

1 **Sexual versus asexual reproduction in an ecosystem engineer: the massive coral *Montastraea***  
2 ***annularis***

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17  
18 Running Title: Asexual dispersal in massive corals

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1 **Summary**

- 2 1. Long-lived sedentary organisms are often assumed to utilise a storage effect whereby the  
3 persistence of a small group of adults can maintain the population when recruitment fails.  
4 However, dependence on storage effects could prove catastrophic if, under changing  
5 climatic conditions, the availability of favourable conditions for recruitment is dramatically  
6 reduced. When a species has multiple reproductive options, a rapidly-changing environment  
7 may favour alternative asexual means of propagation.
- 8 2. Here, we revisit the importance of asexual dispersal in a massive coral subject to severe  
9 climate-induced disturbance. *Montastraea annularis* is a major framework-builder of  
10 Caribbean coral reefs but its survival is threatened by the increasing cover of macroalgae  
11 that prevents settlement of coral larvae.
- 12 3. Samples of *M. annularis* from three sites in Honduras were genotyped using six,  
13 polymorphic microsatellite loci.
- 14 4. A total of 114 unique genets were identified with 8% consisting of two or more clonemates  
15 and an exceptionally large genet at the third site comprising 14 clonemates.
- 16 5. At least 70% of multi-colony genets observed were formed by physical breakage, consistent  
17 with storm damage.
- 18 6. Our results reveal that long-lived massive corals can propagate using asexual methods even  
19 though sexual strategies predominate.

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21 **Keywords:** disturbance, hurricanes, microsatellites, population structure, scleractinian coral

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## 1 **Introduction**

2 Clonal propagation, using fragmentation, budding or the production of asexual planulae, is  
3 considered to be an adaptation to both unfavourable local environmental conditions and relatively  
4 stable habitats. It allows a species to persist when it is unable to complete its sexual reproductive  
5 life cycle (Honney & Bossuyt, 2005) and it enables well-adapted genotypes to become dominant in  
6 the absence of moderate to high levels of disturbance (Miller & Ayre, 2004). Examples of clonal  
7 propagation are found in many invertebrate and plant taxa from both aquatic and terrestrial  
8 environments; coast redwood trees (Douhovnikoff, Cheng & Dodd, 2004), European aspens  
9 (Suvanto & Latva-Karjanmaa, 2005), weedy perennial plants (Ceplitis, 2001), epizoic anemones  
10 (Bronsdon *et al.*, 1997), gorgonians (Coffroth & Lasker, 1998; Gutierrez-Rodrigues & Lasker,  
11 2004) and stony corals (Ayre & Hughes, 2000; Baums, Miller & Hellberg, in press; Le Goff-Vitry,  
12 Pybus & Rogers, 2004). In some species sexual and asexual reproduction contribute equally to  
13 population growth (Sun, Gao & Cai, 2001; Wepler, Stoll & Stocklin, 2006) while in several  
14 species one mode of reproduction clearly dominates over another (Bronsdon *et al.*, 1997; Ceplitis,  
15 2001; Magalon, Adjeroud & Veuille, 2005; Maier *et al.*, 2005). Furthermore, the contribution of  
16 asexual reproduction to recruitment can vary between populations of a single species across its  
17 range (Baums *et al.*, in press; Coffroth *et al.*, 1998; Eckert, 2001; Le Goff-Vitry *et al.*, 2004; Miller  
18 *et al.*, 2004).

19  
20 The balance between sexual and asexual reproduction within a species can be affected by both  
21 biotic and abiotic factors. In marine environments, disturbance events can dramatically alter the  
22 contribution of asexual reproduction to recruitment (Henry & Kenchington, 2004; Le Goff-Vitry *et al.*  
23 *et al.*, 2004; Rasheed, 2004). For example, Le Goff-Vitry *et al.* (2004) documented an increase in the  
24 contribution of asexual reproduction to recruitment in the deep-sea, branching coral *Lophelia*

1 *pertusa* in the presence of intensive fishing trawling. Disturbance may also affect the genotypic  
2 diversity of a species. Hunter (1993) described decreasing levels of genotypic diversity in  
3 populations of the finger coral *Porites compressa* with increasing levels of natural and  
4 anthropogenic disturbance. Interestingly, Coffroth *et al* (1998) observed the greatest genotypic  
5 diversity in populations of the gorgonian, *Plexaura kura*, in low and high disturbance environments  
6 and the lowest genotypic diversity in populations in intermediate disturbance environments. While  
7 these studies demonstrate the occurrence of asexual reproduction within a number of sessile  
8 organisms they focus on species with a branching morphology. The occurrence of asexual dispersal  
9 in large, long-lived organisms with a massive ('mound like') morphology has not previously been  
10 described.

11

12 The importance of asexual methods of dispersal are often found to be strongly related to colony  
13 morphology. For example, scleractinian corals are clonal organisms that dominate shallow reefs and  
14 periodically experience acute disturbance from hurricanes (Bythell, Hillis-Starr & Rogers, 2000).  
15 Whilst all corals grow using asexual budding of individual coral polyps (Jackson, 1977), only  
16 branching species are believed to employ methods of asexual fragmentation for colony dispersal  
17 (Bothwell, 1981; Bruno, 1998; Highsmith, 1982; Lirman, 2000; Smith & Hughes, 1999;  
18 Tunnicliffe, 1981). The branching morphology of these species' makes them particularly  
19 susceptible to wave impacts. Wave energy can break up colonies, distributing fragments across the  
20 reef. In contrast, the dome-shaped morphology of many massive, long-lived coral species fits a  
21 tolerance model for withstanding hurricane disturbance (Massel & Done, 1993). The low relief of  
22 such corals facilitates a laminar flow of water across the colony and minimises the forces of lift that  
23 would otherwise uproot colonies during storms.

1 In the Caribbean, massive, broadcast-spawning coral species such as *Montastraea annularis* recruit  
2 rarely (Bak & Engel, 1979; Mumby, 1999; Smith, 1992); typically, the density of (sexual) recruits  
3 is less than one tenth that of short-lived species that brood their larvae (total 2240 recruits, Mumby  
4 unpubl. data). The apparent scarcity of sexual recruits, combined with a robust morphology and  
5 high longevity, may indicate that massive corals employ storage effects (Edmunds, 2000), whereby  
6 the persistence of a small group of strong adults can maintain the population when recruitment fails  
7 (Murphy, 1968; Warner & Chesson, 1985). Low adult mortality allows such strong year classes to  
8 persist through time until a favourable recruitment period occurs (Warner *et al.*, 1985). However,  
9 rising levels of environmental and ecological disturbance, including the impacts of climate change,  
10 could dramatically reduce the availability of favourable conditions for coral recruitment (Hughes *et*  
11 *al.*, 2003). Under such circumstances asexual means of dispersal may become increasingly  
12 important for successful recruitment and persistence of populations.

13

14 Here, we question the assumption that long-lived, massive coral species do not employ asexual  
15 methods of colony dispersal. Studying one of the dominant reef-building corals in the Caribbean,  
16 *Montastraea annularis (sensu stricto)*, we present genetic evidence that large genets of a long-lived  
17 massive coral do exist.

## 1 **Methodology**

### 2 *Study species*

3

4 *Montastraea annularis* is a dominant framework-builder of Caribbean coral reefs, forming massive  
5 dome-shaped colonies, often over 1 m in diameter. Many colonies within the population are  
6 estimated to be >100 years old with an average growth rate of <10 mm per year (Dustan, 1975;  
7 Gladfelter, Monahan & Gladfelter, 1978). Colonies reproduce sexually by undertaking mass-  
8 spawning events (Szmant, 1991) and are predicted to reproduce asexually via two methods. The  
9 first method is through intra-colony fission caused by partial-colony mortality (Hughes & Jackson,  
10 1985) and the second method is where physical disturbance cleaves the entire colony resulting in  
11 the physical dislodgement of one or more fragments which then reattach nearby (Highsmith, 1982).  
12 During this study, we refer to asexual reproduction in *M. annularis* as the generation of structurally  
13 independent colonies rather than intra-colony fission (Fig. 1).

14

### 15 *Sampling*

16

17 A total of 146 colonies were sampled at three sites (Seaquest – 16°17'39"N, 86°36'00"W; Sandy  
18 Bay – 16°20'02"N, 86°34'04"W; and Western Wall – 16°16'14"N, 86°36'16"W) on the North coast  
19 of Roatan, Honduras in October 2004. Honduras experiences intermediate frequencies of hurricanes  
20 in the Caribbean with a mean interval of ca 17 years (Gardner *et al.*, 2005). Sites were selected  
21 based on their prolific *Montastraea annularis* populations and were located one to three km apart.  
22 Western Wall was located at the western tip of the island and approximately 1 km from Seaquest.  
23 Sandy Bay was a further 2 km east of Seaquest. Each site was located on the forereef at a depth of  
24 ca 5 m and a circular sampling plot was established with a radius of 5 m. Every *M. annularis* colony

1 within each plot was tagged and its distance (to the nearest 5 cm) and bearing (to the nearest 5  
2 degrees) from the centre of the sampling plot (marked by a stake) were recorded. Colony size was  
3 measured as the length and width of the colony to the nearest 5 cm and colony condition was  
4 estimated as percent of live tissue to the nearest 5%. One sample (1 cm x 1 cm) was taken from the  
5 edge of a lobe on each colony using a hammer and chisel. Each sample was split into two  
6 subsamples and placed in a labelled zip lock bag (only one per colony was used for analysis). On  
7 returning to shore each sample was preserved in 80% alcohol and stored at 4° C until extraction.

8

### 9 *DNA extraction*

10

11 DNA was extracted using the DNeasy kit (Qiagen). Approximately 0.5 cm<sup>2</sup> of tissue was scraped  
12 off each sample with a sterile razor blade and placed in a 1.5 ml microcentrifuge tube. Extraction  
13 was performed overnight at 55° C following the manufacturer's instructions. DNA was quantified  
14 using Nanodrop 3.0.0 spectrophotometer. DNA concentrations ranged from 10 to 45 ng µl<sup>-1</sup>.

15

### 16 *Microsatellite Scoring*

17

18 Severance *et al.* (2004) recently identified seven, polymorphic microsatellite loci for *Montastraea*  
19 *annularis*. Of these seven, six amplified well under our laboratory conditions.

20

21 Two 10 µl multiplex polymerase chain reactions (PCR) were performed per sample to assay a total  
22 of six microsatellite loci (M-I and M-II, Table 1). M-I consisted of 0.2µl each of primer pairs  
23 maMS2-5 (3 µM), maMS11 (3 µM), maMS12 (3 µM) and msMS2-8 (2.5 µM), 1 µl 10x PCR  
24 Reaction Buffer (Promega), 1 µl MgCl<sub>2</sub> (25 mM), 0.2 µl of dNTPs (10 mM), 0.4 µl of Taq-

1 Polymerase ( $5 \text{ U } \mu\text{l}^{-1}$ , Storage Buffer B, Promega) and  $4.6 \text{ } \mu\text{l}$   $\text{H}_2\text{O}$ . M-II consisted of  $0.2 \text{ } \mu\text{l}$  each of  
2 primer pairs maMS2-4 ( $3 \text{ } \mu\text{M}$ ) and maMS8 ( $3 \text{ } \mu\text{M}$ ),  $1 \text{ } \mu\text{l}$  10x PCR Reaction Buffer (Promega),  $1.2$   
3  $\mu\text{l}$   $\text{MgCl}_2$  ( $25 \text{ mM}$ ),  $0.2 \text{ } \mu\text{l}$  of dNTPs ( $10 \text{ mM}$ ),  $0.4 \text{ } \mu\text{l}$  of Taq-Polymerase ( $5 \text{ U } \mu\text{l}^{-1}$ , Storage Buffer  
4 B, Promega) and  $4.8 \text{ } \mu\text{l}$   $\text{H}_2\text{O}$ .  $2 \text{ } \mu\text{l}$  of DNA was added to each reaction. Thermal cycling was carried  
5 out with MJ Research PT200 or PT100 cyclers. M-I cycling conditions consisted of an initial  
6 denaturation step at  $95^\circ \text{C}$  for 2 minutes followed by 35 cycles of  $95^\circ \text{C}$  for 1 minute,  $55^\circ \text{C}$  for 1  
7 minute,  $72^\circ \text{C}$  for 2 minutes and a final step at  $72^\circ \text{C}$  for 7 minutes. M-II cycling conditions  
8 consisted of an initial denaturation step at  $95^\circ \text{C}$  for 2 minutes followed by 35 cycles of  $95^\circ \text{C}$  for 1  
9 minute,  $50^\circ \text{C}$  for 1 minute,  $72^\circ \text{C}$  for 2 minutes and a final step at  $72^\circ \text{C}$  for 7 minutes.

10

11 PCR products were visualized using an ABI 3730 (Applied Biosystems) with an internal size  
12 standard (Gene Scan 500-Liz, Applied Biosystems) for accurate sizing. Electropherograms were  
13 analysed using GeneMapper Software 3.0 (Applied Biosystems) and alleles were scored based on  
14 amplicon size. Due to inconsistent scoring only four of the six microsatellites were used in the  
15 following analysis.

16

17 Analyses

18 *Genotyping*

19

20 Of the 146 samples collected, 137 were successfully genotyped. Samples which had identical alleles  
21 at all four loci were identified as clonemates belonging to the same genet. Identical multilocus  
22 genotypes were never shared between sites, only within sites. The probability of identity ( $P_{ID}$ ) was  
23 calculated to give a conservative estimate of the probability that two individuals sampled from the  
24 same population share a multilocus genotype by chance, not by descent (Waits, Luikart & Taberlet,

1 2001). Biased and unbiased  $P_{ID}$  was calculated for each locus by GIMLET (Valiere, 2002) and then  
2 multiplied across loci to give the combined  $P_{ID}$  (Waits *et al.*, 2001). Due to the low probability of  
3 misidentifying colonies as clonemates when they are not, each distinct multilocus genotype was  
4 only included once in the data set in the following population statistical analyses (Baums, Miller &  
5 Hellberg, 2005). Samples were tested for deviations from the expectations of Hardy-Weinberg  
6 equilibrium and the presence of heterozygote deficiencies and excesses were estimated for each  
7 locus within each population using Genepop (<http://wbiomed.curtin.edu.au/genepop>). Estimations  
8 of linkage disequilibrium between loci and calculations of the number of alleles per locus were  
9 conducted using FSTAT (Goudet, 1995). Micro-checker (Van Oosterhout *et al.*, 2004) was used to  
10 test for the presence of null alleles. Tests of Linkage Disequilibrium (data not shown) and  
11 deviations from Hardy Weinberg Equilibrium (Table 4) were not significant following Bonferroni  
12 corrections (test > 0.05). Further tests failed to reveal null alleles for any of the four loci.

13

#### 14 *Genotypic Diversity*

15

16 Genotypic richness, normalized to sample size, was calculated as  $N_g/N$  (Coffroth *et al.*, 1998),  
17 where  $N_g$  is the number of unique genotypes (genets) and  $N$  is the number of colonies genotyped.  
18 Genotypic richness equals one when all colonies in a population are unique and zero when a  
19 population is dominated by a single genet. Genotypic evenness was calculated as  $G_o/N_g$  (Coffroth *et*  
20 *al.*, 1998), where  $G_o$  is the observed genotypic diversity and  $N_g$  is the number of unique genotypes.  
21  $G_o$  was calculated as

22

23

$$G_o = 1/\sum p_i^2$$

24

1 where  $p_i$  is the frequency of the  $i$ th genotype in the population (Stoddart & Taylor, 1988).  
2 Genotypic evenness equals zero in a population dominated by a single genet and one where each  
3 genet is represented by an equal number of individuals.

#### 4 5 *Spatial Distribution of colonies*

6  
7 The spatial distribution of colonies at each site was mapped onto polar plots using the radial  
8 sampling coordinates. XY distances were then calculated for each colony and the pairwise distances  
9 between clonemates and non-clonemates were calculated. To discriminate the mechanism by which  
10 potential clonemates arose, we assumed that storm-induced colony fragmentation must have  
11 occurred when the separation of clonemates exceeded that of normal adult colony size. If the sum of  
12 colony widths and their separation was less than the width of a normal adult colony (52 cm wide  $\pm$   
13 0.025 cm; based on the average size of colonies in the three populations) it was not possible to  
14 discount origins of partial-colony mortality (though severe colony erosion to the colony base only  
15 occurs rarely, Mumby pers. obs.).

#### 16 17 *Size distribution*

18  
19 The size (area, cm<sup>2</sup>; calculated as colony length multiplied by colony width) distribution of  
20 clonemates versus non-clonemates was analysed across sites and within sites using one way  
21 analysis of variance. Data were normally distributed (Anderson Darling test,  $p > 0.05$ ) with  
22 homogeneous variances (Levene's test,  $p > 0.05$ ).

## 1 **Results**

### 2 *Genotypic Diversity*

3 A total of 137 colonies were successfully genotyped from the three sites identifying 114 individual  
4 genets. Over 90% of genets were represented by a single colony (Fig. 2). Small genets of 2-3  
5 colonies comprised an additional 8% of the overall sample and one genet, at Western Wall, was  
6 composed of 14 clonemates (Fig. 2).

7

8 The density of colonies at each site was similar with 48, 53, and 45 colonies / 78.5 m<sup>2</sup> per site at  
9 Sandy Bay, Seaquest and Western Wall respectively. However, the amount of clonal replication  
10 within populations differed significantly between the three sites (ANOVA F = 18.33, p = 0.003),  
11 with the population at Western Wall having a higher degree of asexual recruitment compared to the  
12 populations at Sandy Bay and Seaquest (Table 2; Table 3). The index of genotypic evenness  
13 ( $G_o/N_g$ ) approached a value of 1 for both Sandy Bay and Seaquest indicating that a high proportion  
14 of genets within these populations are represented by a single colony (Table 2). At Western Wall,  
15 however, this index was less than 0.30, indicating that one or more genets were represented by a  
16 large number of colonies, which implies a higher degree of asexual recruitment within the  
17 population (Table 2). Genotypic richness was almost 1 at Sandy Bay and Seaquest (0.88 and 0.92,  
18 respectively), whereas richness fell to 0.67 at Western Wall indicating fewer colonies with unique  
19 genotypes (Table 2).

20

### 21 *Distance between clonemates*

22

23 The distance between clonemates ranged from a minimum of 0.15 m to a maximum of 6.94 m  
24 (Table 3) and did not differ among sites (Mood's  $\chi^2 = 2.04$ , p = 0.360). Conservatively, we estimate

1 that at least 7 (70%) of the clonal replication events involved breakage of the colony and dispersal  
2 of fragments. The large genet at Western Wall was likely formed through the splitting of a single  
3 colony (Fig. 2) but several of the daughter colonies may have split further by partial mortality.

4

#### 5 *Size Distribution*

6

7 No differences were found between the colony size of clonemates and non-clonemates at Seaquest  
8 and Sandy Bay (ANOVA  $F = 0.78$ ,  $p = 0.381$ , for pooled data though same conclusion was borne  
9 out at individual sites). Clonemates were significantly smaller than non-clonemates at Western Wall  
10 ( $0.37 \pm 0.23 \text{ m}^2$  and  $0.74 \pm 0.25 \text{ m}^2$  respectively; ANOVA  $F = 5.40$ ,  $p = 0.025$ ). This result was  
11 caused by the large (14 colony) genet at this site as no differences occurred between clonemates and  
12 non-clonemates of the remaining colonies (ANOVA  $F = 2.27$ ,  $p = 0.143$ ).

1 **Discussion**

2 The recent isolation and development of polymorphic microsatellite loci for *Montastraea annularis*  
3 (Severance *et al.*, 2004) enabled us to quantify the incidence of clones in this long-lived coral  
4 species for the first time. All three sites exhibited clonal replication with 8% of genets comprising  
5 two to three clonemates and a single genet consisting of fourteen clonemates. While sexual  
6 reproduction appears to be the predominant mode of reproduction in *M. annularis*, this massive  
7 coral can propagate asexually in a manner consistent with colony breakage during storms.

8  
9 At least 70% of multi-colony genets observed must have been caused by physical breakage and  
10 dispersal, providing the first genetic evidence of this process in massive corals. In general,  
11 surviving fragments of colonies do not move far from one another or from the parent colony, with a  
12 maximum recorded separation of ca 7 m over the 10 m maximum sampling scale employed in this  
13 study. This mechanism of clonemate creation is likely to involve considerable trauma and  
14 potentially generate damaged areas of tissue that could have elevated susceptibility to disease. If the  
15 formation of clonemates was associated with a decrease in growth rate due to the metabolic expense  
16 of repairing the trauma, the average size of clones would be expected to be less than that of non-  
17 clonemates. However, since the size distributions of clonemates and non-clonemates were generally  
18 indistinguishable (with the exception of the large genet at Western Wall), our data suggest that  
19 clones can still attain normal adult size through asexual reproduction. It will be interesting, in  
20 future, to compare the size-based survival of new clones (ramets) to those generated by sexual  
21 reproduction and recruitment.

22  
23 Whilst this study was based only on a single reef system, our data suggest that sexual reproduction  
24 predominates in *M. annularis*. Given the assumption that this species utilises a storage effect,

1 current changes occurring across reefs of the Caribbean are a potential threat to its long-term  
2 persistence. Recruits are unable to settle on macroalgae (Diaz-Pulido & McCook, 2004) and the  
3 abundance of such plants is increasing markedly in parts of the region (Gardner *et al.*, 2003).  
4 Macroalgal populations can increase when their principal competitor for space (corals) experience  
5 mortality and where grazing levels are unable to prevent dead coral substratum being colonised.  
6 Unfortunately, both factors that favour macroalgal establishment have increased in the last 25 years  
7 or so. Coral mortality rates have increased, largely through outbreaks of disease (Aronson & Precht,  
8 2001) and mass coral bleaching (McField, 1999). Grazing levels have decreased because of a  
9 region-wide mass mortality of the keystone herbivorous urchin *Diadema antillarum* in the early  
10 1980's (Lessios, 1988) and overfishing of herbivorous fishes (Hughes, 1994). Therefore, favourable  
11 conditions for the recruitment of sexual propagules has declined in the Caribbean and may become  
12 worse given anticipated increases in the incidence of mass coral bleaching as the oceans continue to  
13 warm (Hoegh-Guldberg, 2004). Importantly, reductions in the success of sexual reproduction in  
14 massive corals may result in greater importance being placed on asexual methods. The dispersal of  
15 colonies through asexual fragmentation confers several advantages to the population including; (1)  
16 daughter colonies are substantially larger than sexual recruits and, being elevated above the  
17 substrate away from macroalgae, greatly increases their chances of survival, and (2) daughter  
18 colonies do not require macroalgal-free areas in order to settle (i.e. a coral fragment can fall on  
19 established macroalgae and much of the upper coral tissue can continue to grow without macroalgal  
20 contact – PJM pers. obs.).

21

22 We have shown that asexual reproduction can occur within populations of the massive coral *M.*  
23 *annularis*, but the limited scale of this study prevents us understanding the overall importance of  
24 this process in different environments. Corals were sampled within 10 m plots largely because we

1 did not expect clonemates to be distributed over more than a few metres (if at all). However, the  
2 spread of some genets was relatively large and it is likely that several genets extended beyond plot  
3 boundaries. Our observations of the spread of colony fragments are therefore conservative.  
4 Nevertheless, *M. annularis* exhibits an unexpected clonal structure. We are currently working on a  
5 much larger scale project to document the extent of clonal structure in different reef environments.  
6 Intriguingly, the large, 14-colony genet was found at the site with the greatest wave exposure. We  
7 hypothesize, therefore, that the importance of asexual reproduction will be positively correlated to  
8 the incidence of physical disturbance and therefore to the incidence of hurricanes on Caribbean  
9 reefs. It is interesting to note, however, that variation in clonal structure within populations of  
10 *Acropora palmata* is not correlated to the incidence of hurricanes (Baums *et al.*, in press). It has  
11 been suggested that branching corals have adapted to use hurricanes as a means of dispersal  
12 (Highsmith, 1982), whereas the effects of acute disturbance upon massive corals may be more  
13 complex and only significant under particularly stressful conditions. Given current changes in  
14 climatic conditions and unprecedented anthropogenic disturbance to reef ecosystems, it is now  
15 necessary to re-examine the importance of sexual versus asexual reproduction in long-lived coral  
16 species.

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## Tables

Table 1: Characteristics of microsatellite markers for *Montastraea spp.*

Multiplex	Locus	Primer sequence (5'-3')	MgCl <sub>2</sub> (mM)	Annealing temp.(°C)	Product size (bp)	Reference
I	maMS2-5 F	PET-TTGAAGTAAACAGTACGGAAAGG	2.5	55	270–336	Severance et al. 2004
I	maMS2-5 R	TTCATGTAAACCTGTCGCTGTC				
I	maMS11 F	NED-CAGACGGATTAATAGTCTCCCA	2.5	55	308 – 364	Severance et al. 2004
I	maMS11 R	GACGAATTTTGCCGAGTCAC				
I	maMS12 F	VIC-GGACCTAAACGGGAACACAA	2.5	55	240 - 302	Severance et al. 2004
I	maMS12 R	GAAAGGCTATTCAAAGCTGGG				
I	maMS2-8 F	6-FAM-CCCCTTTGTACACATCTTTC	2.5	55	175 - 229	Severance et al. 2004
I	maMS2-8 R	ATGAAGGATAGGCCGCACT				
II	maMS2-4 F	VIC-TGCTTTGACAGCTACGCAAT	3	50	290 - 330	Severance et al. 2004
II	maMS2-4 R	CCGGGAATTTAGCTATTTGG				
II	maMS8 F	6-FAM-TCTTGCCTATCAGCAGAGGAG	3	50	195 – 222	Severance et al. 2004
II	maMS8 R	TCTGCAAACCAATGTACCATCT				

Table 2: Genotypic diversity summary of *Montastraea annularis* colonies sampled at three sites in Honduras. Total area sampled at each reef always equals 78.5 m<sup>2</sup>. Num Col: number of colonies within sampling plot, Col Dens: number of colonies m<sup>-2</sup>, Genet Dens: number of genets m<sup>-2</sup>. N: number of colonies genotyped, N<sub>g</sub>: number of unique genotypes (genets), N<sub>g</sub>/N: genotypic richness, G<sub>o</sub>: observed genotypic diversity, G<sub>o</sub>/N<sub>g</sub>: genotypic evenness.

<b>Region</b>	<b>Reef Name</b>	<b>Num Col</b>	<b>Col Dens</b>	<b>Genet Dens</b>	<b>N</b>	<b>N<sub>g</sub></b>	<b>N<sub>g</sub>/N</b>	<b>G<sub>o</sub></b>	<b>G<sub>o</sub>/N<sub>g</sub></b>
Honduras	Sandy Bay	48	0.61	0.47	42	37	0.88	33.92	0.92
Honduras	Seaquest	53	0.68	0.61	52	48	0.92	43.61	0.91
Honduras	Western Wall	45	0.57	0.37	43	29	0.67	8.15	0.28
<b>Total</b>	3 reefs	146			137	114			
<b>Average</b>		48.7	0.62	0.48	45.7	38	0.82	28.6	0.70
<b>SD</b>		4.04	0.06	0.12	5.51	9.54	0.13	18.3	0.37

Table 3: Clonal structure summary of *Montastraea annularis* colonies sampled at three sites in Honduras. Genet size represents number of individual colonies per genet. Min denotes minimum value, Max denotes Maximum value and SE denotes Standard Error.

Genet size		Sandy Bay	Seaquest	Western Wall
		Frequency of Genet size		
14		0	0	1
3		0	1	0
2		5	2	1
1		32	45	27
<b>Mean number of colonies per genet</b>		1.14	1.08	1.48
<b>Distance between clonemates (m)</b>	<b>Mean <math>\pm</math> SE</b>	1.01 $\pm$ 0.61	3.30 $\pm$ 1.16	2.01 $\pm$ 0.10
	<b>Min</b>	0.15	0.17	0.27
	<b>Max</b>	3.38	6.94	4.60

Table 4: Characteristics of *Montastraea annularis* microsatellite markers for three sites in Honduras. Given are the number of samples per site ( $N$ ), the number of observed heterozygotes ( $H_o$ ), the number of expected heterozygotes ( $H_e$ ) and the number of alleles ( $A$ ) per locus and site. SB: Sandy Bay, SQ: Seaquest, WW: Western Wall. The presence of heterozygote deficits (HD) and heterozygote excess (HE), and their associated p-value, for each locus at each site was estimated using Genepop (<http://wbiomed.curtin.edu.au/genepop/>). The probability of identity ( $P_{ID}$ ) was calculated using GIMLET (Valiere, 2002). Only unique multi-locus genotypes were included in the analysis.

Locus	Characteristic	SB	SQ	WW	$P_{ID}$ Biased	$P_{ID}$ Unbiased
	N					
<b>maMS2-5</b>	$H_e$	33	42	25	0.027	0.024
	$H_o$	34	41	24		
	$A$	15	14	12		
	HD p-value	0.818	0.212	0.364		
	HD SE	0.026	0.026	0.023		
	HE p-value	0.205	0.812	0.672		
	HE SE	0.027	0.023	0.030		
<b>maMS2-8</b>	$H_e$	28	38	23	0.074	0.070
	$H_o$	29	36	23		
	$A$	11	11	11		
	HD p-value	0.521	0.111	0.225		
	HD SE	0.031	0.016	0.022		
	HE p-value	0.518	0.846	0.851		
	HE SE	0.032	0.017	0.019		
<b>maMS2-4</b>	$H_e$	32	36	23	0.062	0.057
	$H_o$	32	37	25		
	$A$	10	13	8		
	HD p-value	0.575	0.726	0.838		
	HD SE	0.018	0.033	0.011		
	HE p-value	0.468	0.215	0.187		
	HE SE	0.019	0.030	0.013		
<b>maMS8</b>	$H_e$	7	10	2	0.703	0.698
	$H_o$	5	9	2		
	$A$	4	5	3		
	HD p-value	0.130	0.196	1		
	HD SE	0.007	0.013	<0.001		
	HE p-value	0.984	0.941	0.982		
	HE SE	0.003	0.007	0.002		
<b>Combined <math>P_{ID}</math></b>					$8.7 \times 10^{-6}$	$6.6 \times 10^{-6}$

1 **Figure Legends**

2

3 Fig. 1: Representation of *Montastraea annularis* colonies on a coral reef highlighting the difference  
4 in scale between intra-colony fission of coral tissue and the generation of new individual colonies  
5 by sexual reproduction or storm-induced asexual fragmentation. Scale: 1cm = 30cm.

6

7 Fig. 2: Polar plots of *Montastraea annularis* populations at (a) Sandy Bay, (b) Sequest and (c)  
8 Western Wall. Each mark represents a colony. Genets represented by a single individual are  
9 indicated in red. Individuals of the same genet are indicated by the same colour. Size classes are  
10 denoted by shape. Radial axis represents distance in m; angular axis represents angle in degrees.  
11 Number of colonies is 42, 52 and 43, respectively. All *Montastraea annularis* colonies present in  
12 the circle were sampled.

13

Fig. 1

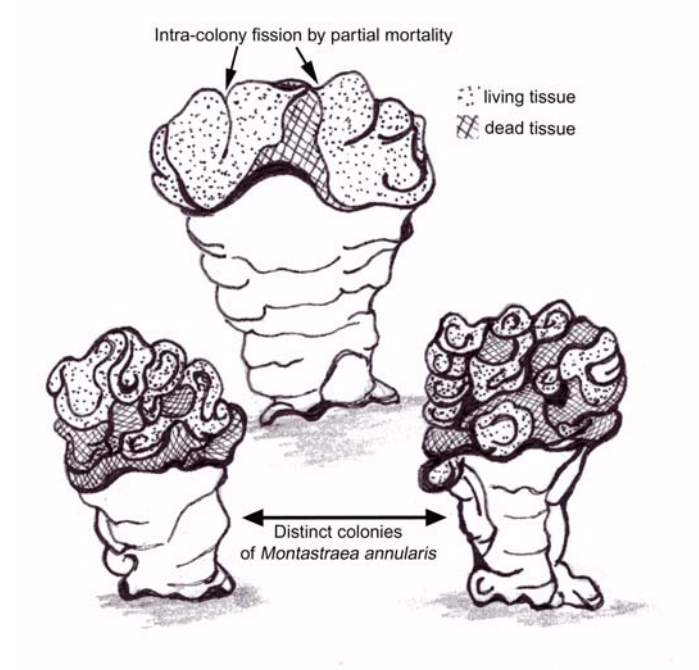
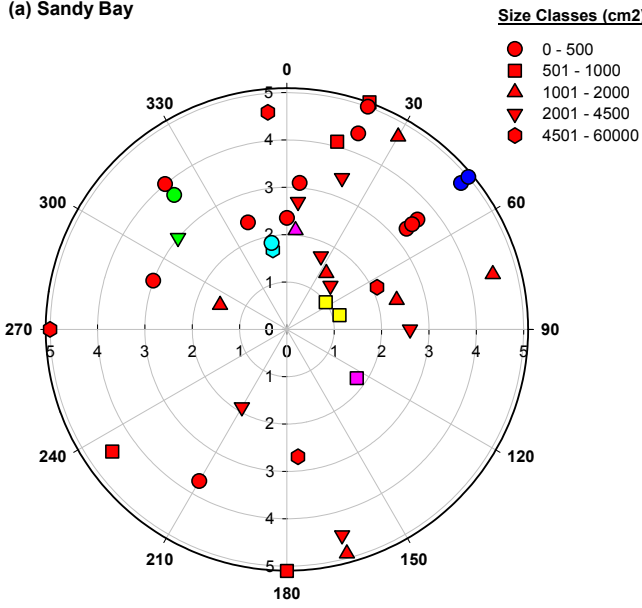
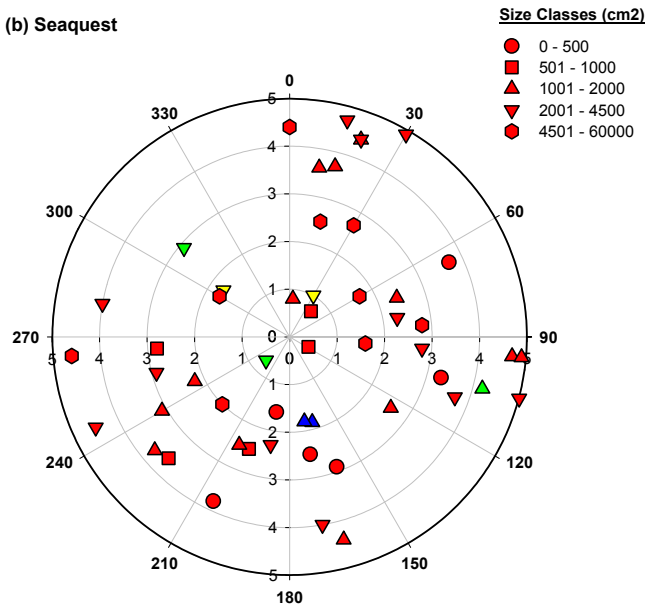


Fig. 2

(a) Sandy Bay



(b) Seaquest



(c) Western Wall

