Somatic Mutation Is a Function of Clone Size and Depth in *Orbicella* Reef-Building Corals

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Abstract. In modular organisms, the propagation of genetic variability within a clonal unit can alter the scale at which ecological and evolutionary processes operate. Genetic variation within an individual primarily arises through the accretion of somatic mutations over time, leading to genetic mosaicism. Here, we assess the prevalence of intraorganismal genetic variation and potential mechanisms influencing the degree of genetic mosaicism in the reef corals *Orbicella franksi* and *Orbicella annularis*. Colonies of both species, encompassing a range of coral sizes and depths, were sampled multiple times and genotyped at the same microsatellite loci to detect intraorganismal genetic variation. Genetic mosaicism was detected in 38% of corals evaluated, and mutation frequency was found to be positively related with clonal size and negatively associated with coral depth. We suggest that larger clones experience a greater number of somatic cell divisions and consequently have an elevated potential to accumulate mutations. Furthermore, corals at shallower depths may be exposed to abiotic conditions such as elevated thermal regimes, which promote increased mutation rates. The results highlight the pervasiveness of intraorganismal genetic variation in reef-building corals and emphasize potential mechanisms generating somatic mutations in modular organisms.

Introduction

The unit of biological organization where genetic variation occurs is a critical component of evolutionary processes and the conceptualization of individuality (Buss, 1983; Okasha, 2006). Individuals are often considered the principal unit of natural selection and are defined by entailing genetic uniqueness, homogeneity, and physiological autonomy (Santelices, 1999). However, numerous organisms violate one or more of these attributes and consequently may highlight levels of selection and ecological interactions not often considered in classical biology (Okasha, 2006).

Genetic variability within an organism can come about due to fusion between distinct individuals (chimerism) and/or through the accumulation of mutations, resulting in genetic mosaicism (Gill *et al.*, 1995; Barki *et al.*, 2002). Generally, only mutations in the germ line are considered to have evolutionary significance, because somatic cells do not typically contribute to gametic formation (Weismann, 1892). However, this may not apply to many organisms, including fungi, plants, and clonal animals, where somatic mutations may be incorporated into asexual daughter cells, gametes, and resulting offspring (Inagaki *et al.*, 1996; Schweinsberg *et al.*, 2014). Hence, in some organisms somatic mutations may alter the unit of organization at which biological processes operate. For example, somatic mutations are known to accumulate in clonal seaweeds and long-lived plants, leading to phenotypic variability within individuals and to intraorganismal selection, which is often referred to as diplontic or somatic selection (Gill, 1986; Santelices *et al.*, 1995). Somatic mutations can reduce organismal fitness, and their accretion is often considered a proponent of extinction in asexual lineages (Gabriel *et al.*, 1993). Alternatively, somatic mutations may be neutral or advantageous, and they have been viewed as a potential mechanism for seaweeds and trees to adapt intragenerationally in response to herbivores and environmental pressures (Gill, 1986; Monro and Poore, 2009; Padovan *et al.*, 2013). While the occurrence and phenotypic consequences of genetic mosaicism have been explored in plants and algae, the implications of intraorganismal genetic variation in clonal animals such as reef corals have only recently become appreciated.

Reef-building corals are largely colonial animals comprised of asexually formed polyps originally derived from a single

sexually produced larva. Thus, a genetic "individual" can be characterized as all modules, physiologically connected or not, originating from the same zygote (Jackson and Coates, 1986). Unfortunately, corals have suffered global losses in abundance over the past several decades due to natural and anthropogenic stressors (Pandolfi *et al.*, 2003; Carpenter *et al.*, 2008). As a result, genetic bottlenecks threaten the resiliency of coral reef ecosystems and may constrain the adaptability of reef corals facing global climate change (Baums, 2008). Somatic mutations have been identified as a potential hidden source of genetic variability that may aid the recovery of declining coral populations (van Oppen *et al.*, 2011), but the evolutionary significance of somatic mutations is difficult to assess because few studies have evaluated their prevalence *in situ* (but see Maier *et al.*, 2012; Schweinsberg *et al.*, 2015; Barfield *et al.*, 2016; Devlin-Durante *et al.*, 2016).

Interspecific differences in the occurrence of somatic mutations have been highlighted in reef corals (Schweinsberg *et al.*, 2015), but identifying biological and/or environmental mechanisms influencing the degree of genetic mosaicism remains elusive. Somatic mutations may be expected to be more abundant in large clonal organisms relative to smaller-statured species because of the increased number of mitotic events required to reach large size (Scofield and Schultz, 2006). However, within closely related species, fragmentation and the degeneration of tissue may muddle the relationship between clonal size and the prevalence of mutations (Babcock, 1991; Ally *et al.*, 2008). Furthermore, correlations between the intensity and spectrum of ultraviolet radiation, water temperature, and ocean depth suggest that individuals occupying shallower habitats may be more susceptible to oxidative stress-induced DNA damage (Lesser, 2010) and perhaps mutation. Although clonal size has been implicated in genetic mosaicism (Gill *et al.*, 1995; van Oppen *et al.*, 2011), and exposure to abiotic stressors is known to incite mutation (Cullis, 1987; Jackson *et al.*, 1998), a relationship between clonal size, oceanic depth, and intraorganismal genetic variation in natural populations has not been previously investigated. Prior studies have identified interspecific differences in the frequency of observed somatic mutations (Schweinsberg *et al.*, 2015), but it is not clear whether this is driven by disparities in the mutational properties of separate markers, environmental effects, or true species variation in the rate of mutation accumulation.

Here, we evaluate the prevalence of intraorganismal genetic variation in two closely related species of scleractinian corals in the *Orbicella* species complex. *Orbicella annularis* and *Orbicella franksi* are considered to be two of the most important framework builders on Caribbean coral reefs, but they have sustained region-wide declines in past decades (Weil and Knowlton, 1994; Hughes and Tanner, 2000; Edmunds and Elahi, 2007). The two species recently diverged between 0.5 and 2.5 million years ago, are gametically compatible and capable of hybridization, and share conserved microsatellite loci (Fukami *et al.*, 2004; Severance *et al.*, 2004; Levitan *et al.*,

2011; Davies *et al.*, 2013). Typically, *O. franksi* colonies are smaller and occur at greater depths than *O. annularis* (Weil and Knowlton, 1994), but at the site of this study, these species are in close proximity and overlap in size and depth distributions. These characteristics provide the unique opportunity to evaluate patterns of intraorganismal genetic variation in the same loci across a broad range of coral sizes and depths. Considering the potential evolutionary significance of somatic mutations in clonal organisms, we aim to assess the pervasiveness of intraorganismal genetic variation in these species and to determine whether the occurrence of somatic mutations is related to clonal size and an individual's previous exposure to abiotic stressors *via* a depth gradient.

Materials and Methods

Sampling

This study was conducted on a permanent transect in Bocas del Toro, Panama (9°19′38″N, 82°12′14″W), previously described by Levitan *et al.* (2011). Briefly, the monitored reef is primarily composed of *Orbicella franksi* (Gregory, 1895) and *Orbicella annularis* (Ellis & Solander, 1786), it is about 100 m long by 30 m wide, and it follows a depth contour of 2.5–8 m. In this location, the two species differ in clonal structure: *O. franksi* individuals are comprised of physically intact colonies, whereas the majority of *O. annularis* colonies have fragmented into groups of asexually produced but spatially separated daughter colonies. The entirety of ramets derived from the same colony is commonly referred to as a genet or clone (Jackson *et al.*, 1985). The species identity, position, and genotype of *O. franksi* colonies and *O. annularis* ramets and clones on this reef have previously been mapped using numerical tags (Levitan *et al.*, 2004, 2011).

To evaluate the prevalence of intraorganismal genetic variation in *O. franksi* and *O. annularis*, 29 genetic individuals encompassing a range of depths and coral sizes were sampled multiple times with a metal core (1.5 cm in diameter), resulting in a total of 193 tissue samples (Fig. 1). Specifically, 18 genetically distinct *O. franksi* colonies were chosen based on depth (3.35–6.71 m) and size (0.05–1.41 m²), and they were sampled 4 times per colony from the north, east, south, and west extents of the coral, resulting in 72 tissue samples. Thus, the distance between cores for a particular coral was dependent on the size of the colony being evaluated. Effort was made to ensure that there was continuous tissue between cores, to avoid the possibility of comparing individuals derived from distinct settlement events.

In *O. annularis*, we sampled independent ramets of fragmented individuals and used the sampling strategy described for *O. franksi* for intact clones. In total, we evaluated 11 *O. annularis* clones based on depth (2.56–4.86 m) and size (0.37–8.76 m²). Due to differences in growth form and number of ramets per genetic individual, *O. annularis* clones were sam-
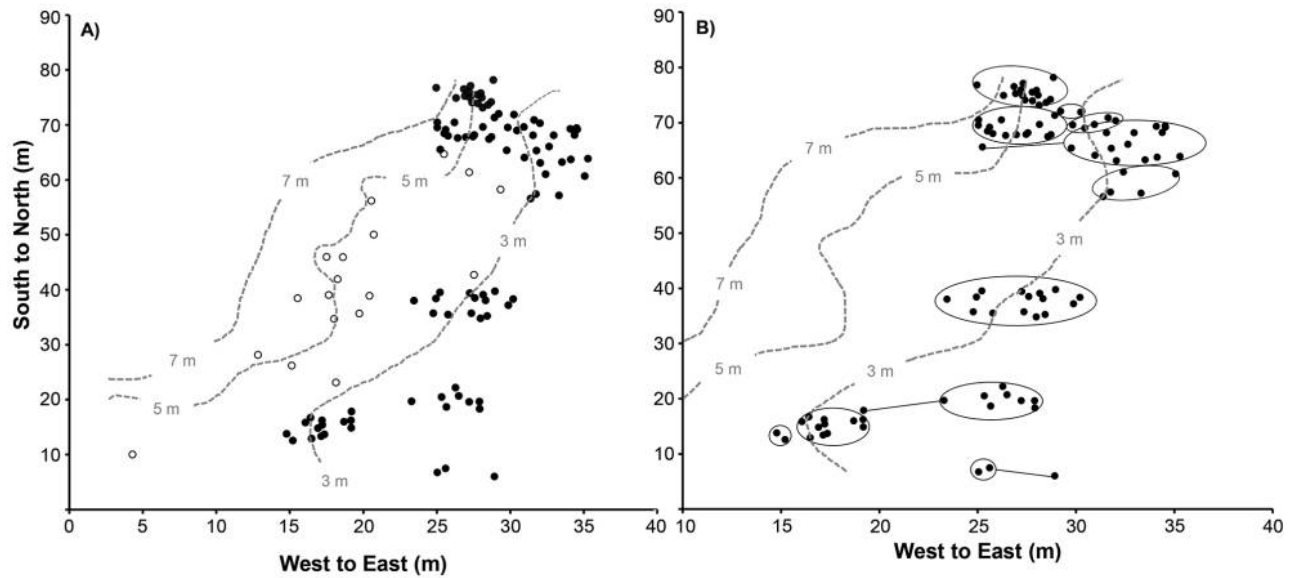
Figure 1. Map of sampled corals. (A) Open circles represent 18 *Orbicella franksi* colonies; filled circles are ramets of 11 *Orbicella annularis* clones. (B) *Orbicella annularis* ramets, ovals, and lines connect ramets originating from the same clone. Dashed gray lines represent depth in each panel.

pled unevenly, with 2–19 cores per clone (average = 11.0, SE ± 1.9), resulting in 121 tissue samples.

Coral size was estimated in *O. franksi* by using measurements of colony height, longest diameter, and perpendicular diameter. Surface area was approximated using these measurements, estimated as an elliptical hemisphere. In *O. annularis*, coral size was estimated using the relative position of mapped ramets along the 100 m × 30 m transect. Ramet position was plotted, and the total area encompassed by each clone was approximated in triplicate, using the tracing function in Image J (https://imagej.nih.gov/ij/). Depth was measured in both species to the uppermost extent of each coral, and the average depth for each independent ramet was used in fragmented *O. annularis* clones.

DNA extraction

Tissue samples were partially digested and fixed in 1.5 mL of CHAOS solution (4 mol L^{-1} guanidine thiocyanate, 0.1% N-lauroyl sarcosin sodium, 10 mmol L^{-1} Tris, pH 8, 0.1 mol L^{-1} 2-mercaptoethanol) (Fukami *et al.*, 2004). Samples in CHAOS were left at room temperature for 72 h before being frozen and stored at −20 °C. Digestion was completed by incubating 500 μL of partially digested tissue in 1.5 mL of extraction buffer (440 μL of 60 mmol L^{-1} Tris, pH 8, 500 μL of 100 mmol L^{-1} ethylenediaminetetraacetic acid, 500 μL 0.5% sodium lauryl sulfate, and 60 μL of 1 mg mL^{-1} proteinase K) at 60 °C for about 12 h.

After incubation, DNA was extracted from 500 μL of digested tissue by using a phenol : chloroform protocol. Digested tissue was combined with 500 μL of 1 : 1 liquefied phenol (J. T. Baker, Center Valley, PA) and chloroform (Sigma-Aldrich, St. Louis, MO), inverted for 2 min, and centrifuged at 12,000 relative centrifugal force (RCF) for 10 min. The aqueous upper phase was transferred to a new tube, combined with 500 μL of chloroform, and the resulting aqueous phase was again transferred to a new tube. DNA was precipitated by the addition of 3 mol L^{-1} of sodium acetate and 95% ethanol, and it was pelleted by centrifugation at 12,000 RCF for 10 min. Pelleted DNA was washed with 75% ethanol and resuspended in 100 μL of double-distilled water. DNA concentrations were quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE), and samples were diluted to 10 ng μL^{-1} for use in polymerase chain reactions (PCRs).

Microsatellite genotyping

Four microsatellite loci were used for genotyping all *O. franksi* and *O. annularis* tissue samples (maMS8, maMS11, maMS12, and maMS2–8). An additional 6 loci were used to genotype all of the 72 *O. franksi* tissue samples and 24 of the 121 *O. annularis* cores (Table 1). The PCR master mixes consisted of 2.4 μL 5X PCR buffer (Promega, Madison, WI), 1.2 μL 1 mmol L^{-1} deoxyribonucleoside triphosphate, 0.15 μL GoTaq polymerase (Promega, Madison, WI), 1.0 μL 10 μmol L^{-1} bovine serum albumin, 1.2–3.5 μL MgCl$_2$ (depending on primer), 1.0 μL fluorescently labeled forward primer, 1.0 μL reverse primer, 1.5 μL DNA (5–10 ng μL^{-1}), and double-distilled water, to bring the total volume to 12 μL. The PCR amplification was conducted under the following cycling parameters: 94 °C for 5 min, 35 cycles of 94 °C for

Summary of 10 polymorphic microsatellite loci used to assess intraorganismal genetic variation in Orbicella franksi *and* Orbicella annularis

Locus (repeat)	Observed range (bp)	n_a	Proportion of mutations	Reference
maMS8[C] (CAA)$_7$	195–204	4	0/181 = 0.00	Severance *et al.*, 2004
maMS11 (GA)$_7$GT(GA)$_5$	308–384	30	33/181 = 0.18	Severance *et al.*, 2004
maMS12[A] (GA)$_{11}$	248–278	16	12/181 = 0.07	Severance *et al.*, 2004
maMS2–8[B] (GTT)$_6$ATT(GTT)$_3$GCT(GTT)$_4$	172–231	14	15/181 = 0.08	Severance *et al.*, 2004
M_fav3[A] (ATG)$_{25}$	143–203	6	0/92 = 0.00	Davies *et al.*, 2013
M_fav5[A] (CGA)$_{17}$	320–413	17	0/92 = 0.00	Davies *et al.*, 2013
M_fav8[C] (CAA)$_{38}$	295–340	12	0/92 = 0.00	Davies *et al.*, 2013
M_fav9[B] (CAAT)$_{21}$	233–299	15	0/92 = 0.00	Davies *et al.*, 2013
M_fav29[D] (CAT)$_{16}$	418–477	14	0/92 = 0.00	Davies *et al.*, 2013
M_fav30[D] (TTTTG)$_8$	227–247	4	0/92 = 0.00	Davies *et al.*, 2013

The top four loci were used on all tissue samples; remaining markers were used on all *O. franksi* samples and a subset of *O. annularis*. Superscript letters A, B, C, and D represent multiplex primer groups. Proportion of mutations represents number of mutations detected divided by number of samples analyzed, with potential chimeras excluded. n_a, number of observed alleles.

40 s, 53 °C (primer M_fav3), 55 °C (primers maMS2–8, maMS8, maMS12, M_fav9, M_fav29, and M_fav30), or 57 °C (primers maMS11, M_fav5, and M_fav8) for 1 min, 72 °C for 1 min, and a final extension time of 72 °C for 10 min. The PCR products from multiple loci were multiplexed (Table 1) with Hi-Di formamide (1 : 20) and 1 μL Genescan 500 ROX (Applied Biosystems, Foster City, CA) and were analyzed with a 3730 Genetic Analyzer with Capillary Electrophoresis (Applied Biosystems, Foster City, CA). Finally, alleles were binned into di-, tri-, tetra- or penta-nucleotide repeats, dependent on locus, and called using Geneious 9 software (Biomatters, Auckland, New Zealand).

Genetic disparities were identified by comparing the genotypic signature of each sample to the most frequent genotype of the coral. Typically, genetic differences represented deviations from the main genotype by a single bin shift (two to five nucleotides, depending on repeat motif) at a single locus, but differences of more than one bin and in more than one locus were detected. Samples containing a genetic deviation were re-amplified with independent PCR reactions and were reanalyzed to exclude allelic differences due to PCR artifacts.

Somatic mutation versus *chimerism*

To evaluate whether genetic disparities represented somatic mutations or were the result of fusion between distinct individuals, we applied a Bayesian clustering analysis, using the program STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). Potential chimeras were identified if cores differed by more than 60% in their cluster assignment probability from other samples of their genet, as suggested by Schweinsberg *et al.* (2015). Runs were conducted with a burn-in period of 100,000 and 100,000 Markov chain Monte Carlo repeats with 3 iterations (*O. franksi* K = 18/*O. annularis* K = 11), using the default settings without a prior. Results from each iteration were merged using CLUMPAK (Kopelman *et al.*, 2015).

Statistical analysis

For each coral not suspected to be a chimera, we calculated the number of cores with at least one mutation and the number of cores that did not contain a mutation relative to the most common genotype of that individual. Thus, a tissue sample could contain mutations in one or two alleles and/or at multiple loci, but it would still be characterized as a single core with a mutation. We modeled the probability of a coral containing mutations by using a binomial generalized linear model (GLM), with number of cores collected, coral species, size, depth, and their interaction terms as predictor variables, and a two-column matrix of the number of cores with and without a mutation as the response variable. All analyses were performed in the R statistical program (R Core Team, 2016). Additionally, the probability of a core harboring mutations at a particular locus was modeled as a function of the number of alleles observed in that microsatellite, using a binomial GLM. Inferences were made using a combination of hypothesis-testing and model-fitting frameworks with Wald's *Z* test statistic (glm command in R) and likelihood ratio testing (lrtest command in the "lmtest" package in R), respectively. Multicollinearity between biotic and abiotic predictors (*i.e.*, coral species, size, and depth) was evaluated using variance inflation factors (vif command in the "car" package in R). Given a significant interaction, prediction lines and Wald-type 95% confidence bands were simulated from the model (visreg command in the "visreg" package in R). When applicable, non-significant predictor variables were removed from the GLM *via* hierarchical model selection to maximize model fit. For all analyses, model assumptions were evaluated as described in Bolker *et al.* (2009).

Results

In total, 58 of the 193 cores taken from both species harbored some genetic difference relative to the respective refer-

ence genotype. Genetic differences were detected exclusively in 3 (maMS11, maMS12, maMS2–8) of the 10 loci, and these 3 markers were amplified in all tissue samples collected (Table 1). The majority of the disparities (43 of 58) represented deviations at a single locus, where remaining loci were identical to other samples from the same clone. The other 15 cores differed at 2 loci but were also identical at remaining markers. All genetic variants in a clone were successfully confirmed with independent PCR amplifications.

Cores containing genetic deviations were found in 13 of the 29 corals tested (45%). Of these, 2 corals had tissue samples that differed by more than 60% in their cluster assignment probability (*Orbicella annularis* clones 1 and 7; Fig. 2). Consequently, we cannot rule out the potential contributions of chimerism to the genetic disparities in these corals, and these individuals were not included in assessments of somatic mutations. Unreported analyses that included these genetic differences as somatic mutations did not alter our findings.

Somatic mutations were detected in 38% (11 of 29) of corals evaluated, and the degree of genetic mosaicism was associated positively with clone size and negatively with coral depth (Fig. 3). Coral species, size, and depth demonstrated some multicollinearity, which can negatively affect model parameterization in multiple regression analyses (Graham, 2003). To explore this, we mean-centered continuous predictors to reduce collinearity prior to conducting a binomial GLM (Table A1; Fig. A1). Qualitatively, the results of analyses with mean-centered predictors were the same as those with the raw data (Table A1), and we consequently report statistical results from analyses with the original data set. Furthermore, comparison of the residual deviance and degrees of freedom in all models suggested an appropriate fit for a binomial GLM.

The probability of a coral containing mutations was not significantly affected by the number of cores collected per individual (GLM: Wald $Z = -0.063$, $P = 0.950$), and it did not vary significantly between the two species (GLM: Wald $Z = 1.050$, $P = 0.294$; Table 2). Also, removing either the number of cores collected (likelihood ratio test [LRT]: df = 1, $\chi^2 = 0.004$, $P = 0.950$) or species (LRT: df = 4, $\chi^2 = 1.090$, $P = 0.300$) from the GLM did not reduce model fit, whereas removing coral size (LRT: df = 2, $\chi^2 = 11.336$, $P = 0.004$) or depth (LRT: df = 2, $\chi^2 = 11.333$, $P = 0.004$) significantly reduced model fit. Thus, the number of cores collected and coral species were removed from the statistical model, resulting in a binomial GLM comprised of size, depth, and their interaction term as predictor variables (Table 3).

The interaction between size and depth was significant (GLM: Wald $Z = 3.639$, $P < 0.001$), and removing the term significantly reduced model fit (LRT: df = 1, $\chi^2 = 18.761$, $P < 0.001$). To evaluate the interaction, we simulated the mutation frequency predicted by the model by using parameter estimates from the binomial GLM (Table 3) and either fixed values of depth or clone size across the range of observed clone sizes and depths (Fig. 4). Mutation frequency was predicted to increase for both shallow (25th quantile of observed depths) and deep (75th quantile of observed depths) corals with increasing size (Fig. 4A). Moreover, the slopes of each prediction were transverse and highlighted the root of the interaction between shallow and deep corals. Mutation frequency was predicted to decrease for both small (25th quantile of observed coral sizes) and large (75th quantile of observed coral sizes) corals with increasing depth in a fairly parallel fashion (Fig. 4B). While the majority of mutations occurred in a single
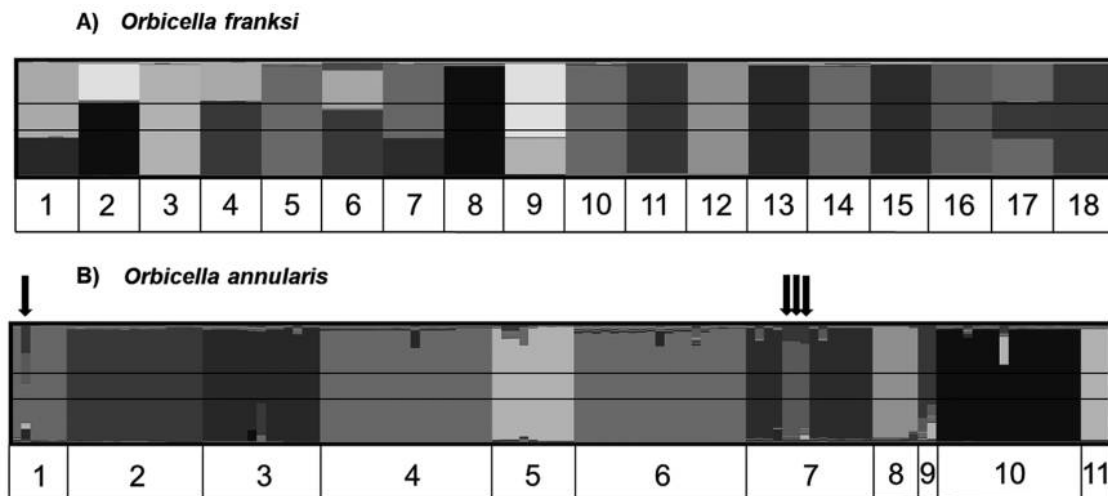

Figure 2. Bayesian clustering analysis used to characterize genetic deviations as either somatic mutations or evidence of chimerism in (A) 18 *Orbicella franksi* colonies and (B) 11 *Orbicella annularis* clones. Each vertical bar represents a single core, and horizontal lines indicate the threshold for characterizing deviations as chimeras or somatic mutations. Arrows indicate cores that have a <40% assignment probability to the respective genet. These corals represent potential chimeras.
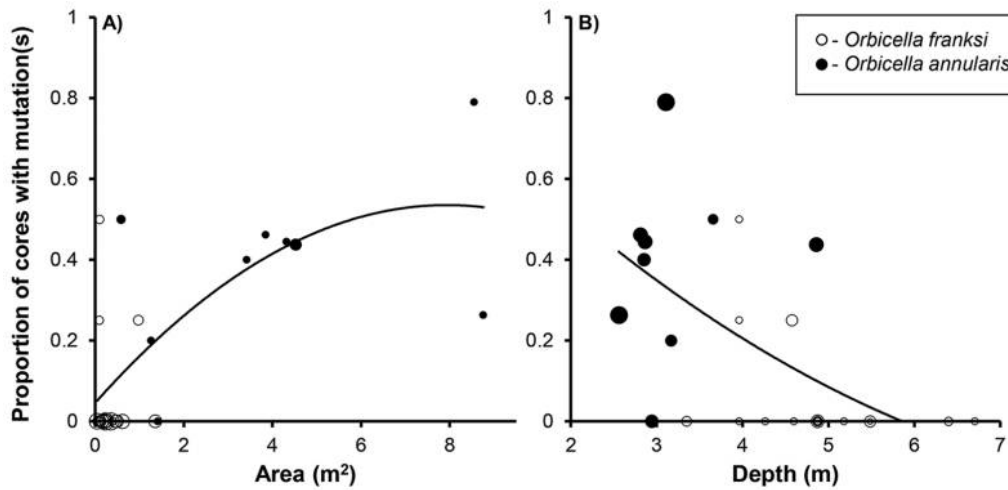
Figure 3.   Proportion of cores with mutation(s) in *Orbicella franksi* (open symbols) and *Orbicella annularis* (filled symbols) as a function of (A) coral size and (B) depth, with potential chimeras excluded. Symbol size in (A) is scaled proportionally to coral depth, where larger symbols are deeper and smaller symbols represent shallower individuals. Symbol size in (B) is scaled proportionally to coral size, where larger symbols represent larger corals and smaller symbols are smaller corals. Curves represent quadratic best fit for visual purposes.

locus (maMS11), unreported analyses that sequentially removed loci from the statistical model did not alter our findings associated with the prevalence of mutations in relation to coral size and depth. Thus, the described mutational patterns were largely consistent across those loci harboring mutations, and they were not entirely driven by any one locus. Six loci (M_fav3, M_fav5, M_fav8, M_fav9, M_fav29, and M_fav30) were amplified in a subset of tissue samples and did not harbor mutations. These loci were included in the study to test for a relationship between locus polymorphism and the frequency of mutation.

All loci evaluated were polymorphic, and the number of alleles observed for each microsatellite ranged from 4 to 30 (Table 1). Mutation frequency for a particular locus was plotted as a function of the number of alleles observed for that microsatellite (Fig. 5). The probability of a microsatellite containing a mutation was positively associated with the degree

of locus polymorphism (GLM: Wald $Z = 8.180$, $P = 0.001$), and removing the predictor variable significantly reduced model fit compared to the intercept alone (LRT: df $= 1$, $\chi^2 = 74.223$, $P < 0.001$).

Discussion

The proliferation of genetic variability within a clonal organism has the capacity to alter the unit of biological organization at which ecological and evolutionary processes operate (Buss, 1983; Gill *et al.*, 1995). We found intraorganismal genetic variation to be common in reef-building corals of the *Orbicella* species complex, with 45% of individuals harboring more than one multilocus genotype. The predominant underlying cause of intraorganismal genetic variation was attributed to somatic mutations, and our results highlight potential mechanisms contributing to this genetic mosaicism. Mutation frequency increased

Table 2

Statistical summary of binomial generalized linear model and Wald Z test statistic evaluating the probability of a coral containing mutation(s) as a function of number of cores collected per individual, coral species, size, depth, and their interaction term

Coefficients	Estimate	SE	Z	P
Intercept	6.038	2.669	2.263	**0.024**
Cores collected	−0.006	0.092	−0.063	0.950
Species	1.222	1.164	1.050	0.294
Size	−1.489	0.531	−2.806	**0.005**
Depth	−2.385	0.854	−2.793	**0.005**
Size × depth	0.563	0.186	3.033	**0.002**

Significant *P*-values are shown in bold.

Table 3

Statistical summary of binomial generalized linear model and Wald Z test statistic evaluating the probability of a coral containing mutation(s) as a function of coral size, depth, and their interaction term

Coefficients	Estimate	SE	Z	P
Intercept	4.120	1.872	2.244	**0.025**
Size	−1.102	0.366	−3.015	**0.002**
Depth	−1.699	0.531	−3.200	**0.001**
Size × depth	0.416	0.114	3.639	**<0.001**

Significant *P*-values are shown in bold.

in corals of greater size and in those occupying shallower depths. We ascribe the increased prevalence of mutations to the greater number of somatic DNA duplication events experienced by larger corals and to abiotic factors correlated with depth, such as temperature, which may influence mutation rates and the degree of genetic mosaicism.

The potential for somatic mutations to produce intraorganismal genetic variation and to influence biological processes has been modeled primarily as a function of the number of mitotic cell divisions experienced by an individual (Orive, 2001; van Oppen *et al.*, 2011; Folse and Roughgarden, 2012). Accordingly, these models predict that the frequency and influence of genetic mosaicism will be most pronounced in relatively large and long-lived organisms such as trees and corals (Gill *et al.*, 1995). In corals, colony size and the amount of asexually produced polyps contained within a clone have been used to estimate the number of somatic mutations accrued within an individual (van Oppen *et al.*, 2011). Moreover, somatic muta-

tions in microsatellite loci have been utilized to approximate clonal age in the reef coral *Acropora palmata* (Devlin-Durante *et al.*, 2016), and the probability of detecting intraorganismal genetic variation in long-lived trees is thought to be primarily dependent on size and/or age (Whitham and Slobodchikoff, 1981; Gill *et al.*, 1995). Our results are consistent with the notion that size, age, and, ultimately, the number of somatic cell divisions accumulated within an individual are important factors influencing the occurrence and degree of genetic mosaicism in clonal organisms. It should be acknowledged that the predictive relationship between size and age can be complicated in modular organisms by indeterminate growth, tissue degeneration, fragmentation, and/or fusion (Babcock, 1991; Ally *et al.*, 2008). However, we maintain that size and number of somatic cell divisions are likely to be correlated, and that, on average, larger clones should experience a greater number of mitotic events relative to smaller ones. Thus, we do not aim to tease apart the contributions of clonal size and age to somatic
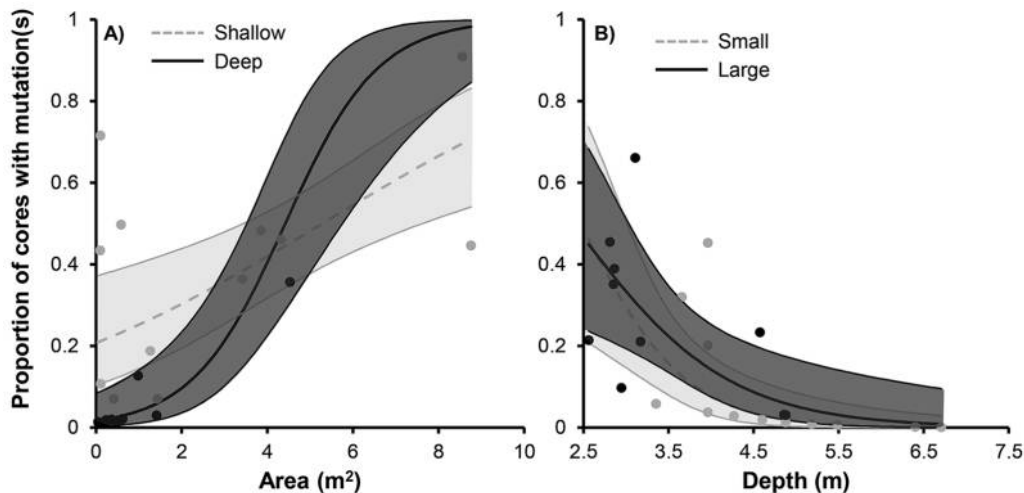

Figure 4. Predictions of the proportion of cores containing mutation(s) in *Orbicella franksi* and *Orbicella annularis* simulated with a binomial generalized linear model incorporating either (A) fixed values of depth and the range of observed coral sizes or (B) fixed values of coral size and the range of observed depths. Fixed values represent either the 25th quantile ("Shallow" and "Small" [dashed line with light gray 95% confidence bands]) or the 75th quantile ("Deep" and "Large" [solid line with dark gray 95% confidence bands]) of measured depths and coral sizes. Symbols represent partial residuals of data falling either below the median (gray) or equal to or greater than the median (black) of observed (A) depths or (B) coral sizes.
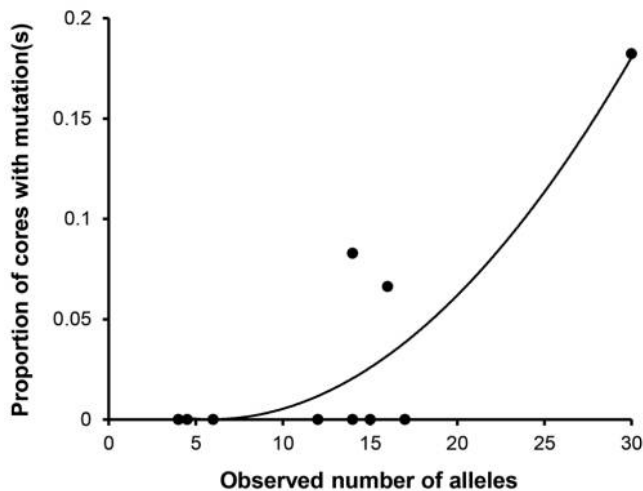
Figure 5. Proportion of cores with mutation(s) in *Orbicella franksi* and *Orbicella annularis* detected by 10 microsatellites as a function of the number of alleles observed per locus. Potential chimeras are excluded. Curve represents quadratic best fit for visual purposes.

mutations; rather, we suggest that larger individuals generally undergo an increased amount of somatic cell divisions and have greater potential to harbor genetic mosaicism as a consequence.

Abiotic conditions such as temperature and ultraviolet (UV) radiation are often correlated with depth in coastal habitats and can induce sublethal stress in marine organisms after prolonged exposure (Dahms and Lee, 2010; Lesser, 2010; Olsen *et al.*, 2013, 2014; Ross *et al.*, 2013). Subjection to elevated temperature and/or UV radiation facilitates the excess production of reduced oxygen intermediates that damage DNA and that can promote mutation (Jackson *et al.*, 1998; Lesser, 2006). Moreover, the physiological response to abiotic stress is often more pronounced in individuals of shallower habitats, where temperature and UV radiation are increased (Shick *et al.*, 1995; Lesser, 2006).

In the same locality as this study, Levitan *et al.* (2014) described a consistent negative correlation between depth and temperature across several years. The authors characterized a bleaching event where host corals expelled their photosynthetic symbiont in response to temperature stress. They found that although mortality was rare, individuals occupying shallower parts of the reef were more likely to bleach and suffered severe losses in reproductive output compared to corals at greater depths. The results of the current study indicate that corals at shallower depths also have a greater propensity to accumulate somatic mutations. Although other factors, such as elevated UV radiation, might contribute to the pattern described here, we find elevated temperature stress to be the most parsimonious explanation for the increase in genetic mosaicism detected with decreasing depth, because mutations induced by UV radiation are often associated with the production of thymine dimers (Cadet *et al.*, 2005). In our study, mutations were not related to the nucleotide composition of the markers. Nonetheless, the described patterns suggest that exposure to abiotic

stress might contribute to the genetic means necessary for reef corals to adapt to increasing sea surface temperatures by producing beneficial mutations. Alternatively, abiotic stress may accelerate the accumulation of deleterious mutations in corals and could exacerbate the negative effects of warming oceans. Primarily, our results highlight the potential for abiotic mechanisms, and perhaps stress, to generate somatic mutations and genetic variation to allow for intraorganismal selection of asexual modules.

Chimerism and genetic mosaicism have been hypothesized to be potential sources of genetic variation that may influence evolutionary processes in long-lived corals (Puill-Stephen *et al.*, 2009; van Oppen *et al.*, 2011). However, the occurrence and degree of intraorganismal genetic variation in reef-building corals have only recently been investigated (Maier *et al.*, 2012; Schweinsberg *et al.*, 2015). In two corals, we could not exclude the potential contributions of chimerism to detected genetic disparities. We caution that although we cannot exclude chimerism as a possibility, our ability to distinguish between fusion and mutation was limited in these cases as a result of the number and degree of polymorphism in the loci evaluated. Nonetheless, mosaicism was found to be the primary cause of genetic discrepancies, with 38% of corals harboring at least a single somatic mutation. Similarly, intraorganismal genetic variation has been reported to vary between 17% and 46% of individuals in 6 different reef-building corals where mosaicism was the predominant underlying mechanism (Maier *et al.*, 2012; Schweinsberg *et al.*, 2015). Moreover, the degree of genetic mosaicism was pronounced in some corals of this study. The ramets of one particular *Orbicella annularis* clone represented 9 unique multilocus genotypes and contained deviations from the most common genotypic signature in 79% of the cores sampled. These results suggest that genetic mosaicism can be appreciable in reef corals and that it at least has the potential to influence their evolution through somatic selection (van Oppen *et al.*, 2011).

The notion that intraorganismal genetic variation may alter the units of selection in reef-building corals is supported in seaweeds and long-lived terrestrial plants, where recent work suggests that mosaics exhibit phenotypic variability and somatic selection in response to environmental pressures (Monro and Poore, 2009; Folse and Roughgarden, 2012; Padovan *et al.*, 2013). A similar mechanism has been proposed for reef corals with intraclonal differences in their susceptibility to ocean acidification and thermal stress (van Oppen *et al.*, 2011). If somatic mutations are proliferated within colonies *via* asexual propagation and if genetically distinct units have differential survival, then modules harboring non-deleterious mutations may be favored (Monro and Poor, 2009). Hence, in heterogenous corals, selection may operate on the scale of genetically distinct modules rather than at the level of the colony. Furthermore, mosaicism has been found to amplify the standing genetic variation in populations of red algae, seagrasses, sponges, and fire corals (Milleporidae) (Santelices *et al.*, 1995; Blanquer

and Uriz, 2011; Reusch and Bostrom, 2011; Schweinsberg *et al.*, 2017), which could be critical in reef corals suffering from recent population bottlenecks (Baums, 2008). Although mosaicism may inflate genetic variation, the majority of new mutations are likely to be harmful (Keightley and Lynch, 2003), suggesting that corals may become more susceptible to reductions in fitness as mutations accumulate (Gabriel *et al.*, 1993).

Studies evaluating intraorganismal genetic variation in natural populations have primarily used microsatellite loci to detect genetic disparities (Puill-Stephen *et al.*, 2009; Blanquer and Uriz, 2011; Reusch and Bostrom, 2011; Schweinsberg *et al.*, 2015). Microsatellite loci are largely neutral, typically have elevated rates of mutation compared to other genes, and can be subject to genotyping errors (Li *et al.*, 2002; Pompanon *et al.*, 2005). Our study found consistent genotypic signatures from repeated samplings and independent PCR reactions, indicating that genotyping error was inconsequential. Elevated mutation rates in microsatellites suggest that our estimates of mutation per locus and tissue sample are likely to be inflated relative to other genes in the genome; but they provide a potential marker for how the likelihood of somatic mutations, across other loci, may vary with individual size and depth. However, considering that our methodologies exclusively targeted insertion and deletion events, we caution that our results may not necessarily be representative of patterns associated with other forms of mutation.

The evolutionary significance of somatic mutations in clonal organisms is profoundly influenced by their heritability through both sexual and asexual pathways (Buss, 1983; Gill *et al.*, 1995). Mutations in the meristem of modular plants and in the soma of seaweeds have discernible importance because they are proliferated through clonal growth and sexual reproduction (Inagaki *et al.*, 1996; Monro and Poore, 2009; Padovan *et al.*, 2013). In corals, somatic mutations can be propagated through asexual fragmentation and have been shown to be transferable through the gametes of some species (Schweinsberg *et al.*, 2014), but the presence or absence of a segregated germ line across cnidarians is currently unclear (Barfield *et al.*, 2016). The extent to which somatic mutations are transmissible to future generations of scleractinians will have important implications for coral reef ecosystems.

The prevalence of genetic mosaicism detected in two species of the *Orbicella* species complex was best explained by disparities in coral size and depth. Characteristically, *O. annularis* encompasses a greater area and occurs at shallower depths than *Orbicella franksi*, which may raise thoughts about how species-specific mutation rates could have contributed to our results. In addition, sampling effort per individual was typically greater in *O. annularis* compared to *O. franksi*, which also could have biased our detection of somatic mutations. However, this study is unique in that the same loci were targeted across closely related species (Weil and Knowlton, 1994; Levitan *et al.*, 2004, 2011), as opposed to utilizing separate markers for each species with distinct mutational properties. Considering that patterns

of mutation in relation to coral size and depth were consistent across multiple loci, it is unlikely that our results were heavily influenced by interspecific differences. Furthermore, our analysis indicates that sampling effort did not significantly influence the probability of a coral harboring mutations, which coincides with other work that found no systematic effect of the number of cores collected per individual and the degree of genetic mosaicism (Schweinsberg *et al.*, 2015). Accordingly, we contend that the detected patterns of genetic mosaicism are a result of the outlined mechanisms rather than artifacts of sampling effort or inherent interspecific differences. Our results also emphasize the importance of marker-specific disparities in detecting somatic mutations. Specifically, insertion and deletion events were more likely to be detected in microsatellite loci that demonstrated a greater degree of polymorphism. We recommend that locus polymorphism should be considered when comparing interspecific differences in the occurrence of genetic mosaicism as a consequence.

Our results suggest that biotic and environmental mechanisms influence the occurrence of somatic mutations and may be important contributors to seemingly interspecific disparities in genetic mosaicism. We hypothesize that larger individuals have a greater potential to accrue somatic mutations because they experience more somatic DNA duplication events. Furthermore, organisms at shallower depths may have a greater propensity to accumulate mutations as a result of increased exposure to abiotic stress. Further research is needed to determine how genetic mosaicism may influence ecological and evolutionary processes in reef-building corals facing global climate change.

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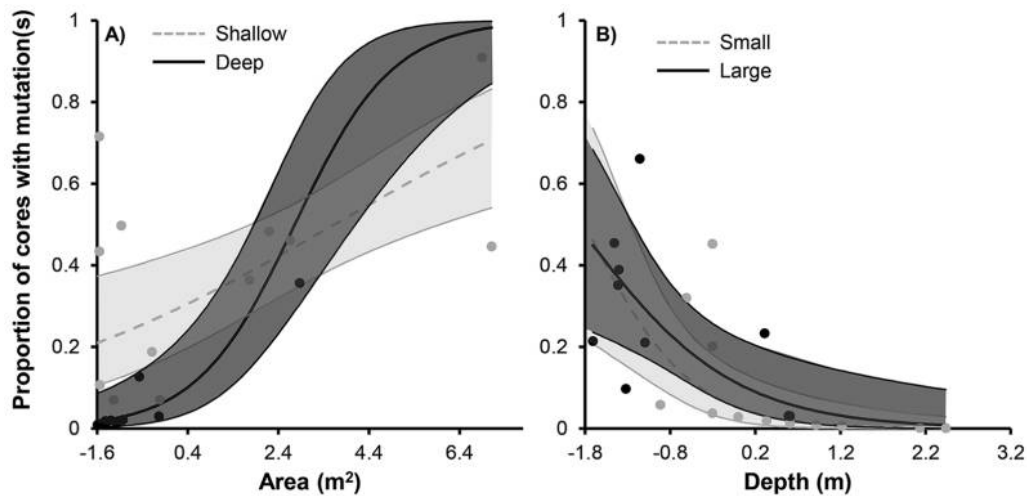
Appendix

Figure A1.   Predictions of the proportion of cores containing mutation(s) in *Orbicella franksi* and *Orbicella annularis* simulated with a binomial generalized linear model incorporating either (A) fixed values of mean-centered depth and the range of observed mean-centered coral sizes or (B) fixed values of mean-centered coral size and the range of observed mean-centered depths. Fixed values represent either the 25th quantile ("Shallow" and "Small" [dashed line with light gray 95% confidence bands]) or the 75th quantile ("Deep" and "Large" [solid line with dark gray 95% confidence bands]) of mean-centered depths and coral sizes. Symbols represent partial residuals of data falling either below the median (gray) or equal to or greater than the median (black) of observed (A) mean-centered depths or (B) mean-centered coral sizes.

Table A1

Statistical summary of binomial generalized linear model and Wald Z test statistic evaluating the probability of a coral containing mutation(s) as a function of number of cores collected per individual, species, mean-centered continuous predictors, and their interaction term

Coefficients	Estimate	SE	Z	P
Intercept	−2.726	1.176	−2.319	**0.020**
Cores collected	−0.006	0.092	−0.063	0.950
Species	1.222	1.164	1.050	0.294
Mean-centered size	0.932	0.333	2.796	**0.005**
Mean-centered depth	−1.487	0.583	−2.550	**0.011**
Mean-centered size × mean-centered depth	0.563	0.186	3.033	**0.002**

Significant *P*-values are shown in bold.