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Asymmetric conspecific sperm precedence in relation to spawning times in the *Montastraea annularis* species complex (Cnidaria: Scleractinia)

N. D. FOGARTY*, M. LOWENBERG*, M. N. OJIMA*, N. KNOWLTON†‡ & D. R. LEVITAN*

*Department of Biological Science, Florida State University, Tallahassee, FL, USA
†Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA
‡Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA

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Abstract

In broadcast spawners, prezygotic reproductive isolation depends on differences in the spatial and temporal patterns of gamete release and gametic incompatibility. Typically, gametic incompatibility is measured in no-choice crosses, but conspecific sperm precedence (CSP) can prevent hybridization in gametes that are compatible in the absence of sperm competition. Broadcast spawning corals in the *Montastraea annularis* species complex spawn annually on the same few evenings. *Montastraea franksi* spawns an average of 110 min before *M. annularis*, with a minimum gap of approximately 40 min. Gametes are compatible in no-choice heterospecific assays, but it is unknown whether eggs exhibit choice when in competition. Hybridization depends on either *M. franksi* eggs remaining unfertilized and in proximity to *M. annularis* when the latter species spawns or *M. franksi* sperm remaining in sufficient viable concentrations when *M. annularis* spawns. We found that the eggs of the early spawning *M. franksi* demonstrate strong CSP, whereas CSP appears to be lacking for *M. annularis* eggs. This study provides evidence of diverging gamete affinities between these recently separated species and suggests for the first time that selection may favour CSP in earlier spawning species when conspecific sperm is diluted and aged and is otherwise at a numeric and viability disadvantage with heterospecific sperm.

Introduction

According to the biological species concept (BSC), maintenance of species boundaries requires reproductive isolating barriers that prevent hybridization and gene flow between heterospecifics (i.e. introgression). These barriers are often categorized as prezygotic, which prevents the formation of a hybrid embryo, or postzygotic, where selection reduces the viability or reproductive capabilities of hybrids (Dobzhansky, 1937; Mayr, 1963; Coyne & Orr, 2004). It has been suggested that reproductive isolating mechanisms that prevent fertilization are the strongest barrier to gene flow between many closely related taxa (Mendelson *et al.*, 2007). Gamete compatibility and fertilization success are determined by gamete recognition proteins that are found on the surface of gametes and evolve rapidly (Metz & Palumbi, 1996; Swanson & Vacquier, 2002; Levitan & Ferrell, 2006; Palumbi, 2009). Divergence of these proteins plays a prominent role in evolution of prezygotic barriers and reproductive isolation (Metz *et al.*, 1994; Swanson & Vacquier, 2002; Tomaiuolo *et al.*, 2007; Palumbi, 2009). Although gametic compatibility has received considerable attention, only recently has conspecific sperm precedence (CSP) or conspecific pollen precedence (CPP) as it relates to plants, been appreciated as an important cryptic prezygotic barrier. Conspecific sperm precedence or CPP is

Correspondence and present address: N. D. Fogarty, Nova Southeastern University Oceanographic Center, 8000 North Ocean Drive, Dania Beach, FL, 33004-3078, USA.
Tel.: 954.262.3630; fax: 954.2624098;
e-mail: nicole.fogarty@nova.edu

defined by the utilization of conspecific sperm/pollen for fertilization when females are exposed simultaneously to conspecific and heterospecific sperm/pollen (reviewed in Howard, 1999; also see Bierne *et al.*, 2002; Geyer & Palumbi, 2005; Ludlow & Magurran, 2006; Mendelson *et al.*, 2007; Howard *et al.*, 2008). Despite the existence of gametic compatibility between two closely related species in the absence of sperm competition (no-choice crosses), during sperm competition trials (choice crosses) conspecific sperm often outcompete heterospecific sperm, providing evidence that CSP may evolve prior to gametic incompatibility (Howard, 1999; Bierne *et al.*, 2002; Geyer & Palumbi, 2005; Willis *et al.*, 2006; Fogarty *et al.*, 2012).

Conspecific gamete precedence is important across a wide variety of reproductive strategies, but perhaps most critical in organisms that broadcast their gametes (i.e. free-spawning marine invertebrates and many plant species; Howard, 1999). Broadcast spawning marine invertebrates, such as corals, share many life-history traits with many plant species (i.e. sessile, often hermaphroditic, external release of sperm/pollen, lack courtship behaviour, asexually propagate; Willis *et al.*, 2006). Broadcasting sperm/pollen into the environment eliminates courtship behaviour and reduces the ability of females to choose mates/males to assure paternity. This results in gamete recognition, and thus, CSP and CPP are potentially prominent mechanism mediating male competition and female choice (Howard, 1999; Palumbi, 2009).

Sympatric broadcast spawning marine species must rely heavily on prezygotic barriers such as temporal or spatial differences in gamete release and/or gametic incompatibility (Palumbi, 1994). Most marine studies test gametic compatibility in the absence of sperm competition (i.e. no-choice crosses) where heterospecific sperm have considerable time to fuse with an egg (Willis *et al.*, 1997; Hatta *et al.*, 1999; Levitan, 2002; Levitan *et al.*, 2004). Over this extended period of time, in the confines of a small experimental chamber, marginally incompatible sperm can eventually fuse with eggs and asymptote at the same level of fertilization as more compatible sperm. However, the rate of fertilization of more or less compatible sperm is expected to differ. These compatibility differences are revealed when sperm from two males are placed in direct competition, and the male producing sperm with lower compatibility loses in competition. This process explains why CSP is often noted in species pairs that show evidence of compatibility in no-choice assays (Bierne *et al.*, 2002; Geyer & Palumbi, 2005; Willis *et al.*, 2006; Fogarty *et al.*, 2012). The potential mechanisms driving CSP include factors that influence collision frequencies such as sperm velocity (Gage *et al.*, 2004; Liljedal *et al.*, 2008), sperm chemotaxis (Riffell *et al.*, 2004), and proteins on the surface of sperm and eggs that determine compatibility (Levitan & Ferrell, 2006; Levitan & Plata Stapper, 2009; Palumbi 2009; Levitan, 2012).

Broadcast spawning corals often spawn in multispecies mass spawning events leading to a high potential for hybridization. Up to 35 species in the Indo-Pacific can release their gametes within a few hours of each other (Willis *et al.*, 1985; Babcock *et al.*, 1986). In acroporid corals, highly synchronized spawning events, heterospecific compatibility, and ambiguous genetic differences between some congeners provide evidence for extensive hybridization and reticulate evolution, that is, where species continually fuse and separate over time through introgressive hybridization (Willis *et al.*, 1997; Hatta *et al.*, 1999; van Oppen *et al.*, 2001; van Oppen *et al.*, 2002; Willis *et al.*, 2006). Yet despite indirect evidence of considerable introgression, only one study has examined CSP in hybridizing Indo-Pacific acroporids (Willis *et al.*, 2006). They found absolute CSP where conspecific sperm sired all of the larvae in 13 of 14 crosses, suggesting a strong prezygotic barrier of CSP (Willis *et al.*, 2006) and alternative explanations for the ambiguous genetic differences between described species. In the Caribbean, *Acropora palmata* and *Acropora cervicornis* mate and form a hybrid originally named *Acropora prolifera* (van Oppen *et al.*, 2000; Vollmer & Palumbi, 2002). These taxa are broadly sympatric and lack temporal isolation in spawning (Szmant, 1986; Fogarty *et al.*, 2012), but choice and no-choice trials demonstrate asymmetries in gametic incompatibility (Fogarty *et al.*, 2012). *Acropora palmata* eggs have semi-permeable barriers to heterospecific fertilization with some evidence of CSP, whereas no prezygotic barriers exist for *A. cervicornis* (Fogarty *et al.*, 2012). Previous studies have also found asymmetry in fertilization in no-choice crosses (Levitan, 2002; Rahman & Uehara, 2004; Evans & Marshall, 2005; Riginos *et al.*, 2006; Lessios, 2007; Fogarty *et al.*, 2012) and in competitive trials (Howard, 1999; Chang, 2004; Harper & Hart, 2005; Mendelson *et al.*, 2007; Martin-Coello *et al.*, 2009; Immler *et al.*, 2011; Fogarty *et al.*, 2012).

Studies of reproductive isolation in Caribbean members of the *Montastraea annularis* complex (Van Veghel & Bak, 1993; Van Veghel, 1994; Knowlton *et al.*, 1997; Szmant *et al.*, 1997; Hagman *et al.*, 1998; Sanchez *et al.*, 1999; Levitan *et al.*, 2004) have shown that spawning times in these corals are remarkably precise and apparently influenced by environmental cues, genetics and neighbouring conspecifics (Levitan *et al.*, 2004, 2011). Individual colonies generally release all their gametes within a minute during a spawning event, whereas at the population level, each species spawns for about an hour with peak spawning occurring over a 20-min period (Levitan *et al.*, 2011). *Montastraea franksi*, on average, spawns 110 min before *M. annularis* and 120 min before *Montastraea faveolata*. At our long-term monitoring site, the average interval between the last spawning *M. franksi* and the first spawning *M. annularis*

is 39 min (SE = 5 min across 13 evenings in which both species spawned). *Montastraea faveolata* is more distantly related to and largely incompatible with the other two species (Fukami *et al.*, 2004; Levitan *et al.*, 2004). In contrast, in the absence of sperm competition, *M. franksi* and *M. annularis* gametes are compatible in no-choice crosses. Although these congeners have the potential to hybridize, other mechanisms associated with the temporal isolation of spawning, including gamete ageing, dilution, and dispersal, make hybridization less likely in the field (Levitan *et al.*, 2004). No-choice crosses demonstrate that *M. franksi* sperm have reduced viability after 2 h, when *M. annularis* eggs would be available for fertilization. Hybridization is thus most likely to occur between *M. franksi* eggs that have gone unfertilized as a result of sperm limitation and *M. annularis* sperm or between *M. franksi* colonies that spawn at the end of their species' spawning distribution and the earliest spawning *M. annularis* colonies (Levitan *et al.*, 2004). Both scenarios require *M. franksi* gametes to float over spawning colonies of *M. annularis*. Field studies demonstrate that gametes can disperse over 500 metres in 100 min; therefore, the likelihood of hybridization depends upon local hydrodynamics and the distribution of *Montastraea* species on nearby reefs (Levitan *et al.*, 2004). Furthermore, a molecular study found little evidence of hybridization between *M. franksi* and *M. annularis* at this site (Levitan *et al.*, 2011). It seems unlikely that temporal isolation alone can provide consistently strong enough reproductive isolation to prevent gene flow between these compatible species. Here, choice crosses were used to examine whether CSP is a cryptic barrier that enhances reproductive isolation between *M. franksi* and *M. annularis*, and how temporal differences in spawning affect CSP.

Materials and Methods

Four days after the full moon in September of 2005 and 2008, twelve ripe *M. annularis* and *M. franksi* coral fragments (approximately 225 cm^2) were collected from Hospital Point in Bocas del Toro, Panama. Corals were placed in tanks with running sea water in an open-air laboratory that received ambient light. On the 5th and 6th day after the full moon, corals were placed in separate buckets at sunset and monitored for spawning. Approximately 100 gamete bundles were collected in 10 mL of sea water from *M. franksi* and *M. annularis* corals. After the bundles broke apart, eggs and sperm were separated by pouring the mixture through 100-μ Nitex mesh (Sefar Canada Inc., Scarborough, Ontario). Sperm was collected with 10 mL of filtered sea water (FSW: 0.45 μm) into a urine cup. Eggs were rinsed free of sperm with four consecutive washes of FSW and placed into a urine cup with 50 mL of FSW. Three replicate egg counts were conducted with egg stock solutions. One millilitre of each sperm stock solution was preserved and later quantified using eight replicate counts with a hemocytometer.

No-choice crosses were conducted between individuals used in each choice cross by adding 1 mL of the sperm suspension and 1 mL of the egg suspension to 48 mL of seawater. For choice crosses, *M. franksi* (500 μL) and *M. annularis* (500 μL) sperm stock were added to a urine cup of FSW and swirled three times. One millilitre of *M. franksi* or *M. annularis* eggs was then added to the urine cup and swirled three times. Fertilization was scored 3 h after gamete introduction. The resulting larvae from the choice crosses were reared for 48 h to ensure a sufficient amount of DNA, and preserved in CHAOS (for recipe see Fukami *et al.*, 2004) until molecular analysis (see below). Although larvae from choice crosses were reared for 48 h to have sufficient DNA for molecular analysis, one potential concern is that postzygotic hybrid inviability influenced the results of this prezygotic study. To test for this in 2005, we conducted a separate experiment rearing larvae from a subset of *M. annularis* and *M. franksi* conspecific and heterospecific crosses. After scoring fertilization, approximately 50 larvae from each cross were added to a six-welled cell culture plate containing FSW (0.2 μm). The sea water was changed every 12 h, and dead larvae and debris were removed with a pipette. An ANOVA was used to determine whether the number of surviving larvae at the time of fixation differed between *M. franksi* conspecific crosses, *M. annularis* conspecific crosses, heterospecific crosses with *M. franksi* eggs and heterospecific crosses with *M. annularis* eggs.

Molecular analysis was conducted on the gamete donors in choice crosses by collecting a small tissue core (1 cm diameter) which was preserved in CHAOS until genotyped. Adult colonies were genotyped using six polymorphic microsatellite markers (Severance *et al.*, 2004). Details on PCR protocols and analysis can be found in Levitan *et al.* (2011). At least two microsatellite loci were used to confirm the maternal allele and identify the paternal allele for each larva.

The number of observed conspecific larvae sired was compared with the number of larvae the conspecific male was expected to sire. The expected values were calculated based on the relative conspecific versus heterospecific sperm concentrations. For example, if conspecific sperm were at twice the concentration as heterospecific sperm, then twice as many conspecific larvae would be expected. A paired *t*-test was conducted for each species of egg donor to determine whether the number of observed conspecific larvae sired differed from the expected value based on the number of available sperm. The proportion of larvae sired by conspecifics is free to vary from 0 to 1. Additionally, we tested the difference in CSP between the two species. A Welch's *t*-test was used to determine

whether the average difference between the observed and expected larvae sired by conspecific males differed between the two species of egg donors.

Results

Spawning times and sperm concentrations differed between the two species. On average *M. franksi* gametes were approximately 2 h old during fertilization experiments (Table 1), whereas *M. annularis* gametes had aged an average of 1 h when fertilization experiments were conducted. Concentrations of *M. franksi* sperm were on average twice as high as *M. annularis* sperm, although in some replicates *M. annularis* had the higher sperm concentration. The ratio of conspecific to heterospecifc sperm ranged from 0.24 to 3.26 for *M. franksi* choice trials and from 0.24 to 1.79 in *M. annularis* choice trials (Table 1). Stock sperm concentrations varied across males averaging 2.3×10^7 ($\pm$ SE 4.99×10^6; Fig. 1). Sperm concentrations differed among individual males (ANOVA, $F_{13,98} = 198.47$, $P \leq 0.001$), but high sperm concentrations were seen in both species. Post hoc comparisons using the Tukey HDS test demonstrated differences among individual males (represented by separate letters in Fig. 1). Egg counts of the egg suspension averaged 457 eggs mL^{-1} ($\pm$ SE 115).

The proportion of eggs fertilized by conspecific or heterospecific sperm in no-choice crosses varied, but two no-choice conspecific crosses with *M. franksi* eggs and two no-choice heterospecific crosses with *M. annularis* eggs suggested incompatible gametes (i.e. fertilization < 0.15). These trials were eliminated from the choice analyses because we wanted to test sperm competition between two compatible crosses to determine whether CSP exists. The average proportion of *M. franksi* and *M. annularis* eggs fertilized by conspecific sperm in the remaining no-choice crosses was 0.69 (SE 0.13) and 0.79 (SE 0.06), respectively (Table 1). Fertilization in the remaining heterospecific crosses averaged 0.89 and 0.75 for *M. franksi* and *M. annularis* eggs, respectively (Table 1). All adults were genotyped at six microsatellite loci, and at least two loci that would distinguish between the maternal and paternal alleles were selected for each cross. There were five unique conspecific and six unique heterospecific males competing in the six *M. franksi* choice crosses. Six unique conspecific and heterospecific male genotypes were represented in choice crosses with *M. annularis* eggs. The number of genotyped larvae per cross ranged from 16 to 37 (Table 1). The maternal allele was confirmed in every larva, and all genotypes were consistent with potential sires.

The number of conspecific larvae that resulted from choice crosses was dependent on the egg donor. There was a significant difference between the two species in the deviation of observed from expected larvae sired by conspecific males (*t*-test: $t_1 = 3.33$, $P = 0.0086$). All choice crosses with *M. franksi* eggs ($n = 6$) demonstrated CSP, five of which showed absolute CSP

Table 1 The concentration of stock sperm (±standard error), the proportion of *Montastraea franksi* (K) and *Montastraea annularis* (A) eggs fertilized in no-choice crosses between conspecifics and heterospecifics, the number of larvae genotyped, the number of conspecific sired larvae, the relative sperm concentrations and gamete ages from competitive crosses. Bold values highlight gametes that were incompatible (< 15% fertilized) and therefore eliminated from the analysis

Year	Cross ♀ (♂K + ♂A)	Proportion of conspecific eggs fertilized (no-choice)	Proportion of heterospecific eggs fertilized (no-choice)	Number of larvae analyzed	Proportion of larvae sired by conspecific sperm	Proportion of conspecific sperm	*M. franksi* sperm age (min)	*M. annularis* sperm age (min)	Difference in sperm age (min)	Egg age (min)
2005	K11(K3 + A26)	0.96	0.89	37	1.00	0.70	72	32	40	72
2005	K19(K9 + A14)	0.49	0.94	24	1.00	0.76	108	50	58	30
2005	K8(K9 + A14)	0.23	0.92	32	1.00	0.77	98	40	58	70
2005	K9(K19 + A23)	0.92	0.92	26	1.00	0.19	100	42	58	178
2005	K8(K19 + A23)	**0.02**	0.87	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2005	K220(K208 + A106)	**0.14**	0.33	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2008	K205(K212 + A103)	0.82	0.95	16	1.00	0.52	186	51	135	184
2008	K208(K205 + A115)	0.72	0.73	20	0.85	0.70	200	56	144	199
2005	A28(K3 + A26)	0.84	0.93	24	0.04	0.30	72	32	40	42
2005	A26(K11 + A28)	0.83	0.84	29	0.72	0.32	72	42	30	32
2005	A28(K9 + A14)	0.96	0.85	30	0.00	0.23	98	40	58	65
2005	A28(K19 + A23)	0.80	0.81	24	0.00	0.19	100	42	58	145
2005	A14(K19 + A23)	0.94	0.82	27	0.04	0.19	100	42	58	120
2005	A29(K19 + A23x)	0.91	**0.01**	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2008	A115(K212 + A103)	0.79	**0.00**	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2008	A106(K205 + A115)	0.37	0.71	34	0.24	0.30	202	58	144	67
2008	A10(K208 + A106)	0.80	0.32	31	0.68	0.64	208	74	134	49

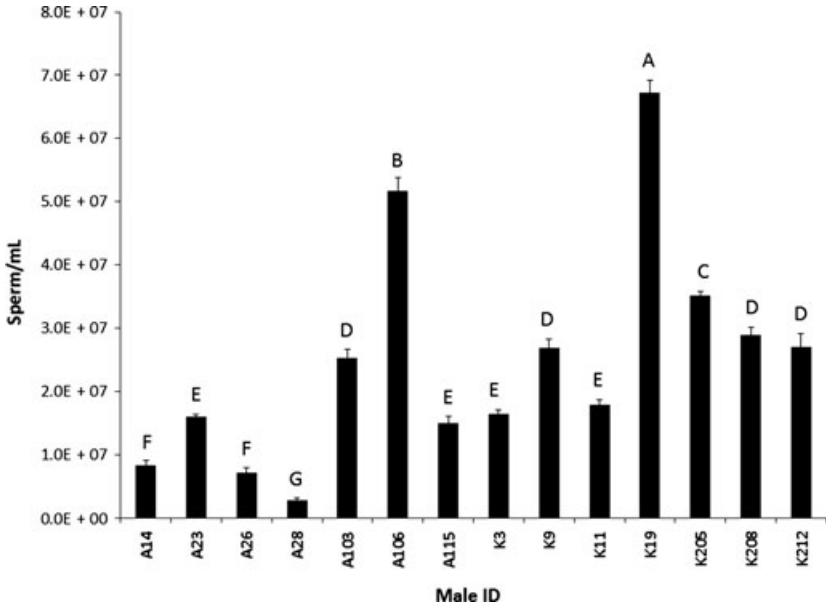

Fig. 1 Sperm concentrations and their associated standard error (represented by bars) from eight replicate sperm counts for each male used in choice crosses. Different letters represent a statistical difference between individual males (ANOVA, Tukey's HDS). A, *Montastraea annularis*; K, *Montastraea franksi*.

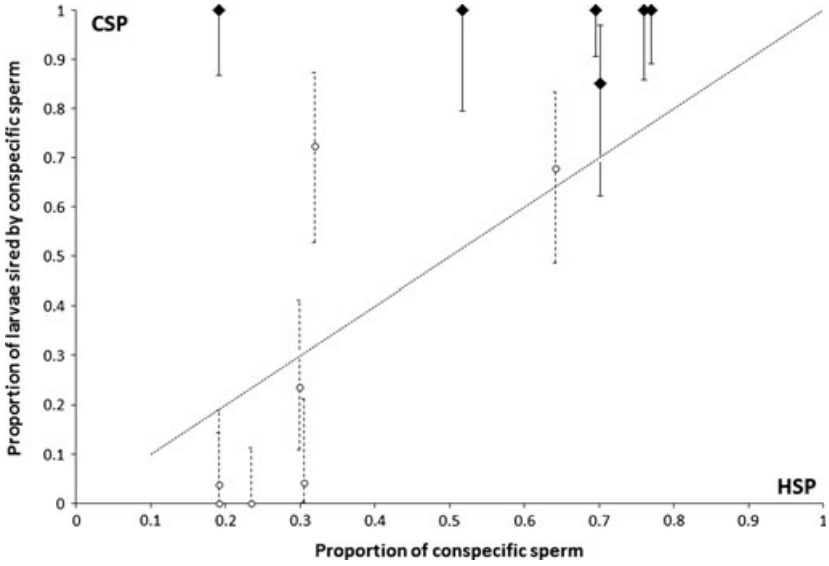

Fig. 2 The proportion of genotyped larvae sired by conspecific sperm from choice crosses as a function of the proportion of conspecific sperm in choice trials. The line represents equal use of *Montastraea franksi* and *Montastraea annularis* sperm. Points to the left of the line demonstrate conspecific sperm precedence (CSP) and points to the right of the line represent heterospecific sperm precedence (HSP). Open circles are *M. annularis*; closed diamonds are *M. franksi*. Error bars represent 95% binomial confidence intervals around proportion of larvae sired by conspecific sperm.

(*M. franksi* sperm sired 100% of the larvae; Fig. 2). The number of observed conspecific larvae resulting from choice crosses with *M. franksi* eggs was significantly different from the null expectation of no CSP (*t*-test: $t_5 = -5.51$, $P = 0.003$); thus, *M. franksi* eggs demonstrate CSP. With *M. annularis* eggs, in contrast, there was no significant difference between the observed versus null expectation (*t*-test: $t_6 = 0.742$, $P = 0.49$); *M. annularis* did not demonstrate CSP.

At the time in which the larvae were sacrificed for molecular analysis, there was no significant difference in larval survival among the individual four cross types (ANOVA, $F_{3,25} = 0.987$, $P = 0.42$). The mean of each cross was *M. franksi* conspecific 0.80 (SE ±0.06), *M. annularis* conspecific 0.73 (SE ±0.04), heterospecific with *M. franksi* eggs 0.82 (SE ±0.05) and heterospecific with *M. annularis* eggs 0.87 (SE ± 0.03). This result indicates that there was no bias in survivorship between conspecific and heterospecific crosses, which would have confounded our estimates of CSP.

Discussion

This study provides further evidence that CSP may evolve prior to gametic incompatibility (Howard, 1999; Geyer & Palumbi, 2005; Ludlow & Magurran, 2006;

Mendelson *et al.*, 2007) and that CSP is often asymmetrical (Howard, 1999; Chang, 2004; Harper & Hart, 2005; Mendelson *et al.*, 2007; Martin-Coello *et al.*, 2009; Immler *et al.*, 2011; Fogarty *et al.*, 2012). It appears that selection for CSP is acting on the first spawning species of multispecies spawning events. Hybridization between early and late broadcast spawning species would occur when the gametes overlap in the water column only after the later species spawns. Under these conditions, the eggs of the early spawning species (*M. franksi*) would already be fertilized or would have a choice of aged and diluted conspecific sperm versus newly spawned heterospecific sperm. Selection might favour CSP in the earlier spawning species, because conspecific sperm are more likely to be at a numerical and viability disadvantage after the later spawning species release gametes. In contrast, the eggs of the later spawning species (*M. annularis*) might have reduced selective pressure for CSP because their conspecific sperm would be fresher and at higher concentrations, favouring conspecific fertilization even in the absence of differential gamete affinities. Although these data do not offer a rigorous test of asymmetrical reinforcement selection for CSP, the direction of the asymmetry noted in CSP in these coral species matches the expectation based on gamete encounter probabilities.

Alternate hypotheses to explain these asymmetries in CSP include (1) genetic drift in gametic affinities that produce stronger CSP in the earlier spawning species by chance and (2) gamete affinities that have evolved to increase intraspecific reproductive success independently of selection against hybridization but that have consequences with respect to interspecific interactions (Levitan, 2002). There are several scenarios that might drive such selection, including increased sperm competition in *M. franksi* relative to *M. annularis*. *Montastraea franksi* asexually propagates much less frequently than *M. annularis*, producing fewer and smaller sized clonemates (Levitan *et al.*, 2011). This results in *M. franksi* being surrounded by more numerous nonclone-mates in close proximity. As these coral species do not self, the increase in the number and proximity of potential mates could lead to higher sperm competition and selection for increased sperm velocity in *M. franksi* (as noted in sea urchins: Levitan, 1993). A consequence of this selection might make them better competitors for intraspecific fertilizations.

We revisited the only two previous coral studies that had examined CSP to determine whether our finding of stronger CSP in the early spawning species is upheld. Fogarty *et al.* (2012) found evidence of CSP in *A. palmata* that spawns on average of 15 min prior to *A. cervicornis*. Despite a lack of strong temporal isolation in Caribbean acroporids, the pattern of CSP in the relatively earlier spawner is consistent. In the Willis *et al.* (2006) review, they summarize findings of absolute CSP in all 11 trials with *Acropora pulchra* eggs, but CSP in two of the three trials with *Acropora millepora* eggs. *Acropora pulchra*, with strong CSP, typically spawns earlier (up to 1hr 40 min) than *A. millepora* (Babcock *et al.*, 1986; van Oppen *et al.*, 2002). Although Willis *et al.* (2006) caution that the one trial lacking CSP could be a misidentification error, another explanation could be attributed to the more equivocal CSP typically found in the later spawning species (Fogarty *et al.*, 2012). As the degree of temporal overlap in spawning times increases, we predict that CSP should become more symmetrical.

Conspecific sperm precedence found in this study provides further evidence of diverging gamete affinities between two coral taxa, *M. franksi* and *M. annularis,* with little evidence of genetic differentiation (Lopez *et al.*, 1999; Medina *et al.*, 1999; Fukami *et al.*, 2004). Previous no-choice experiments demonstrated significant interaction between the direction of compatibility and site, Panama versus Bahamas (Levitan *et al.*, 2004). In Panama, there was a nonsignificant trend of asymmetry where *M. annularis* sperm tended to have slightly lowered ability to fertilize *M. franksi* eggs. Competitive crosses, shown here, often demonstrated the inability of fresher *M. annularis* sperm to outcompete the older *M. franksi* sperm. Significant genetic subdivision between *M. franksi* and *M. annularis* at this site with little evidence of hybridization (Fukami *et al.*, 2004; Levitan *et al.*, 2011) suggests that temporal differences in spawning times coupled with strong CSP in *M. franksi* provide sufficient barriers to prevent gene flow between these species. In the Bahamas, *M. franksi* sperm had reduced success with *M. annularis* eggs, but not the reciprocal (Levitan *et al.*, 2004). Genetic evidence at this site suggests higher levels of introgression and hybridization (Fukami *et al.*, 2004). Although competitive crosses between *M. franksi* and *M. annularis* have not been tested in this region, we predict that CSP will be lacking or incomplete in *M. franksi* eggs. These regional differences in genetic structure and patterns of compatibility suggest regional differences in gamete encounter probabilities, perhaps based on population densities, reef structure, depth distribution or water flow that provide an opportunity to investigate the potential selective pressures that might drive the evolution of gametic incompatibility in sympatric congeners.

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References

Babcock, R.C., Bull, G.D., Harrison, P.L., Heyward, A.J., Oliver, J.K., Wallace, C.C., *et al.* 1986. Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. *Mar. Biol.* **90**: 379–394.

Bierne, N., David, P., Boudry, P. & Bonhomme, F. 2002. Assortative fertilization and selection at larval stage in the mussels *Mytilus edulis* and *M. galloprovincialis*. *Evolution* **56**: 292–298.

Chang, A.S. 2004. Conspecific sperm precedence in sister species of *Drosophila* with overlapping ranges. *Evolution* **58**: 781–789.

Coyne, J.A. & Orr, H.A. 2004. *Speciation*. Sinauer Associates Inc., Sunderland, MA.

Dobzhansky, T. 1937. Genetic nature of species differences. *Am. Nat.* **71**: 404–420.

Evans, J.P. & Marshall, D.J. 2005. Male-by-female interactions influence fertilization success and mediate the benefits of polyandry in the sea urchin *Heliocidaris erythrogramma*. *Evolution* **59**: 106–112.

Fogarty, N.D., Vollmer, S.V. & Levitan, D.R. 2012. Weak prezygotic isolating mechanisms in threatened Caribbean *Acropora* corals. *PLoS One* **7**: e30486.

Fukami, H., Budd, A.F., Levitan, D.R., Jara, J., Kersanach, R. & Knowlton, N. 2004. Geographic differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. *Evolution* **58**: 324–337.

Gage, M.J., Macfarlane, C.P., Yeates, S., Ward, R.G., Searle, J.B. & Parker, G.A. 2004. Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. *Curr. Biol.* **14**: 44–47.

Geyer, L.B. & Palumbi, S.R. 2005. Conspecific sperm precedence in two species of tropical sea urchins. *Evolution* **59**: 97–105.

Hagman, D.K., Gittings, S.R. & Deslarzes, K.J.P. 1998. Timing, species participation, and environmental factors influencing annual mass spawning at the Flower Gardens Banks (Northwestern Gulf of Mexico). *Gulf of Mexico Sci.* **16**: 170–179.

Harper, F.M. & Hart, M.W. 2005. Gamete compatibility and sperm competition affect paternity and hybridization between sympatric *Asterias* sea stars. *Biol. Bull.* **209**: 113–126.

Hatta, M., Fukami, H., Wang, W., Omori, M., Shimoike, K., Hayashibara, T., *et al.* 1999. Reproductive and genetic evidence for a reticulate evolutionary history of mass-spawning corals. *Mol. Biol. Evol.* **16**: 1607–1613.

Howard, D.J. 1999. Conspecific sperm and pollen precedence and speciation. *Ann. Rev. Ecol. Syst.* **30**: 109–132.

Howard, D.J., Palumbi, S.R., Birge, L. & Manier, M.K. 2008. Sperm and speciation. In: *Sperm Biology: An Evolutionary Perspective* (T.R. Birkhead, D. Hosken & S. Pitnick, eds), pp. 367–403. Elsevier Press, New York.

Immler, S., Hamilton, M.B., Poslusny, N.J., Birkhead, T.R. & Epifanio, J.M. 2011. Post-mating reproductive barriers in two unidirectionally hybridizing sunfish (Centrarchidae: *Lepomis*). *J. Evol. Biol.* **24**: 111–120.

Knowlton, N., Mate, J.L., Guzman, H.M., Rowan, R. & Jara, J. 1997. Direct evidence for reproductive isolation among the three species of the *Montastraea annularis* complex in Central America (Panama and Honduras). *Mar. Biol.* **127**: 705–711.

Lessios, H.A. 2007. Reproductive isolation between species of sea urchins. *Bull. Mar. Sci.* **81**: 191–208.

Levitan, D.R. 1993. The importance of sperm limitation to the evolution of egg size in marine invertebrates. *Am. Nat.* **141**: 523–536.

Levitan, D.R. 2002. The relationship between conspecific fertilization success and reproductive isolation among three congeneric sea urchins. *Evolution* **56**: 1599–1609.

Levitan, D.R. 2012. Contemporary evolution of sea urchin gamete-recognition proteins: experimental evidence of density-dependent gamete performance predicts shifts in allele frequencies over time. *Evolution* **66**: 1722–1736.

Levitan, D.R. & Ferrell, D.L. 2006. Selection on gamete recognition proteins depends on sex, density, and genotype frequency. *Science* **312**: 267–269.

Levitan, D.R. & Plata Stapper, A. 2009. Simultaneous positive and negative frequency-dependent selection on sperm bindin, a gamete recognition protein in the sea urchin *Stryongylocentrotus purpuratus*. *Evolution* **64**: 785–797.

Levitan, D.R., Fukami, H., Jara, J., Kline, D., McGovern, T.M., McGhee, K.E., *et al.* 2004. Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the *Montastraea annularis* species complex. *Evolution* **58**: 308–323.

Levitan, D.R., Fogarty, N.D., Jara, J., Lotterhos, K.E. & Knowlton, N. 2011. Genetics, spatial, and temporal components of precise spawning synchrony in reef building corals of the *Montastraea annularis* species complex. *Evolution* **65**: 1254–1270.

Liljedal, S., Rudolfsen, G. & Folstad, I. 2008. Factors predicting male fertilization success in an external fertilizer. *Behav. Ecol. Sociobiol.* **62**: 1805–1811.

Lopez, J.V., Kersanach, R., Rehner, S.A. & Knowton, N. 1999. Molecular determination of species boundaries in corals: genetic analysis of the *Montastraea annularis* complex using amplified fragment length polymorphisms and a microsatellite marker. *Biol. Bull.* **196**: 80–93.

Ludlow, A.M. & Magurran, A.E. 2006. Gametic isolation in guppies (*Poecilia reticulata*). *Proc. R. Soc. Lond. Biol.* **273**: 2477–2482.

Martin-Coello, J., Benavent-Corai, J., Roldan, E.R.S. & Gomendio, M. 2009. Sperm competition promotes asymmetries in reproductive barriers between closely related species. *Evolution* **63**: 613–623.

Mayr, E. 1963. *Animal Species and Evolution*. Belknap Press, Cambridge, MA.

Medina, M., Weil, E. & Szmant, A. 1999. Examination of the *Montastrea annularis* species complex (Cnidaria: Scleractinia) using ITS and COI sequences. *Mar. Biotechnol.* **1**: 89–97.

Mendelson, T.C., Imhoff, V.E. & Venditti, J.J. 2007. The accumulation of reproductive barriers during speciation: postmating barriers in two behaviorally isolated species of darters (Percidae: *Etheostoma*). *Evolution* **61**: 2596–2606.

Metz, E.C. & Palumbi, S.R. 1996. Positive selection and sequence arrangements generate extensive polymorphism in the gamete recognition protein bindin. *Mol. Biol. Evol.* **13**: 397–406.

Metz, E.C., Kane, R.E., Yanagimachi, H. & Palumbi, S.R. 1994. Fertilization between closely-related sea-urchins is blocked

by incompatibilities during sperm-egg attachment and early stages of fusion. *Biol. Bull.* **187**: 23–34.

van Oppen, M.J.H., Willis, B.L., van Vugt, J.A. & Miller, D.J. 2000. Examination of species boundaries in the *Acropora cervicornis* group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. *Mol. Ecol.* **9**: 1363–1373.

van Oppen, M.J.H., McDonald, B.J., Willis, B.L. & Miller, D.J. 2001. The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence. *Mol. Biol. Evol.* **18**: 1315–1329.

van Oppen, M.J.H., Willis, B.L., van Rheede, T. & Miller, D.J. 2002. Spawning times, reproductive compatibilities and genetic structuring in the *Acropora aspera* group: evidence for natural hybridization and semi-permeable species boundaries in corals. *Mol. Ecol.* **11**: 1363–1376.

Palumbi, S.R. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annu. Rev. Ecol. Syst.* **25**: 547–572.

Palumbi, S.R. 2009. Speciation and the evolution of gamete recognition genes: pattern and process. *Heredity* **102**: 66–76.

Rahman, S.M. & Uehara, T. 2004. Interspecific and intraspecific variations in sibling species of sea urchin *Echinometra*. *Comp. Biochem. Phys.* **139**: 469–478.

Riffell, J.A., Krug, P.J. & Zimmer, R.K. 2004. The ecological and evolutionary consequences of sperm chemoattraction. *Proc. Natl. Acad. Sci. USA* **101**: 4501–4506.

Riginos, C., Wang, D. & Abrams, A.J. 2006. Geographic variation and positive selection on M7 lysin, an acrosomal sperm protein in mussels (*Mytilus spp.*). *Mol. Biol. Evol.* **23**: 1952–1965.

Sanchez, J.A., Alvarado, E.M., Gil, M.F., Charry, H., Arenas, O.L., Chasqui, L.H., *et al.* 1999. Synchronous mass spawning of *Montastraea annularis* (Ellis & Solander) and *Montastraea faveolata* (Ellis & Solander) (Faviidae: Scleractinia) at Rosario islands, Caribbean coast of Columbia. *Bull. Mar. Sci.* **65**: 873–879.

Severance, E.G., Szmant, A.M. & Karl, S.A. 2004. Microsatellite loci isolated from the Caribbean coral, *Montastraea annularis*. *Mol. Ecol. Notes* **4**: 74–76.

Swanson, W. & Vacquier, V.D. 2002. Reproductive protein evolution. *Ann. Rev. Ecol. Syst.* **33**: 161–179.

Szmant, A.M. 1986. Reproductive ecology of Caribbean reef corals. *Coral Reefs* **5**: 43–54.

Szmant, A.M., Weil, E., Miller, M.W. & Colon, D.E. 1997. Hybridization within the species complex of the scleractinian coral *Montastraea annularis*. *Mar. Biol.* **129**: 561–572.

Tomaiuolo, M., Hansen, T.F. & Levitan, D.R. 2007. A theoretical investigation of sympatric evolution of temporal reproductive isolation as illustrated by marine broadcast spawners. *Evolution* **61**: 2584–2595.

Van Veghel, M. 1994. Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis*. 1. Gametogenesis and spawning behavior. *Mar. Ecol. Prog. Ser.* **109**: 209–219.

Van Veghel, M.L.J. & Bak, R.P.M. 1993. Intraspecific variation of a dominant Caribbean reef building coral, *Montastraea annularis*: genetic, behavioral and morphometric aspects. *Mar. Ecol. Prog. Ser.* **92**: 255–265.

Vollmer, S.V. & Palumbi, S.R. 2002. Hybridization and the evolution of reef coral diversity. *Science* **296**: 2023–2025.

Willis, B.L., Babcock, R.C., Harrison, P.L. & Oliver, J.K. 1985. Patterns in mass spawning of corals on the Great Barrier Reef from 1981 to 1984. *5th Int. Coral Reef Symp.* **4**: 343–348.

Willis, B.L., Babcock, R.C., Harrison, P.L. & Wallace, C.C. 1997. Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. *Coral Reefs* **16**: S53–S65.

Willis, B.L., van Oppen, M.J.H., Miller, D.J., Vollmer, S.V. & Ayre, D.J. 2006. The role of hybridization in the evolution of reef corals. *Ann. Rev. Ecol. Evol. Syst.* **37**: 489–517.

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