Clonal structure and variable fertilization success in Florida Keys broadcast-spawning corals

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Abstract

Keystone reef-building corals in the Caribbean are predominantly self-incompatible broadcast spawners and a majority are threatened due to both acute adult mortality and poor recruitment. As population densities decline, concerns about fertilization limitation and effective population size in these species increase and would be further exacerbated by either high clonality or gametic incompatibility of parental genotypes. This study begins to address these concerns for two Caribbean broadcasting species by characterizing clonal structure and quantifying experimental pairwise fertilization success. *Orbicella faveolata*, showed surprisingly high and contrasting levels of clonality between two sampled sites; *Acropora palmata* was previously known to be highly clonal. Individual pairwise crosses of synchronously- spawning genotypes of each species were conducted by combining aliquots of gamete bundles immediately after spawning, and showed high and significant variability in fertilization success. Over half of the individual crosses of *O. faveolata* and about one third of *A. palmata* crosses yielded $\leq$ 40% fertilization. Total sperm concentration was quantified in only a subset of *O. faveolata* crosses (range of 1- 6 x 10^7 ml^{-1}), but showed no correlation with fertilization success. We interpret that both parental incompatibility and individual genotypes with low quality gametes are likely to have contributed to the variable fertilization observed with important implications for conservation. Differential fertilization success implies effective population size may be considerably smaller than hoped and population enhancement efforts need to incorporate many more parental genotypes at the patch scale to ensure successful larval production than indicated by estimates based simply on preserving levels of standing genetic diversity.

Introduction

The imperiled status of most Caribbean reef-building corals is attributable to recruitment levels that fail to balance high levels of sustained and acute mortality associated with bleaching, disease and local stressors. Six of the seven Caribbean coral species listed under the United States' Endangered Species Act are broadcast spawners characterized by a complicated sequence of life-history steps: spawning, fertilization, embryonic development in the water column, navigation to appropriate benthic habitat, settlement and subsequent growth/survival. Each of these steps can constitute a bottleneck (Ritson-Williams et al. 2009), though they are often difficult to study. Thus, the relative importance of these potential sequential bottlenecks is poorly characterized.

Adult density and fecundity are, presumably, the primary determinants of larval production. The density of synchronously spawning adults has a strong correspondence with fertilization success (Oliver and Babcock 1992; Levitan et al. 2004) largely due to sperm dilution in open ocean conditions. Since the primary Caribbean reef-building species of the genera *Acropora* and *Orbicella* are largely self-incompatible (Szmant et al. 1997; Fukami et al. 2003; Levitan et al. 2004; Baums et al. 2005), we would expect effective fertilization to correlate with the density of spawning adult genotypes, not necessarily colonies that are often clones of the same genotype. Thus, high clonality of local populations combined with low population densities in these imperiled species would suggest impaired larval production and support a hypothesis of a depensatory population status (Brainard et al. 2011).

The foundation reef-builder, *A. palmata*, is highly clonal in the Florida Keys and shows a trend of declining genotypic richness over recent years (Baums et al. 2005, 2006; Williams et al.

2014). Much less is known regarding clonal structure of *O. faveolata*, though its massive morphology suggests clonal propagation via fragmentation may be minimal. Its columnar congener, *O. annularis*, shows a moderate to high level of clonality (Levitan et al. 2011; Foster et al. 2013) which is significantly related to hurricane incidence. Previous experimental work in Puerto Rico has shown that individual parental genotypes of *A. palmata* provide differential genetic contribution to resultant larval cohorts in mixed crosses (Baums et al. 2013), adding parental incompatibility to the mechanisms that may impair larval production. Previous experience with larval culture in both *A. palmata* and *O. faveolata* in the Florida Keys has yielded high variability in fertilization success among cohorts and years (M.W. Miller., pers. obs.) suggesting a role of parental incompatibility and/or poor gamete performance in both these species.

In this study, we provide novel information on local genetic structure (clonal structure, allelic frequencies, and spatial autocorrelation) of massive *O. faveolata* from two nearby sites in the Florida Keys. With information on genotypic identity of potential *O. faveolata* parents, along with previously genotyped *A. palmata* parents (Baums et al. 2005; Williams et al. 2014), we quantified variation in fertilization success of both species via pairwise crosses. We relate these results to the potential for successful larval production in these imperiled reef-building corals.

Materials and methods

Clonal structure of spawning populations

The clonal structure of *A. palmata* at sites in the upper Florida Keys consists of one to six spawning genotypes at each of the three sites observed for collection of spawn in the current

study (Williams et al. 2014). We mapped and genotyped *O. faveolata* colonies in June 2014 at two sites 3.5 km distant from each other—Horseshoe (25.1388°N, 80.2950°W) and Grecian Rocks (25.1095°N, 80.3063°W)—that were amenable for gamete collection (abundant colonies, shallow and somewhat protected habitats). At each site, transects were run from a fixed point (mooring or stake with known location recorded by handheld GPS) among patches of *O. faveolata* colonies at a recorded heading. Each colony was mapped via the distance along this base transect plus the perpendicular distance from the base transect to the colony on either side. This spatial information, along with GPS waypoints of the transect markers and a subset of distal colonies collected in the field, were used to convert the mapping coordinates to geographic coordinates (latitude, longitude) for each colony.

A small tissue biopsy (~ 2 cm^2) was chiseled from each sampled colony (n = 47 at each site) and a plastic livestock tag was nailed to adjacent substrate for colony identification during subsequent spawning dives. Biopsies were fixed in 95% ethanol upon return to the boat and kept at -80 °C until analysis. Genotyping was conducted as described in Baums et al. (2010) using five microsatellite loci (maMS4, 11, 2–5, 8 and 2–8; Severance et al. 2004). In summary, samples were extracted overnight using the DNeasy tissue kit (Qiagen). PCR products were visualized using an ABI 3730 (Applied Biosystems) automated DNA sequencer with an internal size standard (Gene Scan 500-Liz, Applied Biosystems) for accurate sizing. Electropherograms were analyzed using GeneMapper Software 4.2 (Applied Biosystems).

We assigned all multilocus genotypes that contained the exact same alleles at all five loci to the same genet as in Baums et al. (2010). Three of the *O. faveolata* samples from Horseshoe did not amplify completely. However, the subset of loci that did amplify were identified with a unique combination of alleles from those displayed by the other full multilocus genotypes in the

population. Hence, they were identified as distinct parental genotypes for crossing assays, but were excluded from the population genetic statistics/characterization.

We used GenAlEx version 6.503 (Peakall and Smouse 2006, 2012) to determine the number of different alleles (N_a), the number of effective alleles, ($N_{eff} = \left(\frac{1}{\sum p_i^2}\right)$), observed heterozygosity (H_o = number of heterozygotes/N), expected heterozygosity ($H_e = 1 - \sum p_i^2$), unbiased expected heterozygosity ($UH_e = (2N/(2N - 1)) * H_e$), and the fixation index ($F = (H_e - H_o)/H_e = 1 - (H_o/H_e)$) for each site. p_i is the frequency of the ith allele for the population and $\sum p_i^2$ is the sum of the squared population allele frequencies.

To investigate the spatial structure of clonality, we conducted a spatial autocorrelation analysis using GenAlEx v 6.502 (Peakall and Smouse 2006, 2012). Spatial autocorrelation calculates the pairwise genetic distances among samples for each of a number of distance classes and correlates those with the geographic distance between the sampled colonies. The resulting correlogram depicts the change in correlation (r) between genetic and geographic distance over the range of distance classes; where $r \leq 0$, genetic distance and geographic distance are assumed to be no longer correlated. We calculated pairwise genetic (F_{ST}) and geographic distance (degrees) between all samples, then grouped them into ten 0.005-degree distance classes. We assumed an infinite allele model because not all of the markers fit a strict stepwise mutation model. We used a spatial heterogeneity test for the null hypothesis of no difference in spatial autocorrelation (999 permutations) between two correlograms (Smouse et al. 2008) with the suggested $\alpha = 0.01$ for significance.

Fertilization assays

Spawning and gamete collection

We conducted pairwise crosses to test the hypothesis of similar fertilization success among all parental genets. The imperiled status of these two species and the poor laboratory infrastructure available in the area preclude the collection of corals for spawning in controlled laboratory conditions. We chose to initiate the crossing assays immediately on the boat by combining an aliquot of gamete bundles from each of two parental genotypes in each of three replicated glass vials so we could execute the largest number of replicate crosses with field-collected gametes. We sought to standardize the gamete contribution of the two parents within and among crosses by allocating whole bundles (prior to breakup) into the replicates.

Limited numbers of parental genets of *A. palmata* (Williams et al. 2014) and asynchrony over the five-night window during which they spawn (Miller et al. 2016) results in limited scope of potential pairwise crosses in the upper Florida Keys. In contrast, *O. faveolata* colonies (and genets) are more abundant and spawn more reliably over only two to three nights yielding a much greater scope for pairwise crosses. Thus, parental genets were chosen haphazardly from those spawning on a given night at the study site. We performed three pairwise crosses among three genotypes of *A. palmata* collected from Elbow reef on 14 August 2014. We performed additional, but not all pairwise, crosses among *A. palmata* genets collected from Elbow, Sand Island, and Horseshoe reefs and a single hybrid cross (Horseshoe *A. palmata* x *A. cervicornis;* providing some context for intraspecific variation) on 4 August 2015. We conducted three pairwise crosses among three parental genets of *O. faveolata* collected at Grecian Rocks reef on 17 August 2014, ten pairwise crosses among five parental genets collected from Horseshoe reef on 7 August 2015, and two pairwise crosses among three Horseshoe genets on 5 September 2015. Because our interest was in investigating as many genotypes as possible, no parental genet was crossed on more than one night.

Both species are broadcast-spawning hermaphrodites that release gamete bundles containing both eggs and sperm during specific nights after the full moon in late summer. These buoyant bundles rise to the surface and begin breaking up within 15–30 min after release, allowing mixing among eggs and sperm from different colonies. Fertilization potential declines with sperm age (Levitan et al. 2004; Fogarty et al. 2012a) requiring rapid execution of fertilization assays. Divers used maps, tags, and color-coded subsurface buoys to identify the genotypes of parental colonies from which spawned gamete bundles were collected using tent-collectors. Small jars containing gamete bundles were returned to the boat within 10–15 min.

Implementing pairwise crosses

Upon arrival on the deck, each parental genotype was assigned a number designation in a pre-arranged pairwise crossing array consisting of 13-mL glass vials in a labelled rack grid. Vials were pre-filled with 8 mL of 1-µm filtered reef water which had been stored in Teflon-lined containers to avoid potential contamination. A disposable plastic pipette was used to transfer one drop of bundles from the concentrated surface layer in each collector jar to each replicate glass vial (n = 3) in the array. This procedure was repeated with jars from subsequent genotypes, adding aliquots of bundles to each replicate vial involving that parent. In the case of *A. palmata* crosses in 2015 involving parents from different sites, individual pipette-drop aliquots of bundles were similarly isolated from single parents on the respective boats but in vials containing 4 mL of filtered seawater. Two such vials were combined upon arrival at the field lab (~ 1.5 h later) to yield a similar total volume to the replicate crosses implemented on the boat (i.e., 8 mL plus two drops of bundles).

To minimize the chance of hypoxia developing in high sperm concentrations, gametes were left mixed for approximately 2 h, after which the vials were either topped off with ~ 4 mL

177 additional seawater (2015) or the eggs (both fertilized and unfertilized, along with a small
178 amount of sperm) were pipetted into new vials with 8 mL filtered seawater (2014). The
179 eggs/embryos were left to develop for an additional 4–6 h and then fixed in concentrated zinc-
180 buffered formalin (Z-fix, Anatech Ltd. Baltimore MD, USA) diluted with four parts seawater.
181 For *A. palmata*, fertilization success (% of eggs) was determined by scoring all eggs in each
182 replicate as fertilized (irregular shapes with a translucent texture representing cleaving cells) or
183 unfertilized (round and opaque) under a dissecting microscope. Due to the much higher number
184 of smaller eggs in each replicate for *O. faveolata*, fertilization success was estimated as the
185 average of three sub-aliquots examined from each replicate vial. We tested the hypothesis that all
186 crosses had similar fertilization rates (% of eggs fertilized) by a separate one-way ANOVA for
187 each species (factor was pairwise parental cross, n = 3 replicate vials for each) after verifying
188 statistical assumptions of normality and heteroscedasticity. Because we did not cross the same
189 parents in both years, we did not test for differences between years.

190 **Validation steps**

191 During the assays conducted in 2014, it became apparent that the intent to standardize the
192 gamete contribution from each parent to each replicate cross by using a single drop of bundles
193 was only partly successful on a moving boat. We qualitatively observed that the number of
194 bundles delivered in a drop varied with how densely the bundles were arranged in the buoyant
195 layer of the collector jar, which in turn appeared to be affected by the thickness of the layer (i.e.,
196 abundance of bundles collected) and the movement of the small boat. Thus, we undertook
197 several additional validation steps in 2015 by examining two potential artifacts that might
198 confound fertilization success: (1) potentially skewed contribution from the two parents in the
199 cross (i.e., if there were substantially fewer gametes added from one parent than the other, there

might be fewer between-parent encounters between gametes in that vial); and (2) the absolute sperm concentration in each replicate vial.

To address potential skewed contribution of gametes, we estimated the average number of gametes contributed by quantifying bundles added, and gametes per bundle for a subset of parental genets of each species. First, we quantified the number of eggs and sperm per bundle by segregating replicate individual bundles in individual 2-mL cryovials in 1 mL seawater. After return to the field lab, the eggs were enumerated using a Sedgwick–Rafter counting chamber and stereo-microscope (Olympus SZ61) and total number of sperm was estimated from concentration in replicate diluted sub-samples via an automated cell counter (Cellometer Vision, Nexcelom, Bioscience, Lawrence, MA) equipped with 10X optic magnification. Proprietary, disposable counting chambers (SD-100, Nexcelom Bioscience) were loaded with 20 µL of fixed sperm solution, which spread into a thin layer by capillary action, and inserted into the Cellometer for analysis. The Cellometer Vision software captured images of cells in the counting chamber and analyzed them for cell number within the sampled population. Data from the images were then converted into cell concentration. We tested for variation among parental genets in eggs/bundle, sperm/bundle, and egg:sperm ratio by ANOVA for each species (or Kruskal–Wallis nonparametric ANOVA when parametric assumptions were not met). Lastly, we estimated the number of bundles per pipette drop for these same parents by placing a drop of bundles from each collector jar in replicated vials of buffered formalin fixative so they remained bundled and could be counted back at the lab. We used these estimates for the number of eggs and number of sperm added to each replicate cross for each of the quantified genets and conducted linear regression (each data set passed tests for normality) with the mean fertilization success (i.e., % of eggs fertilized averaged across all crosses involving that parent) to test whether variable or

imbalanced (between the two parents) gamete addition could account for variation in fertilization rate.

We quantified combined sperm concentration in all the replicate vials of the 2015 *O. faveolata* five-parent crossing array. Sperm was immobilized for counting by fixing an aliquot (20 µL) from each replicate fertilization vial with an equal volume of 2x Z-Fix (zinc-buffered formalin, Anatech) at 35 ppt salinity prepared from a 5x concentrate using artificial sea water (Sigma Sea Salts, Sigma Aldrich). An automated cell counter (Cellometer) was used to quantify cell concentration. We plotted sperm concentration against percentage fertilization among all crosses and replicates and examined the relationship by calculating the nonparametric Spearman correlation (data were not normal).

Results

Clonal structure of *O. faveolata*

The degree of clonality was starkly different between the two sites. Grecian Rocks was surprisingly clonal with only ten unique multilocus genotypes identified out of 47 colonies sampled (Ng/N = 10/47 = 0.21). Horseshoe, more in line with expectation, showed few clones with 36 unique multilocus genotypes identified out of 43 colonies genotyped (Ng/N = 36/43 = 0.84). The spatial arrangements of these genets and their ramets are given in Fig. 1.

Allelic frequencies (clones removed) are given for each locus and site in Table 1. In fragmenting species, one might expect samples to be genetically more similar at smaller distance classes. Horseshoe, with higher genotypic and allelic richness, showed little spatial

244 autocorrelation while Grecian Rocks showed positive correlation of geographic and genetic
245 distance for colonies less than 0.02 degrees distance (Fig. 2).

246 **Fertilization assays**

247 Large and significant variation in fertilization success was evident among crosses of
248 different parental pairings in both species, ranging from a mean of less than 10% to over 90%
249 (Fig. 3). Overall, the fertilization rate averaged across all *A. palmata* crosses was 58% (n = 10
250 intraspecific crosses) and 39% for *O. faveolata* (n = 15 crosses). The single hybrid cross (*A.*
251 *palmata* x *A. cervicornis* from Horseshoe) had relatively low fertilization (16%), but not
252 substantially less than two of the ten *A. palmata* intraspecific crosses (9–19%). The frequency
253 distribution of fertilization success (Fig. 3c) is not significantly different between the two species
254 (Kolmogorov–Smirnov test; $p = 0.395$).

255 The five Horseshoe *O. faveolata* genets that were crossed in all combinations showed a
256 high degree of mate-specific variation (e.g., *O. faveolata* parent 4 had ~10% fertilization when
257 crossed with parents 1 or 2, but 80% when crossed with parent 3; Fig. 3b), consistent with
258 parental incompatibility. In addition to apparent incompatibility, there was also a degree of
259 variation that was consistent across mates. There was a factor of 4x variation in mean
260 fertilization success among parental genotypes (i.e., for each, the average of the four crosses with
261 each of the other four parents; 16 to 70%; Fig 3d). This pattern, though not statistically
262 significant (one-way ANOVA; $p = 0.15$), is consistent with a degree of genotype-specific gamete
263 quality or performance.

264 **Validation steps**

Along with substantial variation in the number of bundles delivered from each parent in one pipette drop (Table 2), there was significant genotypic variation in gamete packaging (one-way ANOVA; eggs/bundle, $p = 0.031$; sperm/bundle, $p = 0.006$) among the individual *O. faveolata* genets, as well as gamete ratios (sperm:egg in each bundle, $p < 0.001$). Variation in gamete packaging among *A. palmata* genets was similarly high, but not statistically significant (Fig. 4). We had four *A. palmata* parental genets for which we captured these gamete contribution estimates (Table 2). There was no significant correlation of mean estimated eggs/cross ($r = 0.107$, $p = 0.893$) nor sperm/cross ($r = 0.538$, $p = 0.461$) with the mean fertilization rate of crosses involving that parent (2–4 crosses). Similarly, for *O. faveolata*, (Table 2) the estimates of correlation were not significant (sperm/cross: $r = 0.52$, $p = 0.369$; eggs/cross: $r = -0.942$, $p = 0.218$). Generally under-represented parents (i.e., those with low estimated number of gametes contributed to each replicate cross such as *A. palmata* HS or *O. faveolata* parent 1; Table 2) as well as over-represented parents (such as *O. faveolata* parent 4) each showed variation in fertilization success by a factor of three to eight when crossed with different parents (Fig. 3a,b), further suggesting that the skewed contribution of gametes from the two parents in our crosses does not entirely account for the variable fertilization success observed.

To examine the potential artifact due to absolute sperm concentration (including potential inhibition due to polyspermy at high concentrations), we examined the relationship of fertilization success with total sperm concentration in the array of 10 *O. faveolata* crosses, all implemented immediately upon return of gamete bundles to the boat (Fig. 5). All sperm concentrations were within one order of magnitude ($1–6 \times 10^7$ mL^{-1}) and showed no consistent relationship with fertilization either within or among crosses (Fig. 5). The overall (Spearman)

correlation of total sperm concentration and fertilization success was weak and non-significant (ρ = -0.1, p = 0.595, n = 30 replicate vials).

Discussion

Results of this study, combined with data on declines in population density in both species and inconsistent spawning in *A. palmata* (Miller et al. 2016), increase concerns regarding larval supply as a primary limitation on species recovery. For example, live cover of *O. faveolata* has declined by over 80% since the late 1990s in many sites throughout its range (Brainard et al. 2011), with recent density estimates of 0.074 m^{-2} for spawning-sized colonies (>19 cm diameter) averaged over stratified random surveys conducted 2005–2015 throughout the upper Florida Keys (generated from Florida Reef Resilience Program online query tool, http://frrp.org/cgi-bin/query/start.cgi, accessed 31 January 2017). Levitan et al. (2004) estimated that only 50% of *O. faveolata* colonies at a site spawn on a given night, with an additional 30–50% reduction in the proportion spawning in years following thermal bleaching events (Levitan et al. 2014). This study adds further constraints to larval production in that at least at some sites, there appears to be a high level of *O. faveolata* clonality of the potential spawning parents, and the majority of individual parental combinations yield fertilization success of less than 50% (Fig. 3c). The situation is at least as dire for *A. palmata* with a higher degree of clonality and 30% of synchronously spawning parental combinations yielding <50% fertilization success. The HS *A. palmata* parent showed statistically similar fertilization success in a single hybrid cross (16%) as it did in one intraspecific cross (EL Orange), though it was significantly more successful when crossed with a different *A. palmata* parent (SI Blue; Fig 3a). Interestingly, the HS *A. palmata* genet is a prolific genotype forming a large monotypic stand suggesting that hybrid crossing may

be the most likely route for gametes spawned by this genet due to lack of nearby conspecific mates. Since only one hybrid cross was attempted in this study, variability in hybrid fertilization success is not known, though expected to be high based on extensive Indo-Pacific hybrid crosses (Willis et al. 1997).

The finding of high clonality in the *O. faveolata* patch at Grecian Rocks reef (Ng/N = 0.21) was unexpected. A previous study of clonal structure in the congener, *O. annularis*, at three sites in Honduras revealed Ng/N values of 0.67–0.92 within 10-m diameter plots, and a more extensive study of sites across the Caribbean showed a range of 0.17–0.92, and an overall pooled value of 0.67 (Foster et al. 2007, 2013). However, this species has a columnar or lobed morphology which accommodates fragmentation as confirmed by the strong relationship of clonality with storm disturbance (Foster et al. 2013). *Orbicella faveolata*, in contrast, has a mounding morphology, which makes high levels of fragmentation much more difficult to envision. This result is consistent, however, with anecdotal observations of spawning patterns and occasional fertilization failure on spawning nights with few, and clumped distribution, of spawning adults (M.W. Miller, pers. obs.). We can offer no evidence regarding a mechanism accounting for many clonal colonies at Grecian Rocks but few at Horseshoe; the Grecian Rocks colonies are mostly large (1–1.5 m diameter) and thus the establishment of these colonies is presumed to have occurred long ago, under a less disturbed reef environment.

The methods employed in this study, necessitated by ecological and logistic constraints (small, scattered populations and poor laboratory infrastructure in the area), did not completely control for potential confounding by differences in absolute sperm concentration nor in the relative gamete contribution from the two parents in the cross. However, we contend that parental incompatibility at least contributes to the wide variation in fertilization success among

crosses. First, this interpretation is consistent with incompatibility previously demonstrated in *A. palmata* using methods separating eggs and sperm from each parent (Baums et al. 2013). Incompatibility has also been indicated in studies of congeners *O. franksi* and *O. annularis* (Fogarty et al. 2012b). Second, in the 2015 *O. faveolata* crosses, we observed both relatively high (75–100%) and relatively low (0–25%) fertilization rates across the full range of absolute sperm concentrations present in our assays (~ 1–6 x 10^7 mL^{-1}; Fig 5). This is not consistent with the interpretation that variation in absolute sperm concentration (including potential inhibition by polyspermy at high extremes) accounts for all the variation in fertilization observed in our assays.

Lastly, it is clear that there were, in many cases, differential gamete contributions from the two parents in the cross (Table 2). This differential contribution was consistent for each parent. In other words, some parental genotypes were consistently under-represented (e.g., HS for *A. palmata* or parent 1 for *O. faveolata*; Table 2) due to fewer bundles per drop as well as differential gametes per bundle. If skewed contribution of gametes accounted for the overall patterns of variable fertilization observed, it seems that crosses involving either under-represented or over-represented parents should have shown consistently low success. In contrast, crosses involving the HS *A. palmata* parent varied by a factor of almost three (~22% when crossed with EL Orange but 60% when crossed with SI Blue; Fig 3a) while *O. faveolata* parent 1 showed variation in fertilization by a factor of seven (~5% when crossed with parent 2 up to ~35% when crossed with parent 3; Fig 3b). Meanwhile, over-represented parents (e.g., *O. faveolata* parent 4) showed similarly high variation across different pairings (<10% to 80%; Fig 3b). Again, this pattern is not consistent with a purely artefactual determination of fertilization success by differential gamete contribution. Although the interpretation of strict parental

356 incompatibility in our study may be somewhat confounded by skewed gamete contribution, the
357 highly clonal structure of patches in both species (including singletons alongside clones
358 represented by many large ramets) suggests that skewed contribution is a realistic condition in
359 natural spawning events. Thus, highly variable and often low fertilization success appears to be a
360 realistic expectation of these clonal populations leading to lower larval production and lower
361 effective population size than the census size of the adult population would imply.

362 Our results extend the growing documentation of genotype- or family-specific
363 reproductive traits including timing of spawning, dispersal tendency, gametic compatibility,
364 post-settlement survivorship, and the infecting *Symbiodinium* community (Kenkel et al. 2011;
365 Levitan et al. 2011; Baums et al. 2013; Miller et al. 2016; Quigley et al. 2016). We confirm and
366 expand on previous reports of parental incompatibility in Puerto Rican *A. palmata* (Baums et al.
367 2013), some Pacific acroporids (Willis et al. 1997) and Panamanian *O. franksi* and *O. annularis*
368 (Fogarty et al. 2012b). We confirm that incompatibility occurs at fertilization, which was not
369 clear in the Baums et al. (2013) study in which differential genetic contribution of individual
370 parents to larval cohorts from batch crosses could not be determined until 27 h after fertilization,
371 when each larva contained adequate DNA for genotyping. Gametic incompatibility may also
372 account for at least some of the high variation in observed fertilization success in previous *O.*
373 *faveolata* studies and observations (Levitan et al. 2004; Fogarty et al. 2012a; M.W. Miller, pers.
374 obs.). For example, Levitan et al. (2004) reported approximately double mean fertilization
375 success (~ 80% vs. ~ 40%; and approximately double the standard error) for *O. faveolata* crosses
376 performed in Panama compared with the Bahamas.

377 Parental genotypes also differ in their abundance and ratio of eggs and sperm spawned
378 per polyp (i.e., contained in each bundle). Differences in sex allocation have been previously

379 documented between hermaphroditic spawning coral species (Hall and Hughes 1996), with the
380 ratio of egg volume to sperm volume being conserved within polyps of each species and
381 correlated with colony size, consistent with the theory of deferred female investment to prioritize
382 growth at earlier ages. We did not measure colony size of the parents from which we quantified
383 eggs and sperm per bundle, but all were well within the size of full reproductive capacity. It
384 should be noted that colonies in the sampled populations were subject to severe thermal stress
385 during both the 2014 and 2015 spawning seasons. It is plausible that such a stress may have a
386 carryover effect on gamete characteristics (e.g., abundance or size of eggs produced) in a
387 following year, such as was the case in our 2015 sampling year. In fact, three of the four colonies
388 in which we quantified gamete abundance were rated as 'moderately bleached' (qualitative score
389 of 3 out of 5; DE Williams pers. obs.) while the fourth was not bleached at all during the
390 previous September. The unbleached colony was colony 4 which had significantly fewer eggs
391 per bundle and significantly greater sperm:egg (Fig. 4). Nonetheless, the substantial (though not
392 significant) variation in mean fertilization success (across different partners) among genotypes
393 (Fig. 3d) suggests overall variation in genotypic gamete quality likely also contributes to variable
394 fertilization. Overall, we only performed pairwise crosses of each genotype in a single year, so
395 inter-annual consistency of fertilization success remains to be tested.

396 Each assay in this study involved only two parents as the intent was to detect individual
397 parental incompatibilities. Other studies that have examined fertilization success in crosses with
398 differing numbers of parents (e.g., 2–6) show increasing fertilization in crosses with more
399 parents (Baums et al. 2013; Iwao et al. 2014). It is possible that sperm activation may be more
400 effective in the presence of diverse gamete pools, in which case cumulative fertilization success
401 might be somewhat higher in a mixed pool of parents than is represented in our results.

In addition to increasing concerns regarding the influence of depensatory processes in these imperiled populations, there is an important implication for the growing efforts in population enhancement of Caribbean spawning corals (Lirman and Schopmeyer 2016). Guidelines regarding 'adequate' numbers of genotypes to preserve in any genetic archiving or enhancement effort generally focus on capturing the standing genetic diversity of the population. For example, Shearer et al. (2009) suggest that preserving 10–35 coral genotypes will preserve 50–90% of standing genetic variation in a coral population. In the current situation of rapidly changing ocean conditions, the dire need for rapid adaptation must be paramount in coral restoration and recovery planning. Adaptation will be most successful in populations with high standing genetic diversity, but also requires the recombination of genes via successful sexual reproduction. Our results indicate that many more genotypes may be needed to foster successful sexual reproduction in both these threatened corals than the estimates based on standing genetic diversity alone. Restoration and population enhancement efforts should be based on a more-is-better approach.

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Figure legends

Fig. 1 Map of *Orbicella faveolata* multilocus genotypes and ramets at **a** Grecian Rocks, and **b** Horseshoe, showing the transects used for field mapping. Circles represent colonies sampled; gray represents singleton genotypes (unique from each other) and other colors indicate ramets (clones) of the same genotype within each site

Fig. 2 Correlogram for spatial autocorrelation analysis of *Orbicella faveolata* colonies genotyped at Horseshoe (dotted line; n = 47) and Grecian Rocks (solid line, n = 43) reefs over the first ten

distance classes of 0.005 (decimal degree). r is the correlation of genetic with geographic distance for colonies in each distance class; errors are bootstrapped 95% confidence intervals

Fig. 3 a Fertilization success (mean + SE) of *Acropora palmata* crosses, including three within-site crosses (three parents from Elbow reef) in 2014, and both within- and between-site crosses and one hybrid cross in 2015. Parental designation given as two letters for the site (EL = Elbow, SI = Sand Island, HS = Horseshoe) and one letter for the genet within the site (when needed, only one genet from HS was used). **b** Fertilization success of *Orbicella faveolata* crosses including three pairwise crosses (three parents from Grecian Rocks) in 2014, ten combinations of five parents from Horseshoe in Aug 2015, and two pairwise crosses among three Horseshoe genets in Sept 2015. Dashed line separates 2014 and 2015 crosses. Extrapolation of gamete concentration for some of these is given in Table 2. **c** Frequency (proportion of crosses) of fertilization success for each species. **d** Mean (+ SE) fertilization success for each of five Horseshoe *O. faveolata* genets crossed with each of the other four parents (box in panel **b**)

Fig. 4 Genet-specific characteristics of the number of eggs per bundle (**a**, **d**), sperm per bundle (**b**, **e**), and their ratio (**c**, **f**) for *Acropora palmata* and *Orbicella faveolata*. Genet designations on x-axis are the same ones given in Table 2

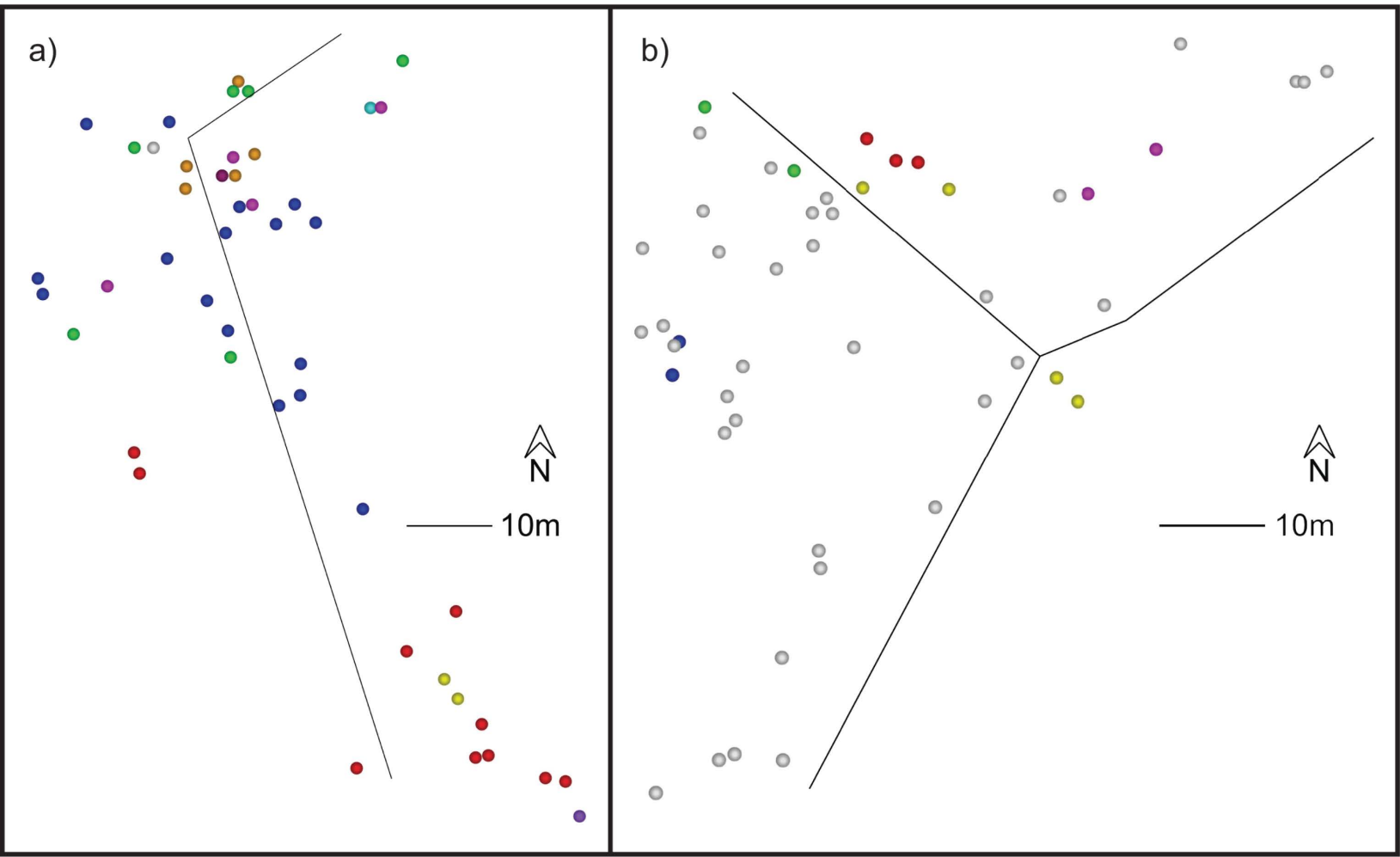
Fig. 5 Relationship of total sperm concentration (number of cells mL^{-1}) with fertilization success for three replicate vials each of ten pairwise parental crosses among five *Orbicella faveolata* genets conducted on 7 August 2015

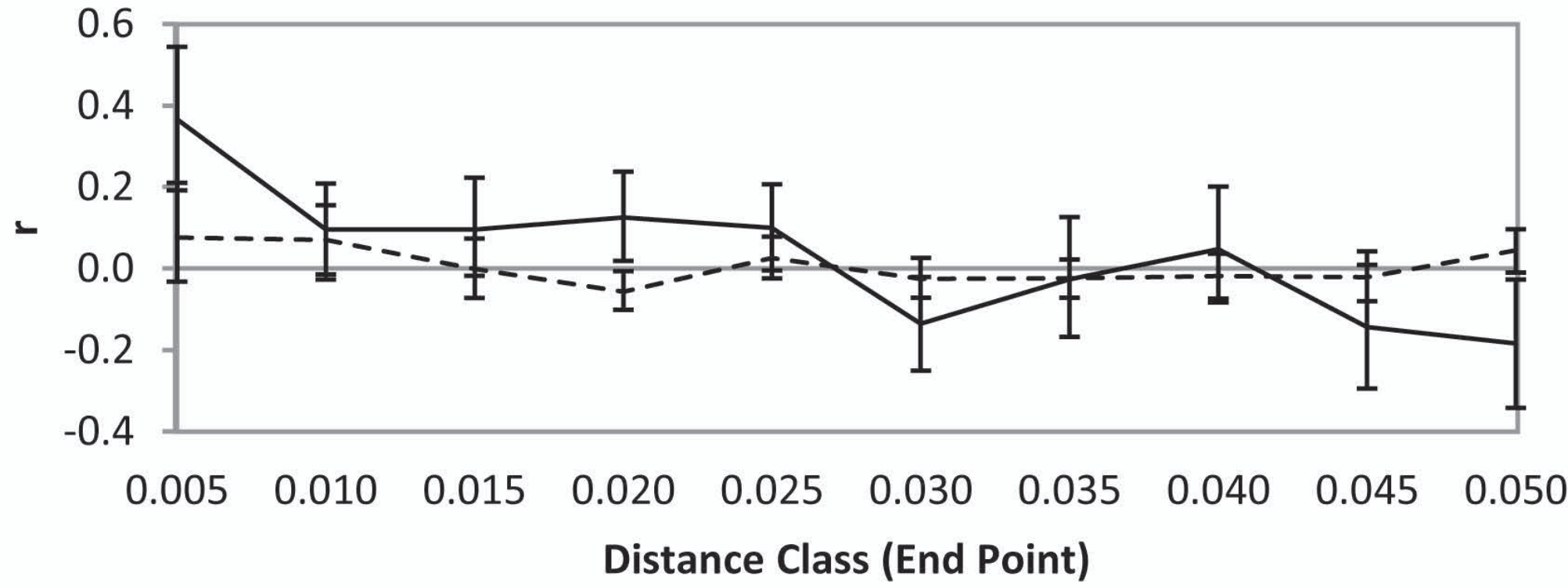
Table 1: Allele frequencies for *O. faveolata* at each site (N= # genotypes, clones removed). Also shown are Na = # different alleles; Ne = # effective alleles; I = Shannon's Information Index; Ho = Observed Heterozygosity; uHe = unbiased Expected Heterozygosity; F = Fixation Index as calculated by GenAlEx v 6.503

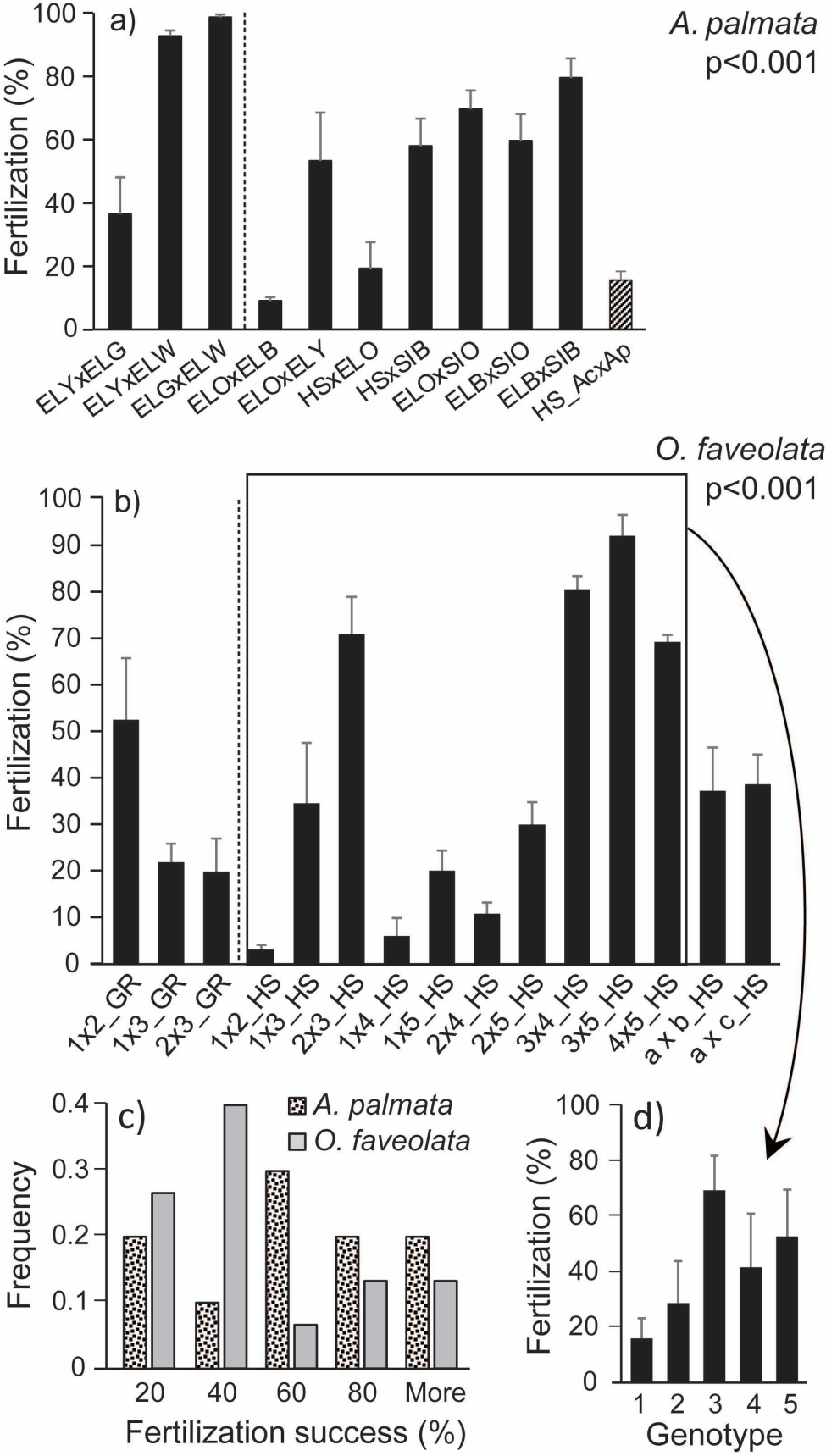
Pop	**Locus**	**N**	**Na**	**Ne**	**I**	**Ho**	**He**	**uHe**	**F**
Horseshoe	**11**	35	6.000	1.310	0.579	0.200	0.237	0.240	0.155
	28	35	6.000	3.149	1.324	0.743	0.682	0.692	-0.089
	4	35	8.000	4.471	1.712	0.657	0.776	0.788	0.154
	5	35	14.000	10.124	2.466	0.886	0.901	0.914	0.017
	8	35	3.000	1.668	0.686	0.343	0.400	0.406	0.144
Mean (SE)			7.4 (1.8)						
GrecianRocks	**11**	10	5.000	1.538	0.778	0.400	0.350	0.368	-0.143
	28	10	5.000	2.985	1.263	0.400	0.665	0.700	0.398
	4	10	5.000	3.279	1.344	0.900	0.695	0.732	-0.295
	5	10	11.000	9.091	2.293	1.000	0.890	0.937	-0.124
	8	10	3.000	2.778	1.055	0.600	0.640	0.674	0.062
Mean (SE)			5.8 (1.3)						

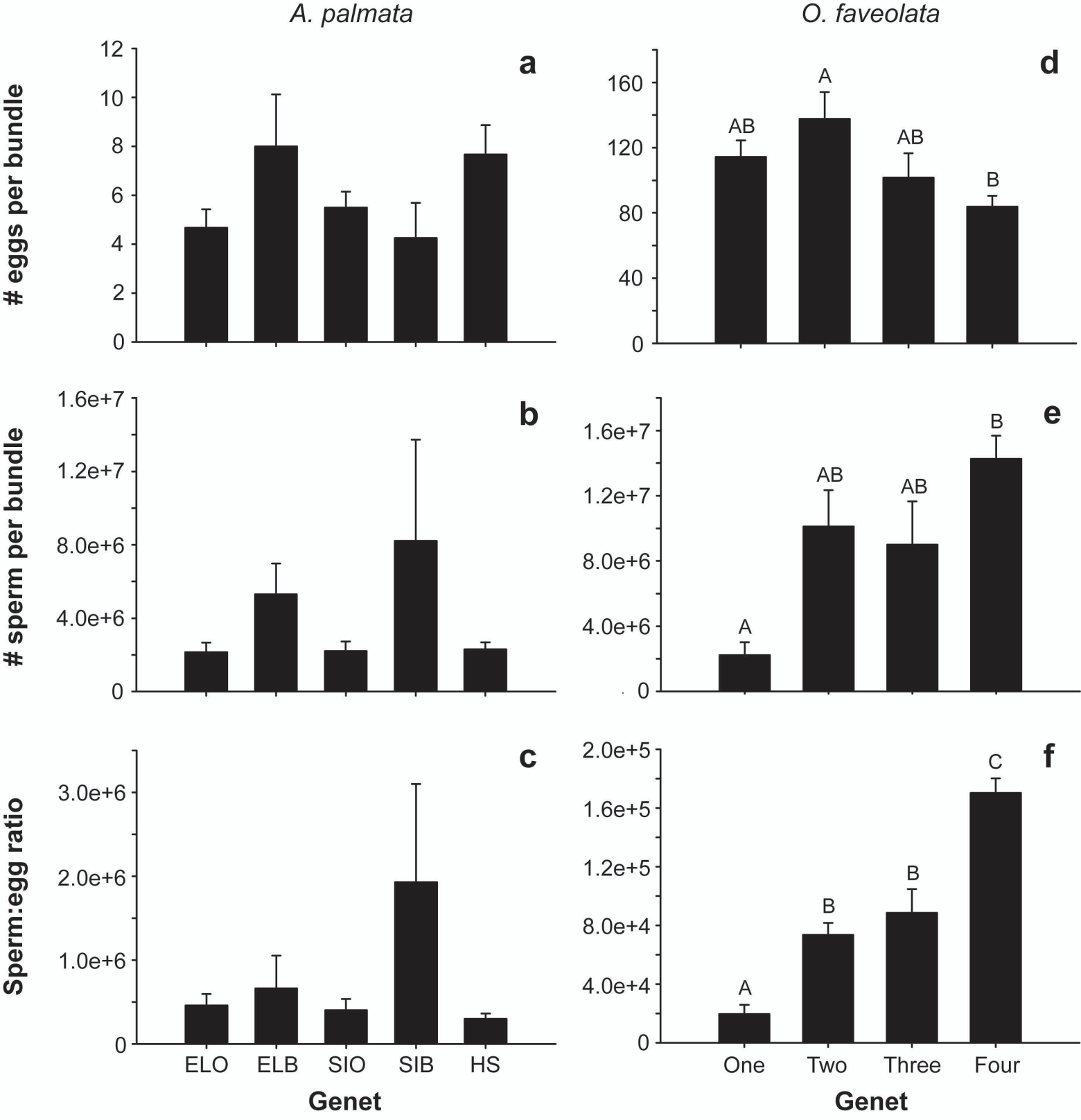
Table 2: Characteristics of gamete packaging and fertilization success for a subset of parents of each species quantified in 2015. Not all parameters were captured for each parental genet (blank cells). One 'drop' of bundles from each parent was added to each replicate fertilization trial. Thus 'eggs/drop' and 'sperm/drop' is the expected number of gametes contributed by that parent to replicate crosses. Mean Fert is the average percent fertilization for that parent among n crosses. Parent designations as given in Fig 3.

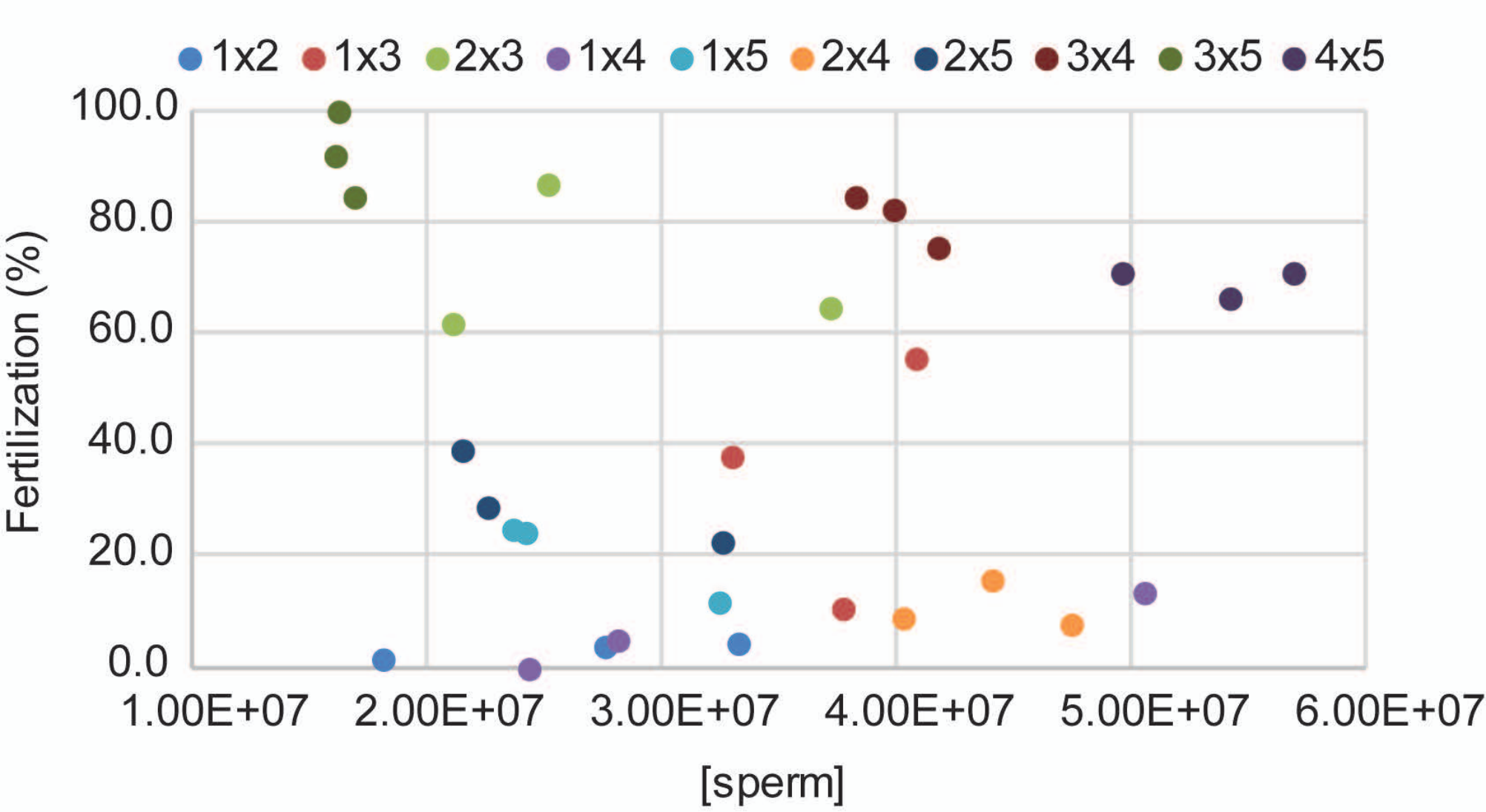
	Parent	bundles/ drop	eggs/ bundle	sperm/ bundle	sperm/ drop	eggs/ drop	Mean Fert (%) / n
O. faveolata	1	18.0	114.3	2228723	40117020	2058	14.9 / 4
	2	13.25	137.7	10117300	134054225	1824	28.8 / 4
	3		101.7	8998700			69.6 / 4
	4	11.25	83.8	14260960	160435800	943	41.7 / 4
	5	6.0		20637200	123823200		52.9 / 4
	a	5.0		8410667	42053333		38.0 / 2
A. palmata	ELO		4.6	2146078			37.7 / 4
	ELB	19.5	8.0	5297575	103302713	156	49.3 / 3
	HS	8.5	7.7	2297360	19527560	65	38.5 / 2
	SIO	18.7	5.5	2206588	41263186	103	64.5 / 2
	SIB	15.4	4.3	8208750	126414750	65	68.6 / 2


a)
N
10m
b)
N
10m


0.6
0.4
0.2
0.0
-0.2
-0.4
r
0.005
0.010
0.015
0.020
0.025
0.030
0.035
0.040
0.045
0.050
Distance Class (End Point)


a)
A. palmata
p<0.001
Fertilization (%)
100
80
60
40
20
0
ELYxELG
ELYxELW
ELGxELW
ELOxELB
ELOxELY
HSxELO
HSxSIB
ELOxSIO
ELBxSIO
ELBxSIB
HS_AcxAp
O. faveolata
p<0.001
b)
Fertilization (%)
100
90
80
70
60
50
40
30
20
10
0
1x2_GR
1x3_GR
2x3_GR
1x2_HS
1x3_HS
2x3_HS
1x4_HS
1x5_HS
2x4_HS
2x5_HS
3x4_HS
3x5_HS
4x5_HS
a x b_HS
a x c_HS
c)
A. palmata
O. faveolata
Frequency
0.4
0.3
0.2
0.1
0
20
40
60
80
More
Fertilization success (%)
d)
Fertilization (%)
100
80
60
40
20
0
1
2
3
4
5
Genotype


A. palmata
O. faveolata
eggs per bundle
sperm per bundle
Sperm:egg ratio
a
b
c
d
e
f
12
10
8
6
4
2
0
160
120
80
40
1.6e+7
1.2e+7
8.0e+6
4.0e+6
3.0e+6
2.0e+6
1.0e+6
2.0e+5
1.6e+5
1.2e+5
8.0e+4
4.0e+4
AB
A
AB
B
A
AB
AB
B
A
B
B
C
ELO
ELB
SIO
SIB
HS
One
Two
Three
Four
Genet
Genet


1x2
1x3
2x3
1x4
1x5
2x4
2x5
3x4
3x5
4x5
100.0
80.0
60.0
40.0
20.0
0.0
Fertilization (%)
1.00E+07
2.00E+07
3.00E+07
4.00E+07
5.00E+07
6.00E+07
[sperm]