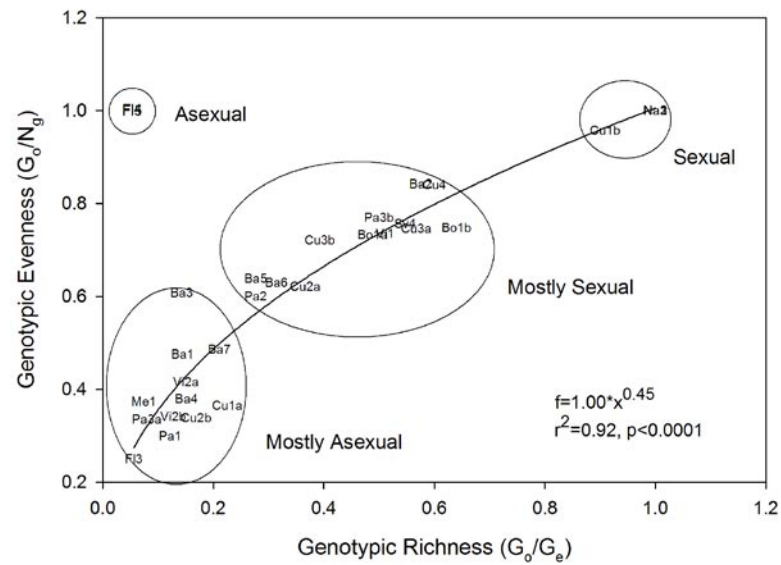


Fig 4.4 Clonal structure of *Acropora palmata* stands ($N = 30$) in the Caribbean. Based on the combination of genotypic evenness (G_o/N_g , how even were ramets distributed amongst the genets?) and genotypic richness (G_o/G_e , how many genets were found?), stands are divided into four groups ranging from asexual to sexual. This grouping is somewhat arbitrary since there is a continuum in the contribution of asexual reproduction to population structure. However, this grouping facilitates discussion. The power function shown was fitted excluding the asexual Florida populations (F14 and F15). The statistic G_o/N_g approaches zero with low evenness (e.g. F13), however it becomes one when the G_o equals N_g (F14 and F15).

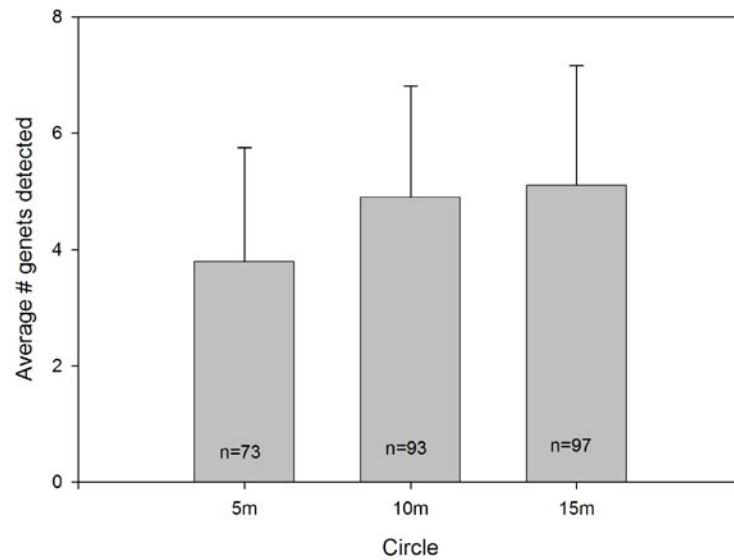


Fig 4.5 *Acropora palmata* sampled on three scales (5 m, 10 m and 15 m radius circles). While more genets were found when sampling within a 15 m radius than within a 10m or 5m radius, the average number of genets detected did not differ between the three spatial scales (one way ANOVA, $p > 0.1$). Included are all sites sampled randomly (Table 4.1). Excluding Florida (since sites there have only one genet at all three scales) does not change the result of the ANOVA. Given are means and 1 SD.

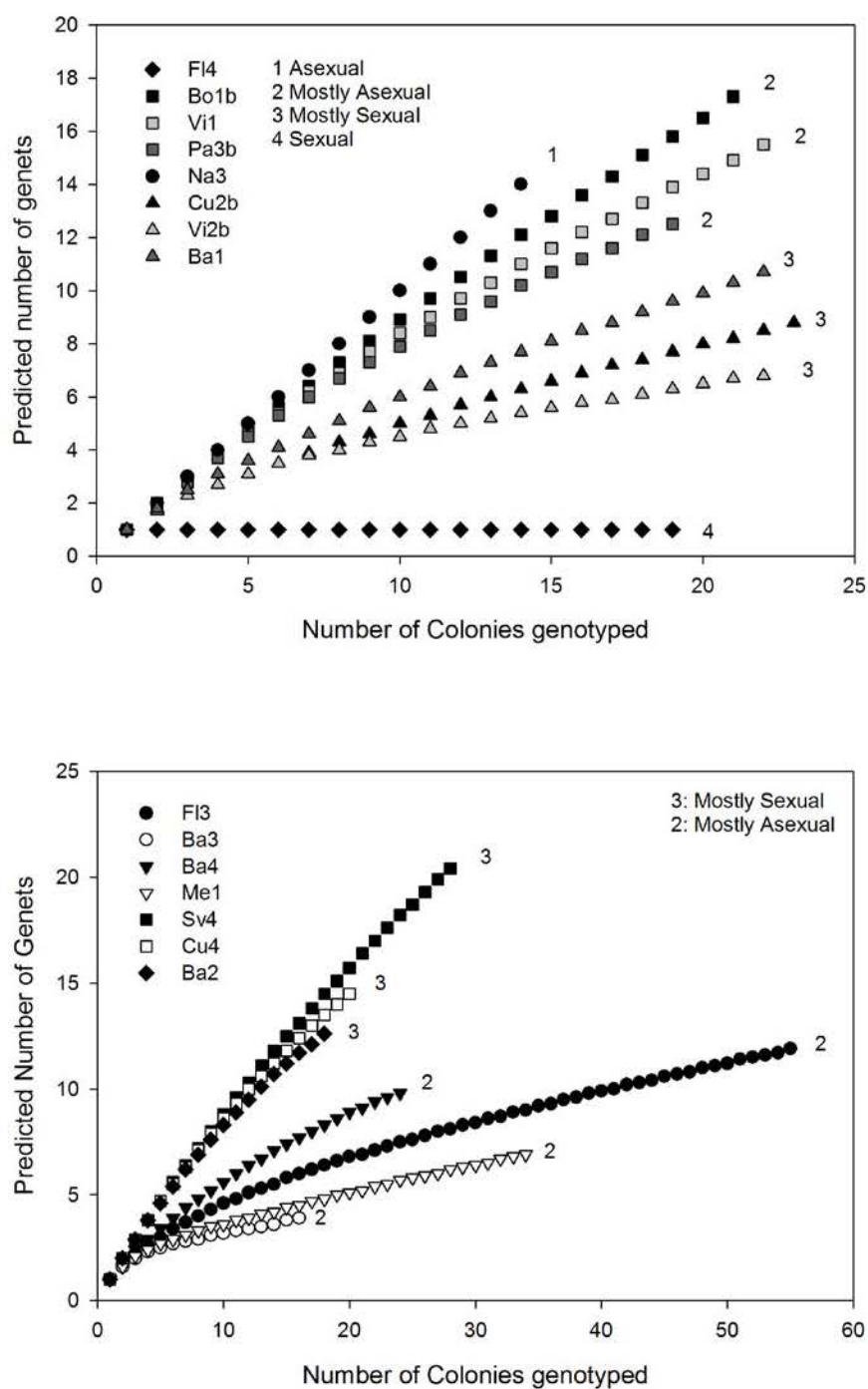
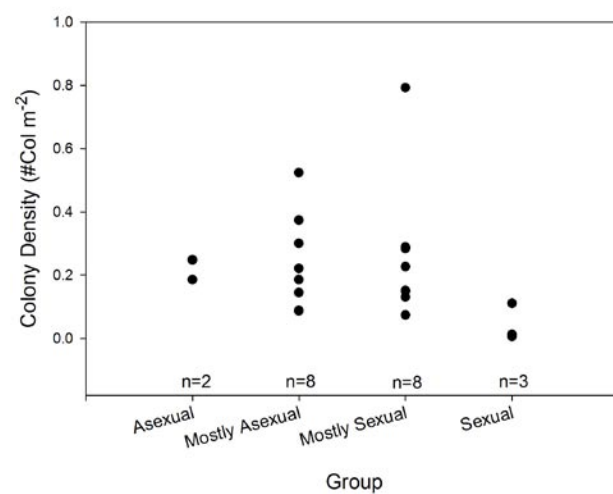
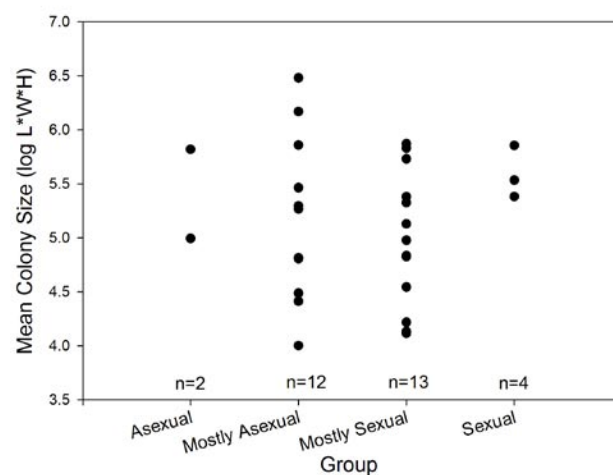


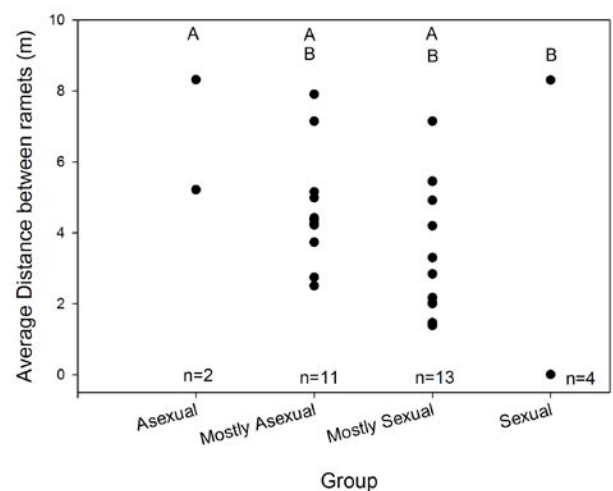
Fig 4.6 Rarefaction analysis of **A)** randomly and **B)** haphazardly sampled *Acropora palmata* stands showing purely asexual (1), mostly asexual (2), mostly sexual (3), and purely sexual (4) reproduction. Not all stands are shown to improve clarity. Reef abbreviations as in Table 4.1.



A



B



C

Fig 4.7 Correlation of ecological parameters with clonal population structure (classified into four groups from asexual to sexual) on small scales (10s of meters). **A)** Colony densities. No significant differences between groups were found (one-way ANOVA, $df = 3$, $F = 1.19$, $p = 0.34$). $N = 18$. **B)** Average mean size of *Acropora palmata* colonies, expressed as the log transformed product of length * width * height (cm) measurements. There is no significant difference between groups (one-way ANOVA, $df = 3$, $F = 0.81$, $p = 0.499$). $N = 31$. **C)** Clonal spread (average distance between ramets in m). Canouan, the site with the largest mean distance between ramets (112 m), was excluded as an outlier. Canouan belongs to the mostly sexual group. Groups are significantly different (one-way ANOVA, $df = 3$, $F = 2.9$, $p = 0.05$). Letters identify similar groups (Tukey's test). $N = 28$.

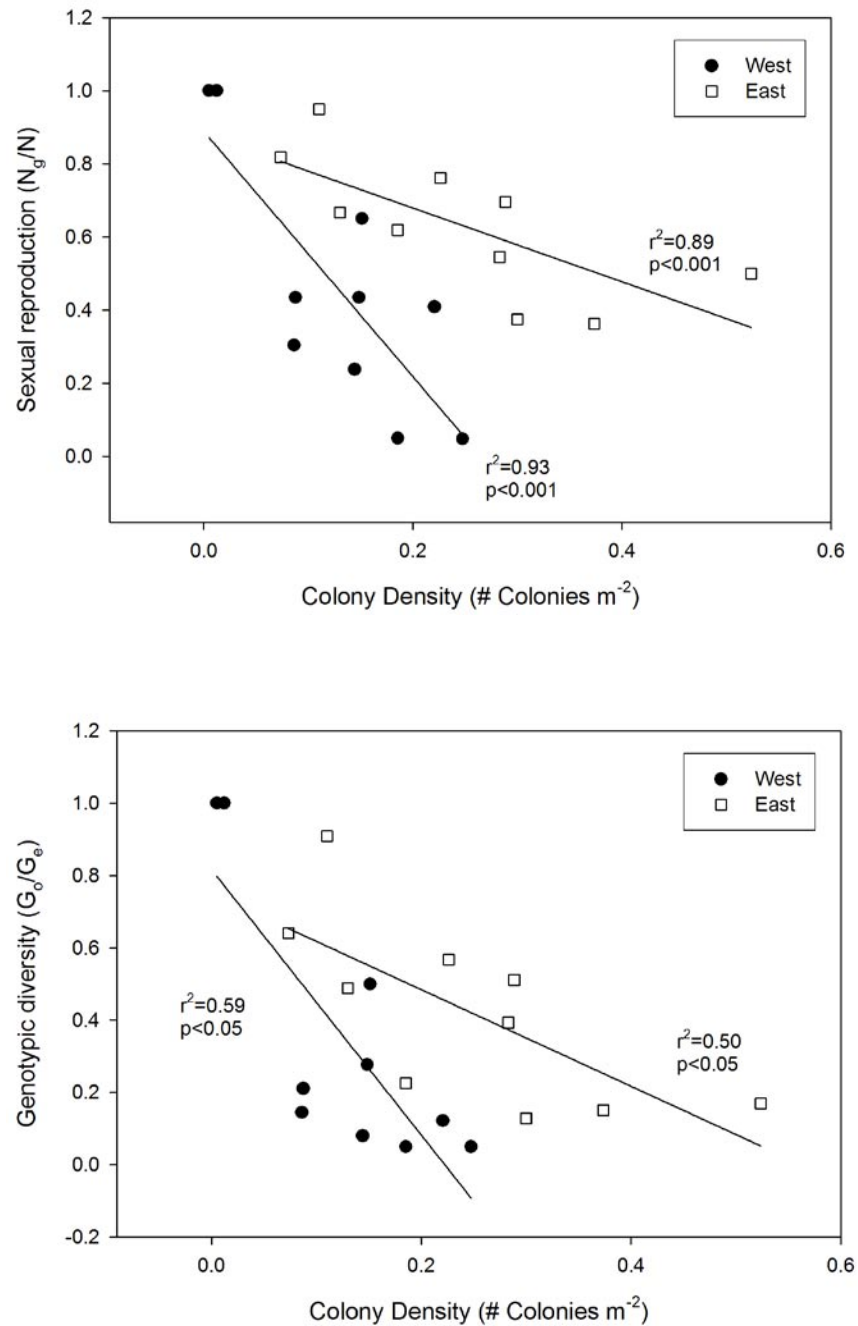


Fig 4.8 Correlation of genetic parameters (genotypic diversity and evenness) with colony density of *Acropora palmata* populations in the two phylogeographic provinces (East and West) of the Caribbean. Higher colony density (# colonies m^{-2}) is associated with A) a higher degree of asexual reproduction (N_g/N) and B) with a lower genotypic diversity (G_o/G_e) within each of the two provinces. P-values for linear least squares regression. N = 10 populations in each subregion.

CHAPTER 5: SUMMARY AND SYNTHESIS

BACKGROUND

Marine populations are often thought to be well connected via long-distance dispersal of planktonic larval stages (Heck and McCoy 1978; Roughgarden et al. 1985; Veron 1995; Caley et al. 1996; Scheltema et al. 1996). Knowledge about the extent to which marine populations are open or closed is essential for both ecological and evolutionary studies of processes influencing marine organisms. Closed populations facilitate adaptation to local conditions because larvae from successful adults recruit to their natal reef, however, absence of exogenous larval input make these populations more prone to extinction. Open populations receive larvae from a common larval pool and so can be reseeded from outside sources should a population decline locally. This has profound implications for the management of marine resources because the sources and sinks of larvae might fall under the jurisdiction of several different nations. Broadcast spawning invertebrates like the Caribbean reef-building coral *Acropora palmata* are expected to be well connected via long-distance transport of their planktonic larval stages.

Genetic markers can be used to assess gene flow between populations. However, only recently have both the high resolution genetic markers and the statistical tools become available that can estimate migrant exchange on ecological time scales. In clonal organisms such as *Acropora palmata* (Fig 1.1), estimates of gene flow need to account for localized asexual reproduction that can skew the genotype frequencies utilized by population genetic models. The contribution of asexuality to population structure is expected to vary across a species' range due to the interplay between sexual recruitment, physical disturbance, and intra-specific competition. In analogy to clonal plants, asexuality might increase in importance at or near a species' range limits. Description of clonal structure patterns might thus elucidate important ecological and evolutionary processes shaping clonal reef-building corals. Understanding these processes becomes urgent in the face of the Caribbean-wide decline acroporid populations have experienced over the past two decades due to disease, hurricanes, predation, and bleaching episodes. Sign of recovery are nearly absent so acroporids are now listed as candidates for the U.S. Endangered Species List (Diaz-Soltero 1999) and a formal review of their status is underway (Bruckner 2002).

To answer questions of gene flow (Chapter 3) and asexual reproduction patterns (Chapter 4), the necessary tools had to be developed: identification of highly polymorphic, Mendelian microsatellite loci (Chapter 2).

CHAPTER 2: MENDELIAN MICROSATELLITE LOCI FOR THE CARIBBEAN CORAL

ACROPORA PALMATA.

Studies of population connectivity and clonal structure in reef-building corals have been hampered by low levels of mitochondrial DNA variation and by an apparent dearth of variable single-copy, nuclear markers. Developing microsatellite markers in *Acropora* has proven especially difficult in Pacific species. In this dissertation, Southern blotting was used to reveal that, indeed, many microsatellite motifs (AAC, AAG, AC) are rare in the genome of the Caribbean species *Acropora palmata* (Fig 2.1, Table 2.2). However, repeats with the motif AAT are both abundant and variable (Fig 2.1). The targeted enrichment protocol used here might help to circumvent problems encountered when developing microsatellites for scleractinian corals and other problematical taxa. Based on this method, eight polymorphic microsatellite markers were developed for *A. palmata*, and crosses were performed to confirm co-dominant inheritance patterns. Five of the eight markers tested show simple Mendelian inheritance (mean observed heterozygosity = 0.88, mean number of alleles per locus = 8.8, Table 2.4, 2.5, 2.7).

Along with outcrossed sexual larvae, individual egg donors also produced triploid and selfed larvae (Table 2.5) that developed normally and survived for 80 hrs, when the experiment was terminated. The occurrence of both self-fertilized and triploid larvae, even at low percentages, underlines the necessity for genetic confirmation of larval origin when interpreting the fertilization success of acroporid crosses. Triploid larvae may be one route to developing variation in ploidy levels among *Acropora* species. Observation of triploid adults would lend further support to this hypothesis.

Microsatellite motifs are not evenly distributed among genomes and have different characteristics within genomes (Primmer et al. 1997). Several groups have been identified as depauperate in microsatellites (e.g. birds, Lagercrantz et al. 1993; Primmer and Ellegren 1998). Even among *Drosophila* species differences in microsatellite abundance have been found (Warner and Noor 2000). Thus, the discovery of polymorphic microsatellite repeats in Caribbean acroporids reported here does not necessarily contradict the apparent lack of microsatellites in Pacific acroporids. Interestingly, Chenuil et al (1997) suggest that an under-representation of dinucleotides in barbel species (*Barbus*) compared to other fish might result from selection pressure to eliminate DNA from polyploidized species via preferential loss of repeat units during slip-strand repair. Comparisons of motif abundance, cell size and DNA content between diploid and polyploid *Acropora* species (Kenyon 1997) might be enlightening.

Preliminary tests of these markers on three Florida populations of *A. palmata* revealed variation among clones within populations (Fig 2.2, 2.3). Seven of the markers amplify DNA from *A. cervicornis* and eight from the hybrid *A. prolifera* suggesting that they may be useful for genetic studies in these equally threatened taxa. These markers should prove to be valuable tools for developing conservation strategies for Caribbean acroporid species and were applied in this dissertation to describe clonal population structure and estimate gene flow in *Acropora palmata*.

CHAPTER 3: MICROSATELLITES DEFINE TWO POPULATIONS OF *ACROPORA*

PALMATA IN THE CARIBBEAN

The extent to which pelagic larvae connect marine populations has been the subject of ongoing controversy, with Caribbean populations being the focus of intense research interest (Roberts 1997; Cowen et al. 2000; Strathmann et al. 2002; Cowen et al. 2003a). The debate centers on both the degree of correlation between surface currents and larval dispersal routes and the time scale over which larval exchange between populations takes place. Theoretically, genetic similarity between populations can be maintained by low levels of migration. Such a small number of migrants, however, would be insufficient to sustain populations in demographic decline. In the face of the depressed state of many tropical benthic organisms such as reef-building corals this question is now of concern to conservation biologists. The microsatellite markers developed in this dissertation (Chapter 2) were used to estimate the population structure and migration rates of the *Acropora palmata*.

A Bayesian clustering approach (Pritchard et al. 2000; Falush et al. 2003) was used to estimate the number of populations present in our samples (Table 3.2, Fig 3.3). This analysis identified two major clusters, a western cluster comprised of samples from Panama, Mexico, Florida, the Bahamas and Navassa and an eastern cluster containing samples from the U.S. Virgin Islands and the Lesser Antilles (St. Vincent and the Grenadines, Bonaire and Curacao). Both F_{st} (0.056) and R_{st} (0.33) estimates were statistically significant and indicate moderate differentiation between the two clusters (Table 3.3, 3.4). Bayesian estimates of migration suggest little to no recent exchange between these clusters (Table 3.7A).

Within each of the two clusters, asymmetric migration patterns were observed (Table 3.7B, Fig 4.4). In the E, *A. palmata* stands in St. Vincent and the Grenadines provide significant amounts of propagules to the US Virgin Islands as well as the Netherlands Antilles while not receiving foreign larval input. Panama does not experience measurable amounts

of immigration from the other localities. The clusters split between the Bahamas and US Virgin Islands in the East, and between Panama and Curacao in the South, congruent with previously suggested biogeographical breaks at the Mona Passage and the Peninsula del Guajira. The detection of two distinct populations of *A. palmata* challenges the assumption of panmixia among Caribbean populations of benthic broadcast spawners. Studies of other coral reef dwellers with a variety of life history characteristics should be investigated to ascertain the generality of these suggested biogeographical breaks in the Caribbean.

Cowen et al (2000) stressed the importance of ascertaining ecologically relevant migration rates between populations as opposed to genetic exchange rates over evolutionary timescales. Traditional population genetic models (F-statistics) could only provide gene flow estimates on the scale of several 1000s of generations. The development of microsatellite markers with their higher information content compared to allozyme markers, prompted efforts to build statistical models that made use of this wealth of information. Assignment tests now allow for the detection of almost real-time migration rates based on genetic markers (Wilson and Rannala 2003). The resulting migration matrices are similar to those obtained from mark and recapture studies that use physical tags (Berry et al. 2004). With these methods in hand, the task of designing appropriate conservation measures for declining marine ecosystems becomes more feasible.

Marine reserves are one of the current tools to manage coral reef organisms. Optimal design strategies, i.e. their placement, number and size, are the subject of much debate (Palumbi 2003). Depending on the desired effect of the reserve (spill-over of larvae to neighboring sites, source for long-distance dispersing larvae to unprotected areas, habitat preservation), the assumption of highly dispersing larvae is more or less important. The existence of two populations of *Acropora palmata* in the Caribbean, possibly separated by a phylogeographical barrier, suggests that marine reserves designed to protect this keystone species will have effects within their respective populations only if the goal is to provide a source of larvae for other imperiled reefs. This leads to an increase in the number of marine reserves necessary (Cowen et al. 2000).

CHAPTER 4: CLONAL STRUCTURE VARIES GEOGRAPHICALLY IN *ACROPORA*

PALMATA

A hierarchical survey of *Acropora palmata* clonal population structure was undertaken at 25 reefs in 8 localities in the Caribbean and western North Atlantic (Fig 4.1). A genetic break divides the 8 localities into a western and an eastern subregion (Chapter 3). Clonal

structure, assayed using the here developed highly polymorphic microsatellite markers, was unpredictable at scales of meters to kilometers. Populations sampled 30-70 m apart on the same reef sometimes varied from being pre-dominantly asexual to sexual, emphasizing the influence of micro-scale processes on *A. palmata* clonal structure (Fig 4.4). Most populations showed intermediate patterns of genotypic diversity. Similar variation was evident between reefs within localities. Only one genet each was found on two reefs in Florida, while a reef just 15 km away showed considerable genotypic variation. Clonal structure became predictable on a Caribbean-wide scale. Highly asexual, genotypically depauperate populations with low colony densities characterized the western subregion, while sexual processes led to genotypically diverse, dense stands in the eastern subregion populations (Table 4.4). Clonal evenness, spread of clones and colony size were similar in both provinces. The contribution of sexual reproduction to population structure (N_g/N , East: $r^2=0.93$, West: $r^2=0.89$, $p<0.001$) and genotypic diversity (G_o/G_e , East: $r^2=0.59$, West: $r^2=0.50$, $p<0.05$) were negatively related with colony density indicating that within provinces, denser populations rely more on asexual reproduction and have lower diversity than less dense populations (Fig 4.8).

The input of sexual recruits might be the main difference between these provinces causing the observed large-scale patterns (Chapter 3). Additionally, local rates of fragmentation, geographical location, exposure and other factors may determine the degree of asexuality within provinces, localities and reefs. Clonal plants sometimes show reduced sexual reproduction at the geographical margins of a species' range (Eckert 2002). In marginal environments, seed production was severely reduced and strongly linked to the failure of sexual recruitment in an aquatic plant (Eckert et al. 1999). In this dissertation, several lines of evidence suggest reduced sexual reproduction in populations of *Acropora palmata* in marginal habitats. Only the two Floridian reefs of 31 reefs investigated were entirely dominated by one clone. During crossing experiments using gametes from a third, mostly asexual reef in Florida, low fertilization rates were observed. Most Pacific acroporids showed fertilization success of $>90\%$ using similar methods (Willis et al. 1997). Moreover, a low percentage of triploid larvae were observed. Triploid organisms, while frequently able to survive and grow, are often incapable of orderly meiosis and thus genetically sterile (Eckert 2002). Future studies should investigate the effects of ecological factors on reproductive output at the margins of *A. palmata*'s range and investigate the possibility of genetic sterility in these populations

CLOSING STATEMENT

This dissertation provided data addressing the current debate about open or closed marine populations. New molecular tools and statistical methods as well as a broad geographical sampling design were essential in describing both gene flow patterns and local modes of reproduction. The discovery of population subdivision in an organism with a long planktonic larval stage within an area marked by strong current flow challenges the notion of open populations in the Caribbean broadcast spawning corals. Additionally, asexual reproduction co-varies with the amount of connectivity between reefs on large spatial scales. The northernmost locality investigated in this study at the margin of the species' range showed signs of reduced sexual vigor. This is in concordance with predictions made for clonal plants and might provide the basis for future studies yielding insights into the ecology and evolutionary potential of clonal coral species.

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APPENDIX



Table A.1 Size structure of *Acropora palmata* stands in the Caribbean for **A)** randomly sampled and **B)** haphazardly sampled stands. Given is the number of colonies measured at each stand (N), the average log of the product of two diameters and the height (all in cm) of each colonies, as well as the standard deviation (SD) and the coefficient of variation (CV) of that measure.

A

Locality	Reef Code	Reef Name	N	Mean	SD	CV
Bahamas	Ba1	Great Iguana	24	6.17	0.51	0.08
	Ba5	Bock Cay	24	4.11	1.24	0.30
	Ba7b	Middle Beach	24	4.81	1.48	0.31
Bonaire	Bo1a	Taylors Made I	24	5.73	1.10	0.19
	Bo1b	Taylors Made II	24	5.38	1.37	0.25
Curacao	Cu1a	Blue Bay I	23	5.46	0.86	0.16
	Cu1b	Blue Bay II	24	5.53	0.67	0.12
	Cu2a	Sea Aquarium	24	4.83	1.26	0.26
	Cu2b	Puntu Picu	24	4.81	1.04	0.22
	Cu3a	Awa Blanca I	23	5.32	1.38	0.26
Florida	Cu3b	Awa Blanca II	24	4.98	1.27	0.26
	FI4	Little Grecian	29	4.99	1.29	0.26
	FI5	Horseshoe	28	5.82	0.65	0.11
Navassa	Na3	Lulu Bay	21	5.38	0.65	0.12
	Pa1	Bocas Del Drago	23	5.26	0.75	0.14
Panama	Pa3a	Bastimentos I	24	5.86	0.56	0.09
	Pa3b	Bastimentos II	24	5.83	0.72	0.12
USVI	Vi1	Johnsons Reef	24	4.13	0.88	0.21
	Vi2a	Hawksnest Bay I	25	4.81	0.89	0.19
	Vi2b	HawksnestBayII	24	4.49	1.07	0.24
	All		484	5.18	0.57	0.11

B

Locality	Reef Code	Reef Name	N	Mean	SD	CV
Bahamas	Ba2	Halls Pond	16	4.21	1.16	0.28
	Ba3	Rocky Dundas	22	4.41	1.06	0.24
	Ba4	Little Darby	25	4.00	1.26	0.31
	Ba6	Black Buoy	32	4.54	1.16	0.26
Curacao	Cu4	Boka Patrick	21	5.87	0.89	0.15
Florida	FI3	Sand Island	52	5.29	0.92	0.17
Mexico	Me1	Chinchorro	39	6.48	0.57	0.09
Navassa	Na1	N Shelf	18	5.85	0.45	0.08
	Na2	NW Point	18	5.85	0.45	0.08
Panama	Pa2	Wild Cayne	18	5.13	0.78	0.15
SVG	Sv4	Canouan	30	4.82	0.81	0.17
All			291	5.13	0.81	0.16

Fig A.1 Size frequency distribution of **randomly** sampled *Acropora palmata* stands in the Caribbean. Site abbreviations indicate the locality (e.g. Ba=Bahamas) followed by the reef number. Refer to Table 4.1 and 4.2 to see a list of all sites.

Fig A.2 Size frequency distribution of **haphazardly** sampled *Acropora palmata* stands in the Caribbean. Site abbreviations indicate the locality (e.g. Ba=Bahamas) followed by the reef number. Refer to Table 4.1 and 4.2 to see a list of all sites.

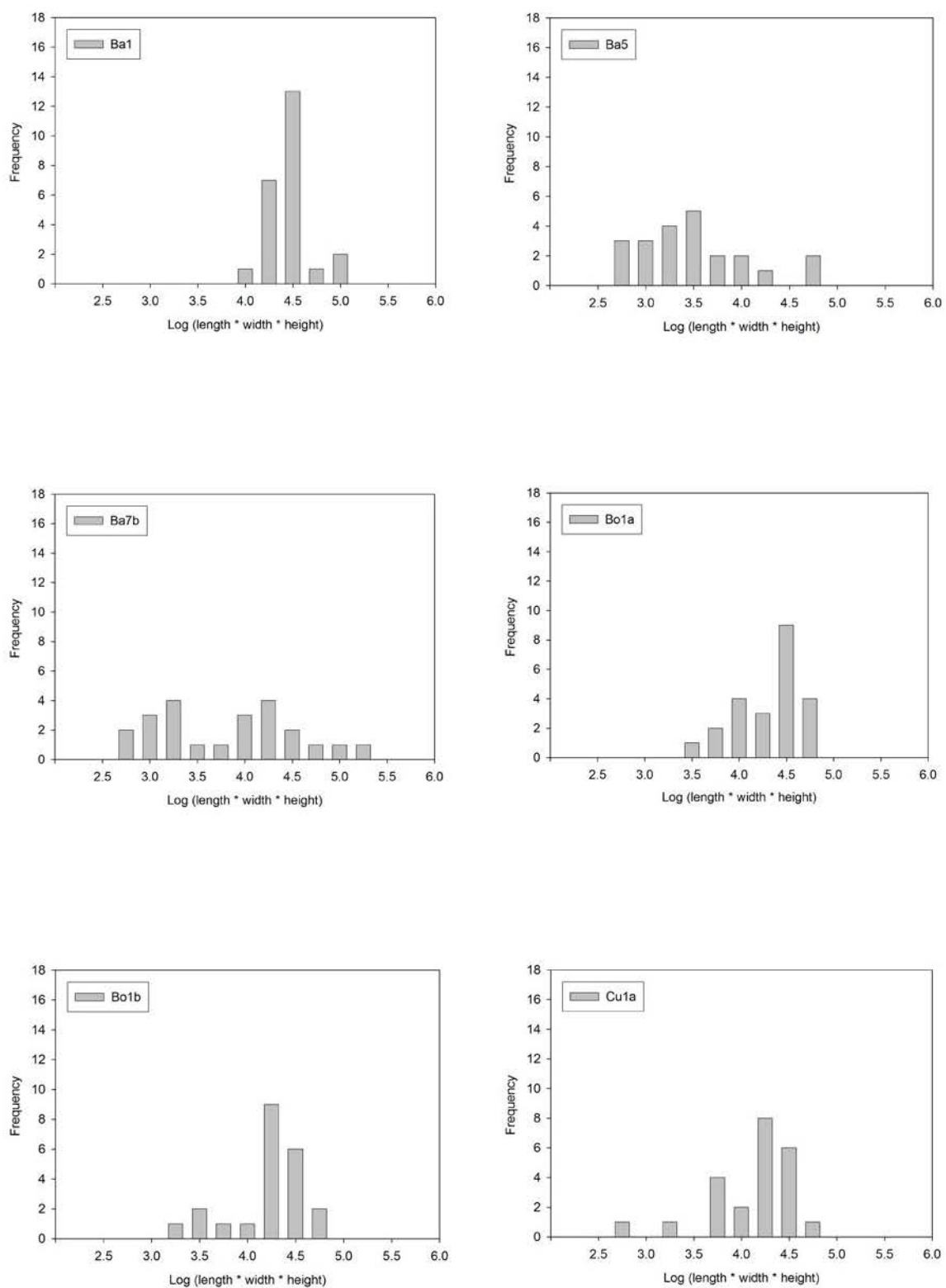


Fig A.1 -1

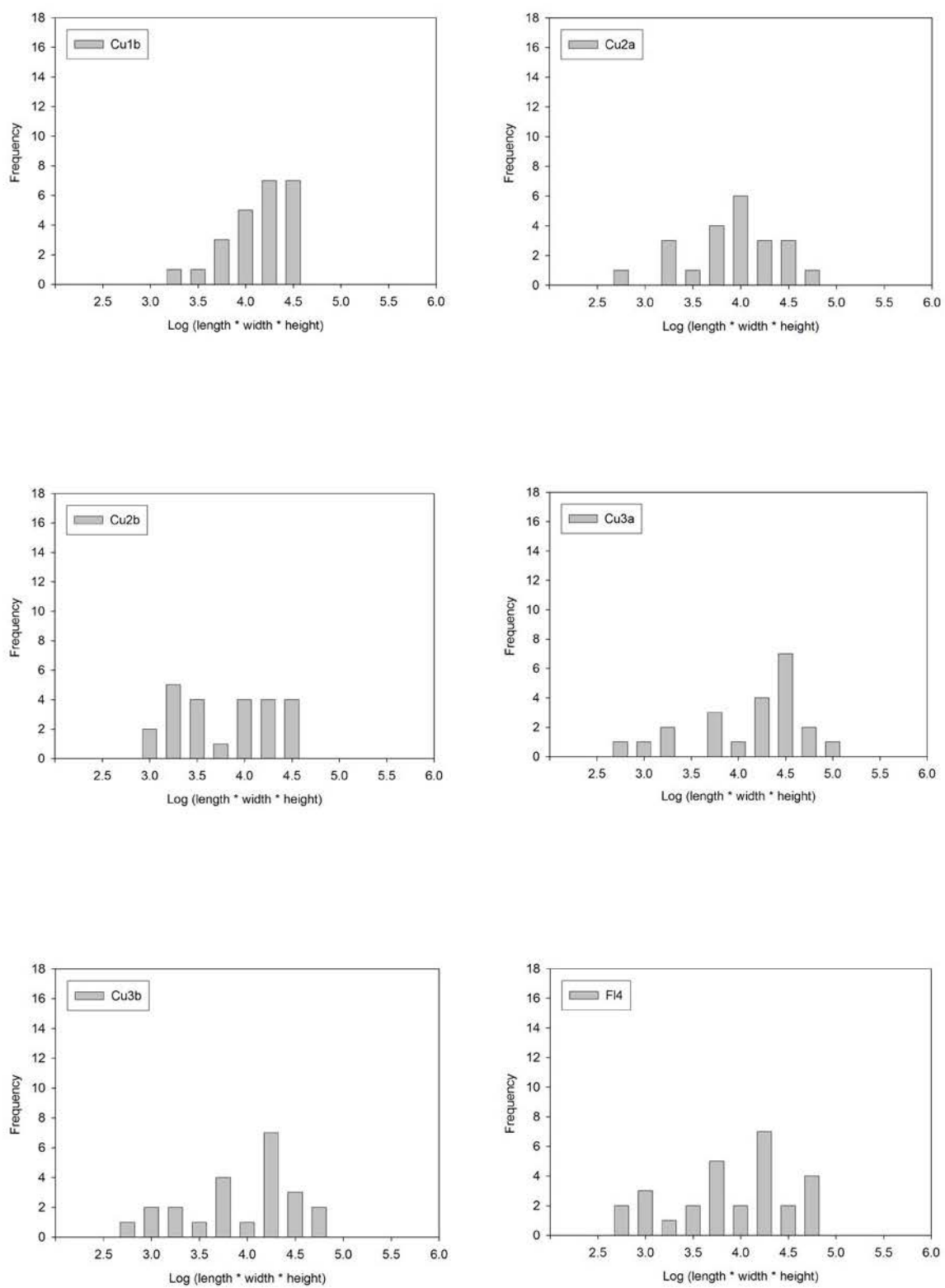


Fig A.1 -2

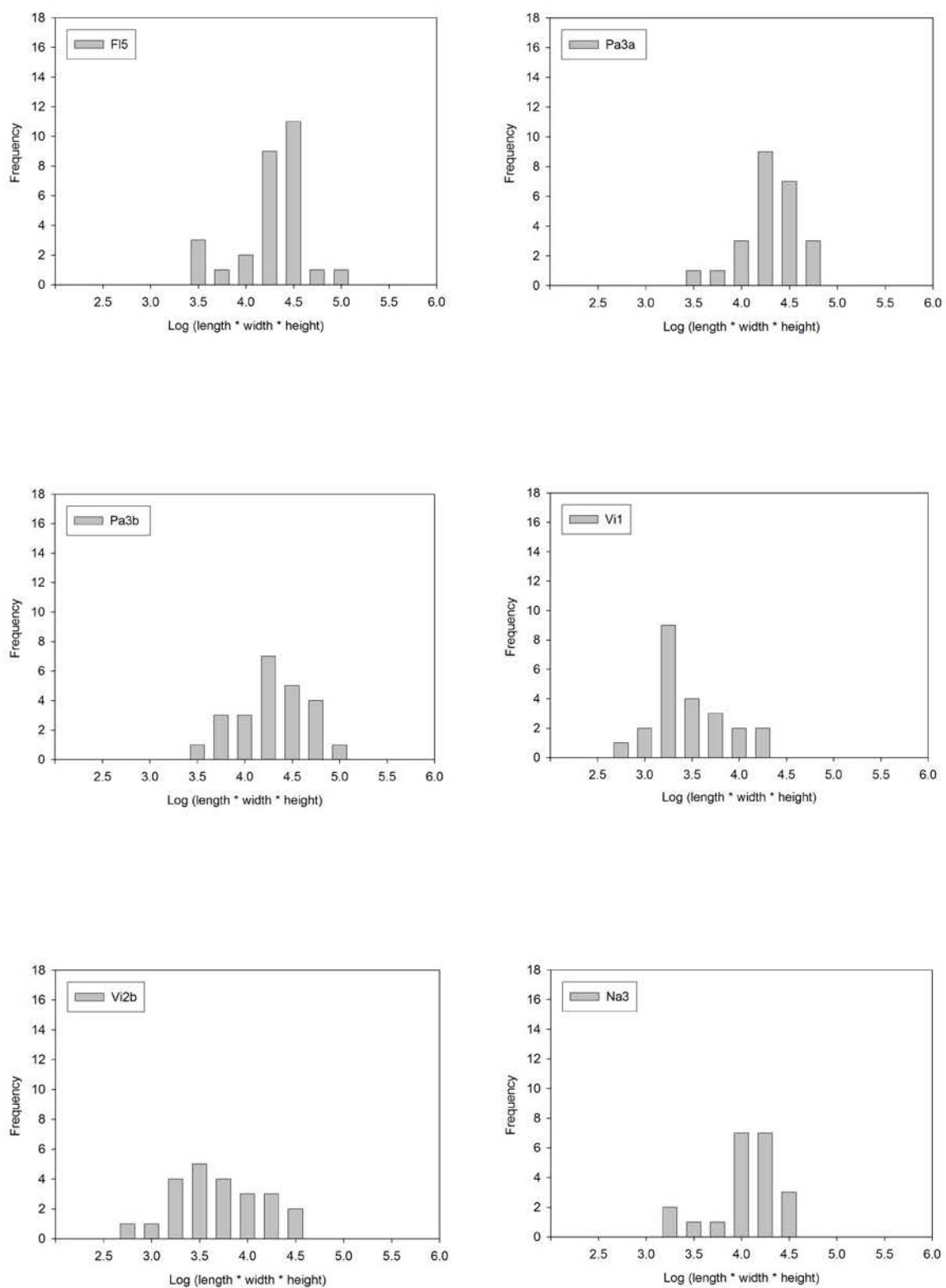


Fig A.1 -3

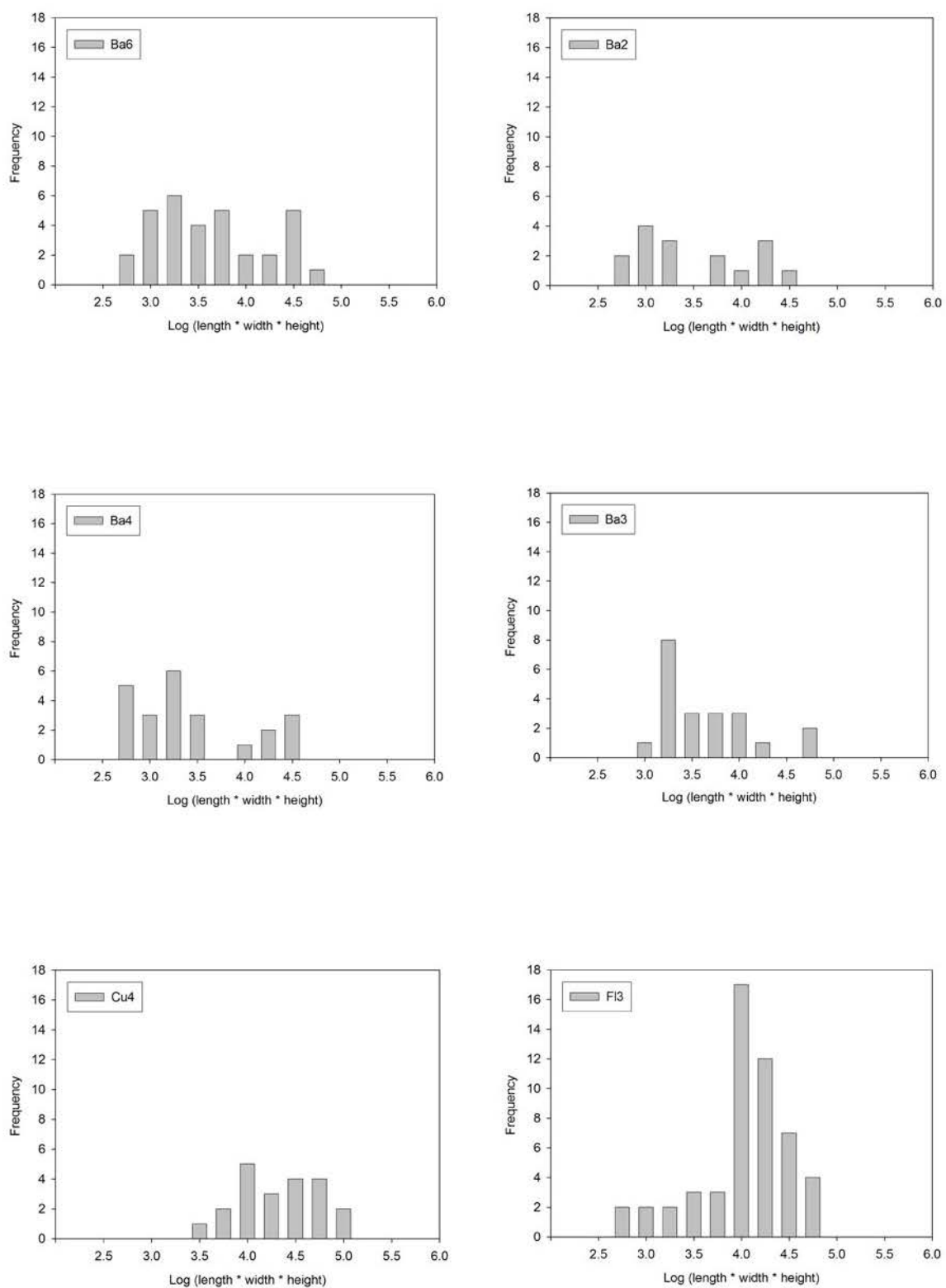


Fig A.2 -1

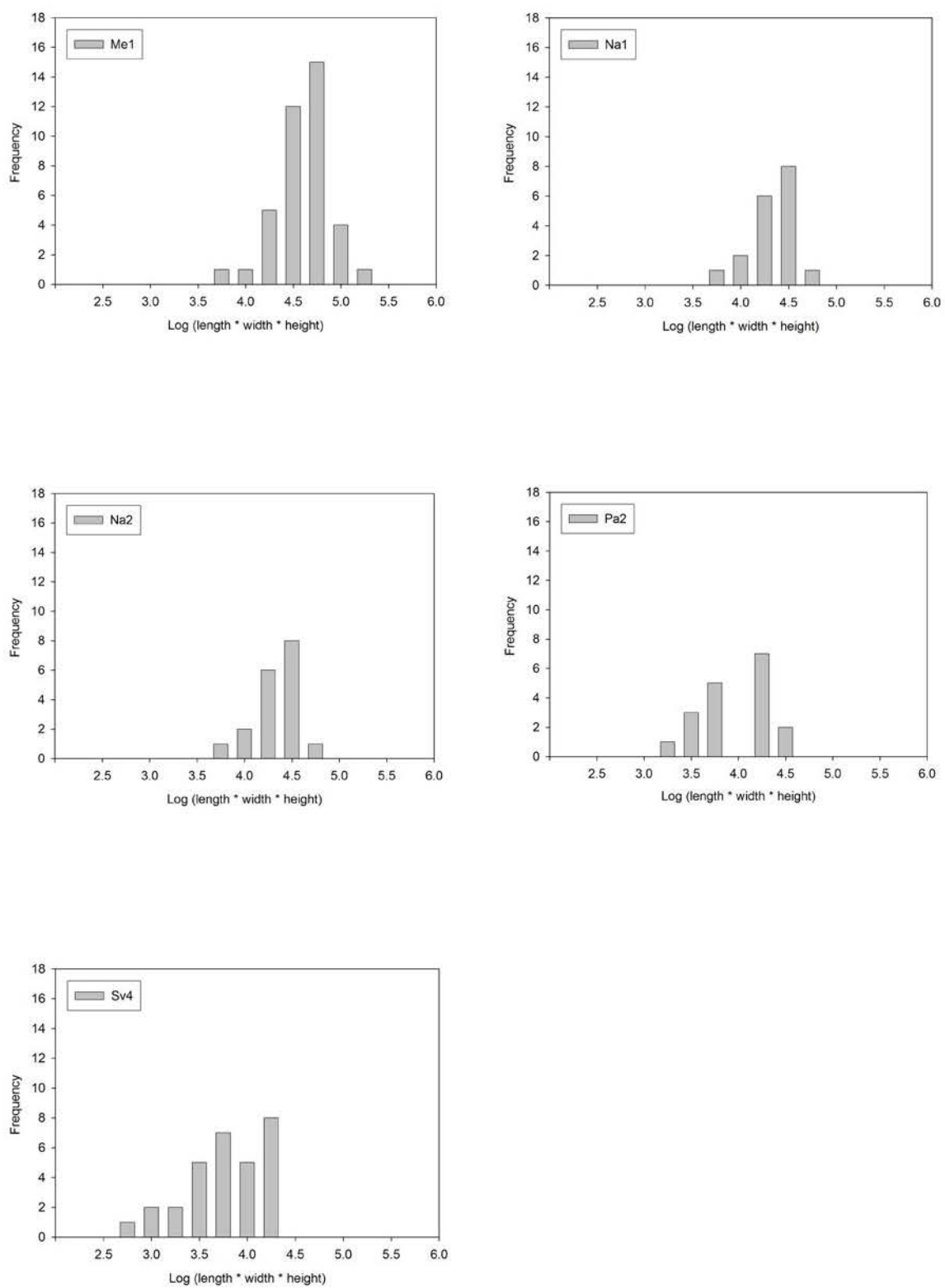


Fig A.2 -2

VITA

Iliana Brigitta Baums was born to Manfred and Christia Baums on 30 August 1974 in Köln, Germany. She has an older sister, Julietta and a younger brother, Ansgar. She was educated in Köln at the Grundschule Lebensbaumweg, and the Heinrich-Mann-Gymnasium from where she received her Abitur in 1993. She was admitted to the Leibniz Kolleg in Tuebingen, Germany, a one-year general studies program in 1993. From 1993 – 1997 she was enrolled at the University of Tuebingen to study Biology and received her Vordiplom from there. She spent the last year of her undergraduate as an exchange student at the University of Miami, USA. Upon return to Germany in the 1997, she pursued her graduate studies in Marine Biology at the University of Bremen.

In August of 1998, Iliana was admitted to the Rosenstiel School of Marine and Atmospheric Science. While enrolled in the Ph.D. program of the University of Miami, she received a Diplom Degree from the University of Bremen in 2000. She was granted the degree of Doctor of Philosophy from the Graduate School of the University of Miami in May 2004.

