

THE RISK OF POLYSPERMY IN THREE CONGENERIC SEA URCHINS AND ITS IMPLICATIONS FOR GAMETIC INCOMPATIBILITY AND REPRODUCTIVE ISOLATION

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Developmental failure caused by excess sperm (polyspermy) is thought to be an important mechanism driving the evolution of gamete-recognition proteins, reproductive isolation, and speciation in marine organisms. However, these theories assume that there is heritable variation in the susceptibility to polyspermy and that this variation is related to the overall affinity between sperm and eggs. These assumptions have not been critically examined. We investigated the relationship between ease of fertilization and susceptibility to polyspermy within and among three congeneric sea urchins. The results from laboratory studies indicate that, both within and among species, individuals and species that produce eggs capable of fertilization at relatively low sperm concentrations are more susceptible to polyspermy, whereas individuals and species producing eggs that require higher concentrations of sperm to be fertilized are more resistant to polyspermy. This relationship sets the stage for selection on gamete traits that depend on sperm availability and for sexual conflict that can influence the evolution of gamete-recognition proteins and eventually lead to reproductive isolation.

KEY WORDS: Fertilization, gamete, polyspermy, reproductive isolation, sexual conflict, sperm availability, *Strongylocentrotus*.

The evolution of gametic incompatibility and its effects on reproductive isolation are based on the affinity between eggs and sperm. Although complete gametic incompatibility has been noted among distantly related taxa, incompatibility is generally a continuum (Hagstrom and Lonning 1967; Strathmann 1981; Minor et al. 1991; Willis et al. 1997; Pernet 1999; Levitan 2002a; McCartney and Lessios 2002) that, in part, is related to the genetic similarity of a mating pair's gamete-recognition proteins (Zigler et al. 2005). Theory suggests that gametic incompatibility and reproductive isolation can evolve through a sexual conflict over fertilization rate. Males under intense sperm competition are selected to have high rates of fertilization to outcompete other males, whereas females under these conditions may be under selection for lower fertilization rates that reduce the risk of polyspermy (the

fusion of more than one spermatozoan with an egg). Under conditions of sexual conflict, novel egg-recognition proteins may be selected that slightly mismatch with sperm, lowering the affinity between sperm and eggs and decreasing the risk of polyspermy (Palumbi 1999; Gavrillets 2000; Haygood 2004). This conflict is thought to be one mechanism that can result in the rapid evolution of gamete-recognition proteins noted in a variety of taxa (reviewed in Swanson and Vacquier 2002). However, this premise hinges on the assumption that mating pairs with matched recognition alleles not only have a higher probability of fertilization but are also more likely to face the risk of polyspermy (see, e.g., Palumbi 1999). Although recent data from intraspecific field experiments suggest that matched alleles are favored under conditions of sperm limitation but perform poorly under conditions of high levels of

polyspermy (Levitan and Ferrell 2006), no comparative data are available within and across species on how the likelihood of fertilization influences the likelihood of polyspermy.

Variation within and among species exists for the influence of sperm availability on fertilization success of externally fertilizing taxa. Some species produce eggs that can be fertilized at low sperm concentrations, whereas others require much higher concentrations (reviewed in Levitan 2006). The existing evidence indicates that the ability to achieve fertilization at low levels of sperm availability is related to the likelihood of conspecific sperm availability. For example, species that spawn at lower population densities are more likely to experience conditions of sperm limitation and may be selected to achieve fertilization with relatively few sperm encounters (Levitan 1993, 1998, 2002b).

At the other end of the continuum of sperm availability, eggs exposed to very high sperm concentrations run the risk of polyspermy. Polyspermy has been noted in a variety of externally fertilizing taxa in the laboratory (Rothschild and Swann 1951; Oliver and Babcock 1992; Styan and Butler 2000; Huchette et al. 2004; Levitan et al. 2004) and field (Brawley 1992; Franke et al. 2002; Levitan 2004). Eggs are protected from multiple sperm fusions by blocks to polyspermy. These blocks include the fast electrical block and the slower cortical reaction (Tyler et al. 1956a,b). Polyspermy occurs when the rates of sperm collision and the rates of fertilization exceed the ability of the egg to block excess sperm.

What is not clear is whether the ease of fertilization and the susceptibility to polyspermy are related, within and across species. Although the mechanisms that might increase the ease with which eggs are fertilized differ from those that prevent polyspermy, these traits may be correlated because any factor that increases the rate of sperm-egg fusion might allow the rapid fusion of several sperm before a block to polyspermy can be established. Alternatively, greater ease of fertilization might lead to adaptations for a very efficient block to polyspermy. All other things being equal, eggs should be selected for both fast fusion under sperm limitation and efficient blocks to polyspermy when sperm are abundant. However, trade-offs may exist because of the inherent constraints that fast fusion might place on rapid blocks to polyspermy. Species that are subject to different levels of sperm availability may be selected either for fast fusion, which makes eggs easier to fertilize but more susceptible to polyspermy, or for slow fusion, which makes them more resistant to polyspermy.

The sea urchins *Strongylocentrotus purpuratus*, *S. franciscanus*, and *S. droebachiensis* vary in their egg traits and susceptibility to fertilization under sperm limitation (Table 1). *Strongylocentrotus purpuratus* has the smallest eggs, which lowers the sperm collision rate, and an egg surface that reduces the chance of fertilization given a spermatozoan collision; *S. droebachiensis* has the largest and easiest-to-fertilize eggs (Levitan 1993, 1998, 2002b). This pattern of egg traits also predicts a reciprocal re-

Table 1. Gamete traits of *Strongylocentrotus droebachiensis* (Sd), *S. franciscanus* (Sf), and *S. purpuratus* (Sp). Egg diameter in mm (Egg size), estimated proportion of sperm collisions that results in a fertilization event (Fertilizability), average log amount of congeneric sperm needed to fertilize 50% of eggs in the laboratory (Hybrid resistance), velocity of sperm in mm/s (Sperm velocity), slope of the relationship between sperm half-life as a function of log sperm concentration—higher slopes are sperm that have a more pronounced reduction in half-life with sperm dilution (Sperm longevity), relative performance of gametes to achieve fertilization under sperm-limited conditions in the sea (Field performance), and relative degree of sperm availability based on population density and nearest neighbor distances in Barkley Sound, British Columbia, Canada (Sperm availability).

Trait	Sd	Sf	Sp	Reference
Egg size	0.145	0.135	0.084	1
Fertilizability	0.1667	0.0512	0.0559	1
Hybrid resistance	2.47	6.39	7.34	4
Sperm velocity	0.088	0.130	0.145	1
Sperm longevity	0.308	0.391	0.457	1
Field performance	High	Medium	Low	2,3
Sperm availability	Low	Medium	High	2,3

References: 1. Levitan 1993, 2. Levitan 1998, 3. Levitan 2002b, 4. Levitan 2002a.

lationship among these species in susceptibility to polyspermy. However, sperm velocity, which influences rates of fertilization (Levitan 2002b), is inversely related to egg size and ease of fertilization in these species. This reciprocal pattern might reflect a sexual conflict (females selected to produce eggs with low affinity for sperm to avoid polyspermy, whereas males are selected to have fast sperm and rapid fertilization to compete with other males) and confound the relationship between ease of fertilization and polyspermy across these species. We therefore examined the relationship between ease of fertilization and polyspermy in a laboratory study of these three congeneric sea urchins.

Methods

Evidence of polyspermy was quantified in three species of cooccurring sea urchins: *S. purpuratus* (purple sea urchin), *S. franciscanus* (red sea urchin), and *S. droebachiensis* (green sea urchin). Individuals of these species were collected from the Deer Island Group in Barkley Sound, British Columbia, Canada, in March 2005 for an initial study and then in May and June 2006 for a follow-up study. Individuals were kept in an open seawater system and fed macroalgae for no more than two weeks before use in laboratory crosses. The initial 2005 study was conducted during spawning season for all three species. Conspecific crosses as well as hybrid crosses using *S. droebachiensis* eggs were examined (all

other heterospecific crosses were attempted but resulted in fertilization rates too low to be analyzed). The follow-up 2006 study was conducted later in the spawning season, when *S. purpuratus* and *S. franciscanus* were still in prime spawning season but after the spawning season of *S. droebachiensis* (Strathmann 1987; Levitan 2002b). The purpose of the latter study was to examine further the two species with more similar fertilization kinetics. In 2006, antibiotics were used in the embryonic cultures to decrease mortality not associated with polyspermy. The two studies involved slightly different methods and were therefore analyzed independently. We used the gametes from a single male and a single female sea urchin for each cross. Sea urchins in conspecific crosses were used only once (70 trials). Eggs from a subset of these individuals were also used with sperm from other species (16 trials).

LABORATORY CROSSES

On each experimental day, we injected sea urchins with 0.55 M KCl to induce spawning. We collected sperm off the aboral surface of each male sea urchin and kept them in a glass dish on ice to prolong viability. We collected eggs by inverting each female sea urchin in a glass bowl filled with 1 μ L filtered seawater and diluting to a stock concentration of 4000–6000 eggs/mL. Gametes from each individual cross were tested over a range of sperm concentrations from severe sperm limitation (typically 0% fertilization) to severe conditions of polyspermy (typically 0% of fertilized eggs surviving). A 0.11-mL aliquot of dry sperm was added to a scintillation vial filled with 10.89 mL of filtered seawater. We serially diluted this mixture by taking 1 mL and adding it to 8 mL of filtered seawater five times in *S. purpuratus* and *S. franciscanus* (final dilutions = 10^2 – 10^7) and six times in *S. droebachiensis* (final dilutions = 10^2 – 10^8). The additional sperm dilution was added to the *S. droebachiensis* trials because this species successfully fertilizes at lower sperm concentrations. In all trials, we added 1-mL aliquots of the egg stock solution to each vial within 2 min of sperm dilution. Egg and sperm concentrations were measured in all stock suspensions. After the gametes were introduced, we swirled the vials three times and left them in a seawater table at ambient water temperature (10–11°C).

We determined fertilization after 3 h by counting approximately 250 eggs and scoring the number of eggs undergoing development. Often *S. franciscanus* eggs failed to raise vitelline envelopes at extreme sperm concentrations; those eggs were scored as “fertilized and killed by excess sperm” if lower concentrations of sperm in that replicate produced 100% fertilization.

In 2005, we conducted an additional set of trials to examine the susceptibility of eggs to polyspermy from heterospecific sperm. Previous work has established that both *S. purpuratus* and *S. franciscanus* sperm can fertilize *S. droebachiensis* eggs (Levitan 2002a). These trials used the methods outlined above but

compared *S. droebachiensis* eggs exposed to conspecific sperm and to sperm from the other two congeners.

LARVAL CULTURES

We placed all scored eggs and embryos in beakers with 100 mL of filtered seawater and kept those beakers in a flowing seawater table to maintain the cultures at ambient sea temperatures. In these cultures, the exact numbers of fertilized and unfertilized eggs were known. When very few eggs were fertilized (< 10%) at very low sperm concentrations we did not establish cultures because the sample size was too low to provide a precise measure of survivorship. In the 2006 trials, we added antibiotics to the cultures (penicillin and streptomycin as per Strathmann 1987). This treatment generally increased the mean and decreased the variance in embryonic survivorship.

After 48 h, we counted the developing larvae in each culture. We divided the number of developing larvae by the number of fertilized eggs (as scored after 3 h) to determine the raw embryonic survivorship. We adjusted these survivorship values by dividing them by the highest survivorship value for that set of cultures in a replicate (the series of sperm dilutions). This adjustment removed differences in replicate culture survivorship not associated with sperm concentration. The adjusted survivorship values were logit transformed for increased linearity and examined with an ANCOVA. This estimate, the changes in embryonic survivorship of fertilized eggs associated with variation in sperm concentration, was used as our estimate of polyspermy. This estimate allowed comparison of species that differed in the stage at which polyspermy killed eggs and provided a cumulative estimate of polyspermy for a full population of eggs.

An alternative method of estimating polyspermy is microscopic inspection of fixed eggs for multiple sperm fusions (Brawley 1992; Franke et al. 2002; Levitan 2004), but it provides only a snapshot estimate at a single time. This alternate method requires that eggs be fixed a few minutes after sperm and eggs are mixed. At that time, some eggs might not yet have been fertilized, fertilized eggs might not have raised vitelline membranes or show signs of fertilization, sperm might not have fully penetrated the egg membrane, or sperm may have entered and no longer be visible (Franke et al. 2002). All these factors tend to produce an underestimate of the degree of polyspermy.

FITTING DATA TO THE FERTILIZATION KINETICS MODEL

The estimates of fertilization and developmental success were fit to a fertilization kinetics model (Styan 1998) that incorporates the effects of polyspermy at high sperm concentrations. The model predicts the proportion of monospermic fertilizations as a function of gamete concentration, the collision constant (a function of egg cross-sectional area and sperm velocity, set for each

species from published values; Levitan 1993), the fertilization constant, the interaction time of sperm and eggs, and the interval between fertilization and the establishment of an effective block to polyspermy. The empirical data on developmental success (the fraction of eggs fertilized and developing successfully, used as the proxy for monospermic fertilizations) and the empirically derived parameter values of gamete concentration and sperm–egg contact time were used in a nonlinear regression (SAS) to generate best-fit estimates of the fertilization constant and the time to block polyspermy. Once these values were estimated, the equation was solved for the F_{25} (the sperm concentration at which 25% of eggs are fertilized) and P_{25} (the sperm concentration at which 75% of the eggs die by polyspermy). A low F_{25} value indicates that eggs are more easily fertilized at lower sperm concentrations, and similarly a low P_{25} value indicates eggs are more susceptible to polyspermy at lower sperm concentrations.

The Styan model does not force autocorrelation between the predicted level of fertilization and polyspermy. The estimates of F_{25} and P_{25} are independent, because the data are fit to the empirical model by varying the time required to establish the polyspermy block. To test this independence, we conducted simulations of the model using fabricated data, in which the ease of fertilization and the susceptibility to polyspermy were either dependent or independent of each other. We then used Styan's model to predict the values of F_{25} and P_{25} . When the ease of fertilization was held constant and the susceptibility to polyspermy varied, the correlation between F_{25} and P_{25} was zero. The F_{25} and P_{25} values were only correlated when easy to fertilize eggs were also susceptible to polyspermy (unpubl. simulations).

Results

DEVELOPMENTAL SUCCESS

The initial 2005 study indicated that developmental success of zygotes decreased with increasing sperm concentration (Fig. 1). An ANCOVA tested a main effect of species, a covariate of sperm concentration, and their interaction. The interaction was not significant (slopes of different species did not differ, $P = 0.77$), so it was removed from the model. Species ($P < 0.0001$) and sperm concentration ($P < 0.01$) were significant. Pairwise tests of least square means indicated that the sperm concentration that reduced developmental success in *S. droebachiensis* was approximately one order of magnitude lower than those reducing success in the other two species ($P < 0.01$ in each case); this species was susceptible to developmental failure at the lowest sperm concentrations. *Strongylocentrotus franciscanus* and *S. purpuratus* did not differ significantly ($P = 0.92$).

In the follow-up 2006 study, an ANCOVA revealed a significant interaction between the slopes of these two latter species ($P < 0.0001$; Fig. 2). *Strongylocentrotus franciscanus* exhibited

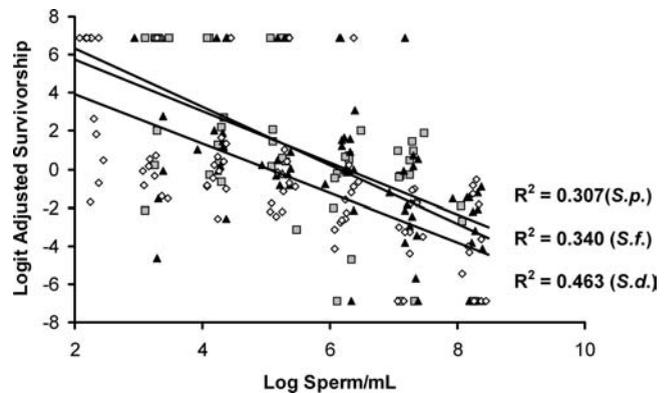


Figure 1. 2005 trials demonstrating the relationship between sperm availability and logit-transformed survivorship of eggs fertilized by at least one spermatozoan. Conspecific crosses are indicated by black triangles (*Strongylocentrotus purpuratus*, *S.p.*), gray squares (*S. franciscanus*, *S.f.*), and open diamonds (*S. droebachiensis*, *S.d.*).

a sharper decrease in developmental success at higher sperm concentrations than did *S. purpuratus*. Independent regression analysis indicated a significant negative relationship between sperm concentration and developmental success ($P < 0.0001$), and in *S. franciscanus* this relationship included a significant quadratic component ($P < 0.0001$ for polynomial component); *S. franciscanus* showed an accelerating decrease in developmental success at the highest sperm concentrations.

In general, *S. franciscanus* tended to show signs of developmental failure a short time after exposure to very high levels of sperm concentration. At concentrations above 10^8 sperm/mL, eggs of this species often failed to raise vitelline envelopes or failed to develop past the raising of the envelope. In contrast, *S. purpuratus* and *S. droebachiensis*, although exhibiting the extremes in their susceptibility to overabundant sperm, tended to

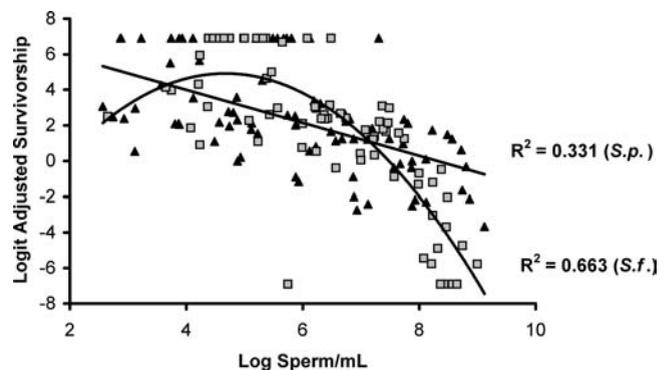


Figure 2. 2006 trials demonstrating the relationship between sperm availability and logit-transformed survivorship of eggs fertilized by at least one spermatozoan. Conspecific crosses were conducted between two species, indicated by black triangles (*S. purpuratus*) and gray squares (*S. franciscanus*).

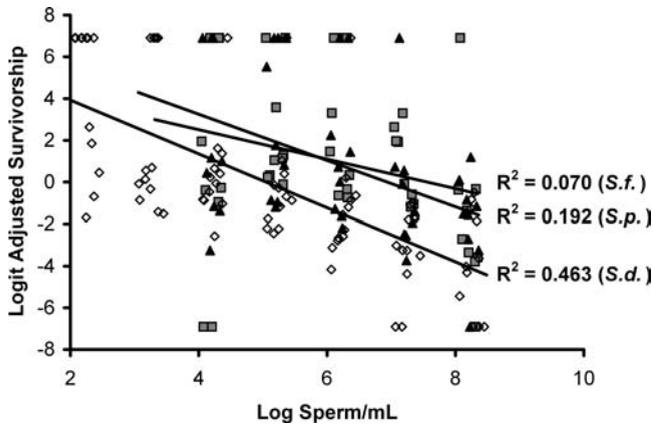


Figure 3. 2005 trials demonstrating the relationship between sperm availability and logit-transformed survivorship in conspecific and heterospecific crosses involving *S. droebachiensis* eggs and either *S. droebachiensis* males (open diamonds), *S. franciscanus* males (gray squares), or *S. purpuratus* males (black triangles).

initiate development even at high sperm concentrations before differentially failing at 48 h. The significant curvilinearity of the *S. franciscanus* response at high sperm concentrations, compared to the other two species, probably reflects this differential response at high sperm concentrations.

Eggs of *S. droebachiensis* were more susceptible to developmental failure (at up to 48 h) with conspecific sperm than with heterospecific sperm (Fig. 3). An ANCOVA tested a main effect of sperm donor species, a covariate of sperm concentration, and their interaction. The interaction was not significant (slopes of different species did not differ, $P = 0.52$), so it was removed from the model. The remaining factors, sperm donor species ($P < 0.0001$) and the covariate ($P < 0.0001$), were highly significant. Pairwise tests of least square means indicated that eggs suffered reduced developmental success at concentrations approximately one order of magnitude lower sperm concentration when exposed to conspecific sperm than when exposed to heterospecific sperm ($P < 0.001$ compared with both heterospecific donors). No difference was detected between the two heterospecific sperm donor species ($P = 0.82$).

RELATIONSHIP BETWEEN EASE OF FERTILIZATION AND SUSCEPTIBILITY TO POLYSPERMY WITHIN AND AMONG SPECIES

In all conspecific trials, sperm concentration was varied such that at the lowest sperm concentrations, fertilization of eggs averaged less than 5% and at the highest sperm concentrations fertilization (fusion by at least one spermatozoan) averaged over 95%, but at these high sperm concentrations most of these embryos failed to develop (Fig. 1). Each replicate fertilization trial in 2005 was fitted to the Styan fertilization kinetics model and then solved for

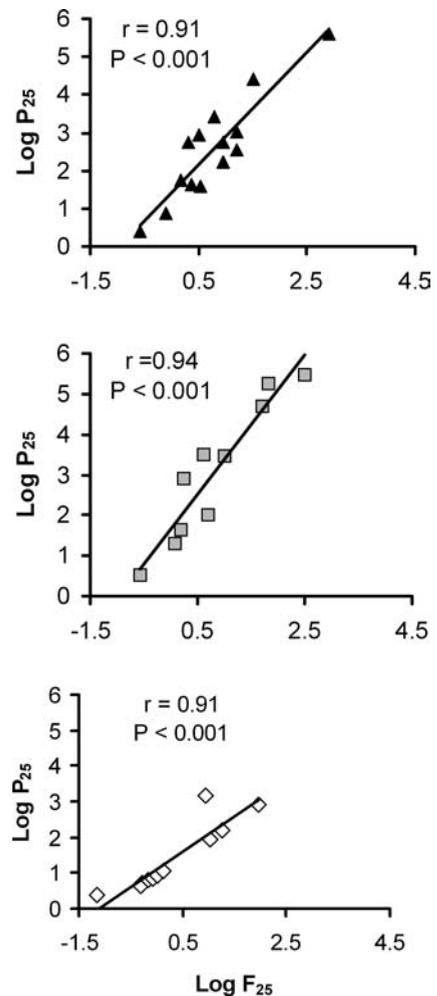


Figure 4. Within-species correlations between ease of fertilization (F_{25}) and susceptibility to polyspermy (P_{25}) for three congeners: *S. purpuratus* (black triangles), *S. franciscanus* (gray squares), and *S. droebachiensis* (open diamonds).

F_{25} (the sperm concentration at which 25% of eggs are fertilized) and P_{25} (the sperm concentration at which 75% of the eggs have died by polyspermy and 25% survive). Within each species, the correlation coefficients between the level of sperm needed to fertilize and that causing developmental failure exceeded 0.9 (Fig. 4). Females within each species varied in how their eggs responded to sperm availability. Females producing eggs that were fertilized at low sperm concentrations were also susceptible to polyspermy at relatively low sperm concentrations.

Consistent with the analysis of developmental success, an ANOVA of the P_{25} values indicated a significant species effect ($P = 0.015$). Tukey pairwise comparisons revealed that *S. droebachiensis* has a significantly ($P = 0.015$ with *S. franciscanus*) and marginally significantly ($P = 0.078$ with *S. purpuratus*) lower threshold for polyspermy than did the other species.

Discussion

THE RELATIONSHIP BETWEEN EASE OF FERTILIZATION AND SUSCEPTIBILITY TO POLYSPERMY

Within, among, and across (hybrid) species, ease of fertilization at low sperm concentrations was related to the susceptibility to polyspermy. Within all three species, females that produced eggs that were fertilized at low sperm concentrations were also the most likely to experience developmental failure at relatively low sperm concentrations (Fig. 4). Among species, the rank order of ease of fertilization (*S. droebachiensis*, *S. franciscanus*, and *S. purpuratus*, respectively) based on both laboratory and field studies (Table 1) also matches the rank order of susceptibility to polyspermy (Figs. 1, 2). In addition, hybridization assays indicate that although hybrid sperm can be less likely to fertilize *S. droebachiensis* eggs (Table 1), they are also less likely to result in polyspermy (Fig. 3). Although the mechanisms responsible for sperm–egg fusion rates (e.g., rate of collisions and match of recognition proteins) and blocks to polyspermy (electrical block and cortical reaction) are different, a high collision rate or high gamete affinity can result in overcoming eggs with sperm before a polyspermy block can be established.

Additional support for the notion that within-species variation in fertility translates into variation in the risk of polyspermy comes from field studies. In induced spawning events with *S. franciscanus* under natural conditions, mates with matched gamete recognition protein genotypes had relatively high reproductive success under conditions of low spawning density and sperm limitation, but relatively poor success under conditions of high spawning density in which polyspermy was common (Levitan and Ferrell 2006). High affinity between sperm and eggs was beneficial under sperm-limited conditions but detrimental when sperm were oversaturated. This produced a pattern of frequency-dependent selection that was positive (common genotypes selected) at low spawning densities and negative (rare genotypes selected) at high densities.

Field studies also support the relationship between ease of fertilization and polyspermy among species. Under field conditions, *S. purpuratus* had 25% and 40% fewer eggs fertilized at low spawning densities compared to *S. franciscanus* and *S. droebachiensis*, respectively (Levitan 2002b), but showed no sign of polyspermy at densities (150 males/m² in Levitan 2002b and unpublished data) far exceeding the densities in which *S. franciscanus* exhibited polyspermy (5 males/m² in Levitan 2004).

The overall pattern of ease of fertilization and polyspermy in these species match the predictions based on gamete traits (egg size and egg receptivity; Table 1). These patterns also match the predicted selective pressures given their differences in spawning density; the rarest species (*S. droebachiensis*) living under the most sperm limiting conditions produces the eggs easiest to fertil-

ize and the most clumped species (*S. purpuratus*) found under the most sperm competitive conditions produces the eggs most resistant to polyspermy (Levitan 2002b). These patterns are consistent with density-dependent selection on gamete traits in which there is a trade-off between being successful at one level of sperm availability at the cost being maladaptive at another (Levitan 2002a,b, 2004; Levitan and Ferrell 2006).

Additional evidence that one mechanism influencing fertilization rate influences another comes from a study of heterospecific fertilization. For both *Strongylocentrotus droebachiensis* and *S. purpuratus*, females that produced eggs more easily fertilized by conspecific sperm were also more easily fertilized by heterospecific sperm (Levitan 2002a). This suggests that mechanisms that increase the likelihood of fertilization at low sperm concentrations (e.g., more sperm collisions or a higher density of egg receptor proteins) will have cascading effects on gamete compatibility across species, because any factor that makes it easier to fertilize an egg provides more opportunities for heterospecific sperm to overcome partial incompatibilities (Levitan 2002a).

In our experiments, some fraction of reduced developmental success may have been caused by other factors, besides polyspermy, associated with sperm availability. At extreme sperm concentrations, eggs were at times obliterated; the cause of egg death may have been too many collisions with sperm rather than excess fusion events. Regardless of whether the eggs perished before or after sperm fusion, if excess sperm kills eggs, it sets the stage for a sexual conflict.

It is also possible that some fraction of reduced developmental success at higher sperm concentrations was caused by factors independent of sperm killing eggs. If lower quality eggs require higher sperm concentrations to be fertilized and also have lower developmental success (Marshall et al. 2002) it could result in a relationship between fertilization and developmental failure unrelated to polyspermy. However, this scenario would result in a negative relationship between F₂₅ and P₂₅, because poor-quality eggs would require more sperm to achieve fertilization (high F₂₅) and also show lower survivorship (low P₂₅). This is opposite to our observations (e.g., Fig. 4) and therefore would obscure rather than falsely magnify the importance of polyspermy.

SEXUAL CONFLICT, SELECTION ON GAMETE RECOGNITION PROTEINS, AND REPRODUCTIVE ISOLATION

The degree of sexual conflict in external fertilizers depends on the availability of sperm (Franke et al. 2002; Levitan 2004, 2005; Levitan and Ferrell 2006). When individuals are scattered and sperm availability is low, both males and females are selected to have gametes with high fusion rates. However, as population density increases and sperm become overabundant, sexual conflict may arise over the ease of fertilization. Although polyspermy is

costly to both males and females, it is never advantageous for a male to produce sperm with a low fusion rate when sperm from several males compete for fertilizations. Females may therefore be selected to have lower affinity for sperm under these conditions whereas males are selected to have sperm with a high affinity for eggs. Consistent with this notion, *S. purpuratus*, the species that lives at the highest levels of crowding and sperm availability of these three congeners, produces the smallest, most sperm resistant eggs, and the fastest, most competitive sperm (Levitan 1993).

This conflict can result in selection on the blocks to polyspermy and the reproductive proteins that mediate gametic compatibility. Selection for efficient blocks to polyspermy at high levels of sperm availability would ease the conflict between males and females because excess sperm would be prevented from causing developmental failure and sperm with the highest affinity for eggs would still be selected. However, unless the block is perfect over the natural range of sperm availability, conflict will exist. The constraints preventing a perfect block to polyspermy are the limits of adaptation for fusion with only one spermatozoan during simultaneous sperm collisions and the trade-off of making eggs so difficult to fertilize that no sperm succeed. Because each egg has its own independent fate, under moderate levels of sperm availability some eggs can be sperm limited whereas others face the risk of polyspermy (Styan and Butler 2000; Franke et al. 2002; Levitan 2004; the present study). Eggs are selected to balance these opposing risks and as a result are exposed to the cost of polyspermy and sexual conflict.

A second mechanism for reducing the level of polyspermy is to lower the collision rate. This could be done behaviorally, if females did not spawn when sperm were overabundant, or by producing eggs less likely to be found by sperm. Although females appear to use sperm as a cue (Starr et al. 1990, 1992) and spawn less frequently than males (Levitan 2002b) there is no indication that females withhold spawning because of overabundant sperm. In contrast, a reduction in egg size, accessory structures surrounding eggs, or sperm attractant chemicals can reduce the collision rate. Theory and empirical evidence support the notion that target size influences fertilization rate and that there is selection for large target size under sperm-limited conditions and small target size under polyspermic conditions (reviewed in Levitan 2006).

A final mechanism for reducing the risk of polyspermy is selection in favor of mutations to the egg receptor proteins that result in a slight mismatch with sperm. The result can be "chase away" selection, as sperm proteins are selected to "chase" the evolution of these novel egg receptor proteins (Rowe et al. 1994; Holland and Rice 1998; Palumbi 1999; Gavrilets 2000; Haygood 2004). This mechanism is thought to explain the often-observed rapid evolution of gamete-recognition proteins (Swanson and Vacquier 2002). Evidence for this mechanism has been found in *S. francis-*

canus, where the most successful mating pairs at high density were common, competitively superior male genotypes, mated with rare female genotypes that could avoid the risk of polyspermy through reduced gamete affinities (Levitan and Ferrell 2006).

The notion that differences in the gamete-recognition proteins among males and females can reduce the risk of polyspermy is supported by the present comparison of conspecific and heterospecific fertilization. These species differ in their sperm binding proteins (Biermann 1998), and heterospecific crosses resulted in lower likelihood of polyspermy (Fig. 4). Although overall genetic divergence is a poor predictor of heterospecific compatibility in sea urchins, increased divergence in sperm binding lowers the probability of heterospecific fertilization (Zigler et al. 2005). Divergence in gamete-recognition proteins both within and among species seems to reduce the likelihood of fertilization at low sperm concentrations and polyspermy at high sperm concentrations. This result implies that within-species processes that reduce the risk of polyspermy can have cascading effects on the rapid evolution of gamete-recognition proteins and reproductive isolation.

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LITERATURE CITED

- Biermann, C. H. 1998. The molecular evolution of sperm binding in six species of sea urchins (Echinoida: Strongylocentrotidae). *Mol. Biol. Evol.* 15:1761–1771.
- Brawley, S. H. 1992. Fertilization in natural populations of the dioecious brown alga *Fucus ceranoides* and the importance of the polyspermy block. *Mar. Biol.* 113:145–157.
- Franke, E. S., R. C. Babcock, and C. A. Styan. 2002. Sexual conflict and polyspermy under sperm-limited conditions: in situ evidence from field simulations with the free-spawning marine echinoid *Evechinus chloroticus*. *Am. Nat.* 160:485–496.
- Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* 403:886–889.
- Hagstrom, B. E., and S. Lonning. 1967. Experimental studies of *Strongylocentrotus droebachiensis* and *S. pallidus*. *Sarsia* 29:165–176.
- Haygood, R. 2004. Sexual conflict and protein polymorphism. *Evolution* 58:1414–1423.
- Holland, B., and W. R. Rice. 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* 52:1–7.
- Huchette, S. M. H., J. P. Soulard, C. S. Koh, and R. W. Day. 2004. Maternal variability in the blacklip abalone, *Haliotis rubra* Leach (Mollusca: Gastropoda): effect of egg size on fertilisation success. *Aquaculture* 231:181–195.
- Levitan, D. R. 1993. The importance of sperm limitation to the evolution of egg size in marine invertebrates. *Am. Nat.* 141:517–536.
- . 1998. Does Bateman's principle apply to broadcast-spawning organisms? Egg traits influence in situ fertilization rates among congeneric sea urchins. *Evolution* 52:1043–1056.

- . 2002a. The relationship between conspecific fertilization success and reproductive isolation among three congeneric sea urchins. *Evolution* 56:1599–1609.
- . 2002b. Density-dependent selection on gamete traits in three congeneric sea urchins. *Ecology* 83:464–479.
- . 2004. Density-dependent sexual selection in external fertilizers: variances in male and female fertilization success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. *Am. Nat.* 164:298–309.
- . 2005. The distribution of male and female reproductive success in a broadcast spawning marine invertebrate. *Integr. Comp. Biol.* 45:848–855.
- . 2006. The relationship between egg size and fertilization success in broadcast-spawning marine invertebrates. *Integr. Comp. Biol.* 46:298–311.
- Levitan, D. R., and D. L. Ferrell. 2006. Selection on gamete recognition proteins depends on sex, density, and genotype frequency. *Science* 312:267–269.
- Levitan, D. R., H. Fukami, J. Jara, D. Kline, T. M. McGovern, K. E. McGhee, C. A. Swanson, and N. Knowlton. 2004. Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the *Montastraea annularis* species complex. *Evolution* 58:308–323.
- Marshall, D. J., C. A. Styan, M. J. Keough. 2002. Sperm environment affects offspring quality in broadcast spawning marine invertebrates. *Ecol. Lett.* 5:173–176.
- McCartney, M. A., and H. A. Lessios. 2002. Quantitative analysis of gametic incompatibility between closely related species of Neotropical sea urchins. *Biol. Bull.* 202:166–181.
- Minor, J. E., D. R. Fromson, R. J. Britten, and E. H. Davidson. 1991. Comparison of the bindin proteins of *Strongylocentrotus franciscanus*, *Strongylocentrotus purpuratus*, and *Lytechinus variegatus*—sequences involved in the species specificity of fertilization. *Mol. Biol. Evol.* 8:781–795.
- Oliver, J., and R. Babcock. 1992. Aspects of the fertilization ecology of broadcast spawning corals—sperm dilution effects and in situ measurements of fertilization. *Biol. Bull.* 183:409–417.
- Palumbi, S. R. 1999. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proc. Natl. Acad. Sci. USA* 96:12632–12637.
- Pernet, B. 1999. Gamete interactions and genetic differentiation among three sympatric polychaetes. *Evolution* 53:435–446.
- Rothschild, L., and M. M. Swann. 1951. The conduction time of the block to polyspermy in the sea-urchin egg. *Exp. Cell Res.* 2:137–137.
- Rowe, L., G. Arnqvist, A. Sih, and J. Krupa. 1994. Sexual conflict and the evolutionary ecology of mating patterns—water striders as a model system. *Trends Ecol. Evol.* 9:289–293.
- Starr, M., J. H. Himmelman, and J. C. Theriault. 1990. Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science* 247:1071–1074.
- . 1992. Isolation and properties of a substance from the diatom *Phaeodactylum tricorutum* which induces spawning in the sea urchin *Strongylocentrotus droebachiensis*. *Mar. Ecol. Prog. Ser.* 79:275–287.
- Strathmann, M. 1987. Phylum Echinodermata, class Echinoidea. Pp. 522–534 in M. Strathmann, ed. *Reproduction and development of marine invertebrates of the northern Pacific coast*. Univ. of Washington Press, Seattle, WA.
- Strathmann, R. R. 1981. On barriers to hybridization between *Strongylocentrotus droebachiensis* (O. F. Muller) and *Strongylocentrotus pallidus* (G. O. Sars). *J. Exp. Mar. Biol. Ecol.* 55:39–47.
- Styan, C. A. 1998. Polyspermy, egg size, and the fertilization kinetics of free-spawning marine invertebrates. *Am. Nat.* 152:290–297.
- Styan, C. A., and A. J. Butler. 2000. Fitting fertilisation kinetics models for free-spawning marine invertebrates. *Mar. Biol.* 137:943–951.
- Swanson, W. J., and V. D. Vacquier. 2002. Reproductive protein evolution. *Annu. Rev. Ecol. Syst.* 33:161–179.
- Tyler, A., A. Monroy, C. Y. Kao, and H. Grunfest. 1956a. Membrane potential and resistance of the starfish egg before and after fertilization. *Biol. Bull.* 111:153–177.
- Tyler, A., A. Monroy, and C. B. Metz. 1956b. Fertilization of fertilized sea urchin eggs. *Biol. Bull.* 110:184–195.
- Willis, B. L., R. C. Babcock, P. L. Harrison, and C. C. Wallace. 1997. Experimental hybridization and breeding incompatibilities within the mating systems of mass spawning reef corals. *Coral Reefs* 16:S53–S65.
- Zigler, K. S., M. A. McCartney, D. R. Levitan, and H. A. Lessios. 2005. Sea urchin bindin divergence predicts gamete compatibility. *Evolution* 59:2399–2404.

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