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*

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ABSTRACT: Sperm competition and female choice are fundamentally driven by gender differences in investment per offspring and are often manifested as differences in variance in reproductive success: males compete and have high variance; most females are mated and have low variance. In marine organisms that broadcast spawn, however, females may encounter either sperm limitation or sperm competition. I measured the fertilization success of male and female *Strongylocentrotus franciscanus* over a range of population densities using microsatellite markers. Female fertilization success first increased and then decreased with mate density, limited at low density by sperm limitation and at high density by polyspermy. Mate density affected variance in fertilization success in both males and females. In males, the variance in fertilization success increased with mate density. In females, the pattern was more complex. The variance in female success increased similarly to males with increased mate density but then decreased to low levels at intermediate densities, where almost all eggs were fertilized. As density increased further, the female variances again increased as polyspermy lowered average fertilization success. Male and female variances differed only at intermediate densities. At low densities, both sexes may be under selection to increase fertilization success; at intermediate densities, males may compete; and at high densities, both sexes may be under selection to increase success by increasing (males) or decreasing (females) likelihood of fertilization during sexual conflict. Only within a narrow range of densities do patterns of sexual selection mirror those typically noted in internally fertilizing taxa.

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Sexual selection, the selection that arises from differences in mating success (Arnold 1994*b*), can strongly influence the evolution of traits within a species and the evolution of reproductive isolation among species (Andersson 1994). Most research on sexual selection has focused on situations where sexual selection is clearly more intense for one sex than the other. Because they often invest less in each successful mating, males are generally regarded as competing for mates, and females are seen as choosing among potential mates (Birkhead and Møller 1998). Male competition can result in high variance in male reproductive success because some males are more successful than others, and it can result in low variance in female reproductive success because most females are mated (Bateman 1948).

The differing selection on males and females can also lead to sexual conflict, which occurs when one sex (usually males) gains fitness while the other sex loses fitness with each additional mating (Parker 1979). Mating can be costly to both males and females (Partridge and Farquhar 1981) or only to females (Chapman et al. 1995), but regardless, males generally have a higher optimal mating rate because they invest less in each mating and produce offspring with each additional mating, whereas females often do not (Holland and Rice 1998). This conflict is thought to result in chase-away selection, in which males are selected to induce females to mate, but females are selected to resist mating (Arnqvist and Rowe 1995; Holland and Rice 1998; Gavrilets et al. 2001).

Broadcast spawning, the release of both eggs and sperm into the sea for external fertilization, is the likely ancestral mating strategy (Franzen 1956; Jagersten 1972; Parker 1984; Wray 1995; but see Rouse and Fitzhugh 1994) and remains a widespread reproductive mode (Giese and Kanatani 1987), yet we know little about how sexual selection

and sexual conflict have influenced its evolution (Levitan 1998*b*; Franke et al. 2002). Patterns of sexual selection may differ greatly from those of other reproductive strategies because heterogeneities of adult distribution and water movement can lead to high variation in the relative concentration of eggs and sperm in the water (see, e.g., Denny and Shibata 1989; Benzie et al. 1994). The result is either sperm limitation or sperm competition (Levitan and Petersen 1995; Yund 2000) and is likely to influence the intensity and nature of sexual selection (Levitan 1998*b*).

Although data on sperm competition exist for externally fertilizing fish (Petersen and Warner 1998) and pair-spawning horseshoe crabs (Brockmann et al. 1994), these animals spawn in very close proximity and do not face, to the same degree, the problems of sperm dilution and heterogeneous gamete concentrations noted in sessile or sedentary marine invertebrates (Levitan 1998*b*). Similarly, some data exist on sperm competition in brooding invertebrates (Grosberg 1991; Yund and McCartney 1994), but these species seem, through sperm filtration, storage, and selfing (Bishop 1998), to avoid sperm limitation to a large extent (Yund 2000). At present, no data are available on sperm competition and reproductive variances in sessile or sedentary external fertilizers, the group most likely to experience large fluctuations in egg and sperm concentrations and to exhibit patterns of sexual selection different from those of internally fertilizing taxa (Levitan 1998*b*).

Here I present data on the ways in which variance in fertilization success depends on population density. Previous studies have noted that sexual selection can depend on the ecological context (e.g., food supply, sex ratio, hunger, and density; see Rowe et al. 1994). The results here indicate that males and females only differ in fertilization variances at intermediate densities, where the more typically studied sexual-selective patterns of sperm competition and female choice may be common. In contrast, at low and high densities, male and female variances are more similar, yet reflect the very different selective regimes imposed by sperm limitation and sexual conflict, respectively.

Methods

Field Experiment

The mean and variance of male and female fertilization success were measured during 35 experimental spawning events of the sea urchin *Strongylocentrotus franciscanus* in Barkley Sound, Vancouver Island, Canada. Experiments were conducted along a 500-m expanse of rocky shoreline at the mouth of Bamfield Inlet (48°50.5′N, 125°08.5′W). The substratum was rock and boulders down to about 8 m, then sand and silt to a depth of 40 m within about 100 m of shore. The shallow subtidal (down to about 3 m at low tide) was occupied by the kelp *Macrocystis integrifolia*; below that the rocky surfaces were covered with coralline algae and occupied primarily by *S. franciscanus*. I chose a single site for this study to eliminate variation arising from confounding factors such as topography and wave exposure (see Levitan 2002*a* for estimates of female reproductive success over a range of sites and conditions). All experiments were conducted between early May and mid-June (2000–2002). Mature gametes can be collected from this species from early February through early July (Strathmann 1987; D. R. Levitan, unpublished data).

Water flow was measured during each dive with an S4 current meter (InterOcean). The meter was placed 0.5 m off the bottom to record the flow velocity and direction (north-south and east-west vectors) at 0.5-s intervals for the duration of the experiment. These data were used to calculate the rate of advection (distance a parcel of water moved, calculated over the full experimental time period). This measure of flow provides information on the interval during which sperm reside over a spawning aggregation and explained a small but significant amount of variation in female fertilization success in a previous study (Levitan 2002*a*).

For each event, I induced between six and 32 sea urchins to spawn by injecting them with KCl. Previous work found no differences in female fertilization success between natural and KCl-induced spawning as a function of population density (Levitan 2002*a*), so the induced and natural spawning events did not differ greatly in spawning intensity or the quality of the gametes released (i.e., their ability to fertilize). Natural and induced spawning events did not differ in degree of aggregation, and the degree of aggregation did not change before, during, or after natural spawning events (Levitan 2002*a*). While there may be subtle differences in patterns of fertilization between natural and induced spawning events, these differences have not been observed.

After injection, each individual was tagged with a numbered latex band stretched around its body, and individuals were scattered over a range of densities (0.1–10/m^2) that overlap with those of naturally occurring populations. Published estimates of *S. franciscanus* densities in British Columbia range from 0.03 to 17/m^2 using 10-m^2 plots at 17 locations (Rumrill 1987); from 2 to 32/m^2 using 20-m^2 plots at 25 locations (Bureau 1996); and from 0 to 29/m^2 using 1-m^2 plots at one site (Bernard and Miller 1973). In a previous study, the naturally occurring density of male urchins able to be induced to spawn with an injection of KCl ranged from 0.1 to 7 males/m^2 in 25-m^2 plots across 31 locations (Levitan 2002*a*). Two published observations of natural *S. franciscanus* spawning events were at densities of 1 and 3 spawning males/m^2 (Levitan 2002*a*). These two observations of spawning indicated that approximately

30% of individuals spawned, thus the 0.1–10 spawning urchins/m^2 tested here coincides fairly well with the overall density estimates of <1 to 30 urchins/m^2 noted in the literature for this region.

The positions of all individuals were mapped 30 min after the initiation of spawning, and eggs were collected in the water column above each spawning female with a plankton pump containing 12 independent filter chambers (Levitan 2002*a*). On the three occasions when more than 12 females spawned, 12 females were haphazardly chosen for egg collection.

From the map positions, the densities of males and females were calculated as the number of spawning males or females divided by the area the animals occupied. The area was calculated as that of a circle with diameter equal to the distance between the two most distantly spaced spawning individuals (Levitan 2002*a*). This method was chosen over an arbitrary quadrat size estimate so that the effects of the number of spawning animals could be distinguished from those of the density of spawning animals (i.e., so that I could compare few and many animals spawning at high and low densities).

After all egg collection, all tagged animals were collected and brought to the laboratory. Tube feet were collected from all these adults and placed in 95% ethanol for microsatellite genotyping. Three hours after completion of the field experiment, at least 250 eggs from each female sampled were examined for evidence of early development. Embryos collected from each female were then cultured for 3 days, without food, before individual larvae were frozen for paternity analysis (Levitan 2002*b*).

In the spring of 2003, six additional spawning events were conducted, with the modification that samples of eggs were fixed in formalin within 2–3 min of collection. These trials were conducted at the higher end of the density range in order to determine whether polyspermy is a possible consequence of spawning at high densities. These samples were analyzed for the presence of mono- and polyspermic fertilizations. Egg samples were rinsed with phosphate buffer saline, made permeable with 98% methanol, rinsed a second time, stained with 0.004 μM solution of Hoechst dye 33342, rinsed a third time, and mounted in distyrene: tricresyl phosphate: xylene (DPX; see Franke et al. 2002 for details). The number of sperm fused to each egg was determined by examination under a fluorescence microscope. The occurrence of multiple sperm fusions was defined as the presence of more than one sperm fused to, or completely inside, the egg cell membrane. A confocal microscope was used to examine a subsample of eggs with sperm fused to the surface, and it revealed that those sperm were partially penetrating the egg cell membrane. Twelve samples were collected on each sampling date, and an average of 41 eggs per sample were examined. No embryos from this subset of experiments were investigated for paternity.

Parentage Analysis

All adults and 20 larvae per female from the main experiment were genotyped on the basis of microsatellite loci (McCartney et al. 2004). DNA from each individual larva was extracted by the addition of 10 μL of larval extraction buffer (790 μL ddH_20; 100 μL 10 × polymerase chain reaction [PCR] buffer; 100 μL proteinase K solution, 25 mg/mL; 10 μL TWEEN 80) to the microcentrifuge tube containing the larva. The samples are then placed in a thermocycler for 60 min at 65°C, then for 15 min at 95°C. The extracted DNA was diluted 2 : 11 with sterile pure water and stored at −80°C.

Adult tissue was extracted according to a CTAB/PCI extraction protocol (as in Levitan and Grosberg 1993). Diluted DNA (from either adult tissue or whole larvae) was added to a standard PCR cocktail (5.9 μL autoclaved ddH_20, 1.0 μL 10 × PCR buffer, 1.0 μL 1 mM dNTPs, 0.5 μL 10-μM fluorescently labeled forward primer, 0.5 μL 10-μM reverse primer, 0.75 U Taq polymerase) and amplified as follows: 95°C for 5 min; then 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min; then 75°C for 25 min.

After amplification, PCR products from three compatible loci (i.e., different-sized fragments or different fluorescent labels) were mixed with HiDi Formamide (1 : 12) and analyzed with an ABI Prism 3100 automated sequencer equipped with Genescan software. The data were further analyzed with Genotyper software that, by applying a series of filters to remove anomalies such as stutter bands, determines DNA fragment length within one nucleotide.

To identify inherited markers and assign paternity, I scored the alleles of six to 15 loci for each potential parent and then chose the three (minimum) most diagnostic loci for that particular set of parents. The larvae were then tested with those loci. The first step in identifying paternity of each larva was to confirm that the larva shared an allele with the female urchin the larva was collected near. Then nonmaternal alleles were matched to the pool of potential males. Typically three to six loci were enough both to identify (exclude all but one set of parents) and to confirm (by presence of at least two diagnostic alleles in both the larvae and the adults) the parentage of larvae.

Model Fit for Sperm Limitation and Polyspermy

To determine whether the pattern of fertilization with density was best explained by sperm limitation at low densities and polyspermy at high densities, I fit these data to fertilization kinetics models that either did (Styan 1998) or did not (Vogel et al. 1982) allow for the effects

of polyspermy. These models predict fertilization as a function of gamete concentration, egg size, sperm velocity, interaction time of sperm and eggs, and depending on the model, the effectiveness of the polyspermy block. I modified the models by substituting male density for sperm concentration. The relationship between sperm concentration and male density ($\ln \text{sperm}/\mu\text{L} = 1.4506 \times \ln \text{males}/\text{m}^2 + 6.4212$, $R^2 = 0.9988$) was estimated from the relationship between sperm concentration and fertilization success in the laboratory (Levitan 1993) and the relationship between male density and fertilization success from an earlier field study also conducted in Barkley Sound (Levitan 2002*a*). In the fertilization kinetics models, egg concentration, egg size, sperm velocity, and contact time were held constant for determination of the fraction of variation explained by male density.

Calculation of Male and Female Fertilization Success

Calculation of female fertilization success is the proportion of her eggs fertilized. Calculation of male fertilization success was based on paternity share across all females in a spawning event. Twenty larvae from each female were genotyped on the basis of microsatellite loci. The product of the paternity share (e.g., the proportion of larvae sired by that male out of 20) and the female fertilization success (proportion of her eggs fertilized) was the male fertilization success for that individual female. The total fertilization success of a male in a spawning event was the sum of his successes across all females. This measure of fertilization success uses the same units for both males and females, so a fertilization success of "1" is equivalent to the total number of eggs released by a single female. Fertilization success in a female can vary between 0 and 1, whereas a male's can vary between 0 and the complete fertilization of all females by a single male (e.g., 5.0 if there were 5 females in a particular event). Variation in fertilization success among males or females within each spawning event was standardized by dividing the variance by the square of the mean fertilization success for that sex and event (Arnold and Wade 1980).

Variation in female egg production was not considered because fecundity (the number of eggs produced) is not usually considered to be under sexual selection (Arnold 1994*b*). In addition, laboratory tests indicate that an eightfold manipulation of egg density had no significant effect on the fraction of eggs fertilized (Levitan et al. 1991). Variation among females in the number of eggs released should not greatly influence the fraction of eggs fertilized.

Results

Average Female Fertilization Success and Male Density

The average fertilization success for a spawning event depended on the density of spawning males (fig. 1). Fertilization success was lower at low densities, reached a peak at intermediate densities of 1–3 males/m^2, and then declined at high densities. This result is consistent with reduction of female fertilization success by sperm limitation at low densities and by polyspermy at high densities (fig. 1). The model allowing polyspermy explained a greater proportion of the variance ($R^2 = 0.793$) than did the model not incorporating polyspermy ($R^2 = 0.733$). More importantly, the model that did not include polyspermy could not explain any of the data at the high densities, where fertilization was reduced.

The subsample of spawning events used to investigate the pattern of single and multiple sperm fused to eggs indicates a similar pattern of an increase and then a decrease in monospermic fertilizations as a function of male spawning density (fig. 2*A*). The number of multiple sperm fusions to eggs increased with increasing male spawning densities (fig. 2*B*). These trials where samples were fixed almost immediately are not directly comparable to the full data set where samples were investigated after 3 h because the former is only looking at a snapshot of time (sperm entering the egg become difficult to track several minutes later) while in the latter there was no distinction made between multicellular embryos that might or might not have been developing abnormally because of polyspermy.

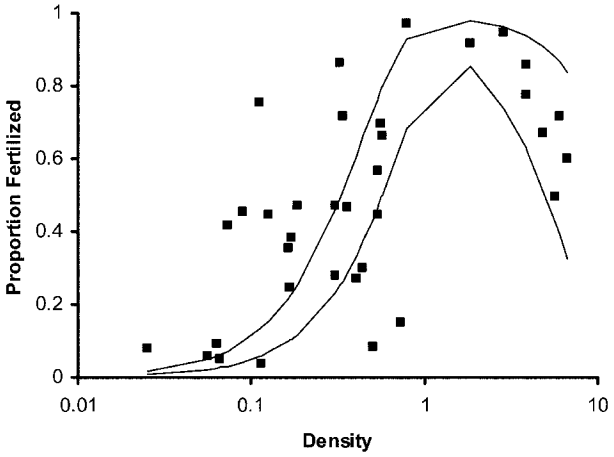

Figure 1: Fertilization success as a function of density. The average proportion of eggs fertilized for all females (range 2–12) in 35 independent experimental spawning events. Upper and lower 95% confidence intervals on the mean were calculated with a fertilization kinetics model (Styan 1998) that incorporates the effects of polyspermy at high male densities.

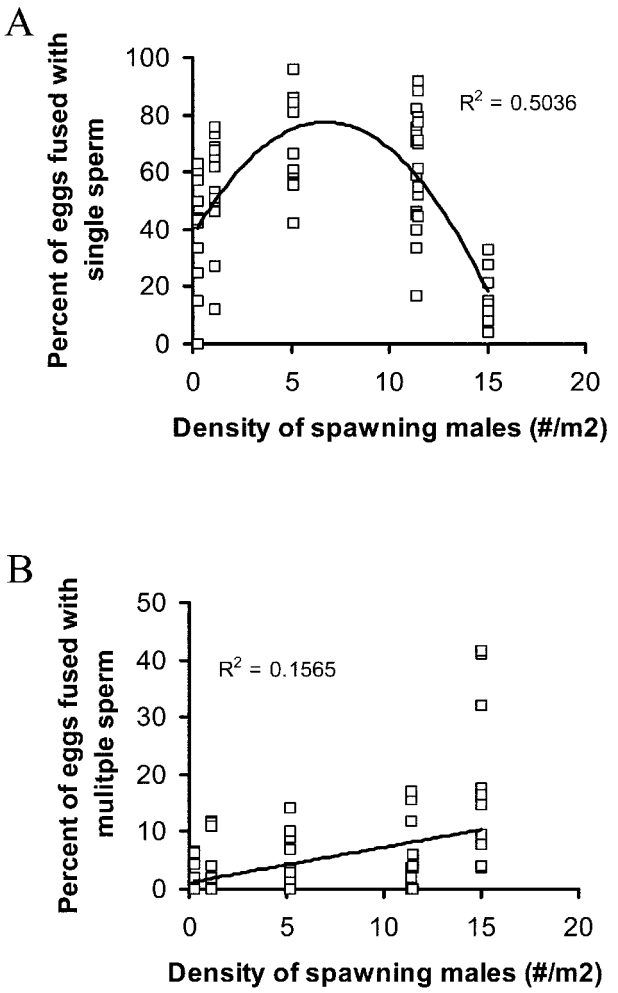

Figure 2: Mono- and polyspermic fusions to eggs as a function of male density. *A*, Percentage of eggs fused with a single sperm. *B*, Percentage of eggs fused with multiple sperm.

The critical finding is that at higher densities both methods indicate lower levels of developmental success and that this can be attributed to polyspermy.

Although polyspermy in sea urchins is often manifested in abnormal cleavage patterns (Ernst 1997), laboratory studies have shown that as sperm densities increase, a greater proportion of polyspermic eggs (as noted by sperm staining techniques) show no cleavage or raised vitelline envelope (Franke 2003). In *Strongylocentrotus franciscanus*, laboratory experiments testing fertilization at sperm densities above 1×10^7/mL (such that the water is cloudy) often result in development halted at the one-cell stage, deformed embryos, or eggs that do not raise the vitelline envelope and show no obvious distinction from control eggs held without sperm. Eggs from these same females generally produce >95% normal-looking embryos when exposed to sperm densities one or two orders of magnitude lower (D. R. Levitan, unpublished data). In the field trials conducted at high population densities, dense sperm clouds often surrounded the spawning urchins, suggesting sperm densities similar to those resulting in polyspermy in the laboratory.

Other Factors Influencing Average Female Fertilization Success

The residuals of the relationship between male density and mean fertilization success were tested in a multiple regression on the number of males spawning (natural log transformed), rates of advection (natural log transformed), and day of the year. The number of males spawning had no significant effect on the residuals of average female fertilization success ($P = .543$). Increased water flow resulted in reduced mean female fertilization success (fig. 3*A*). Peak fertilization was noted mid-season (fig. 3*B*). Together, 46% of the variation in residuals could be explained by these two factors (table A1 in the online edition

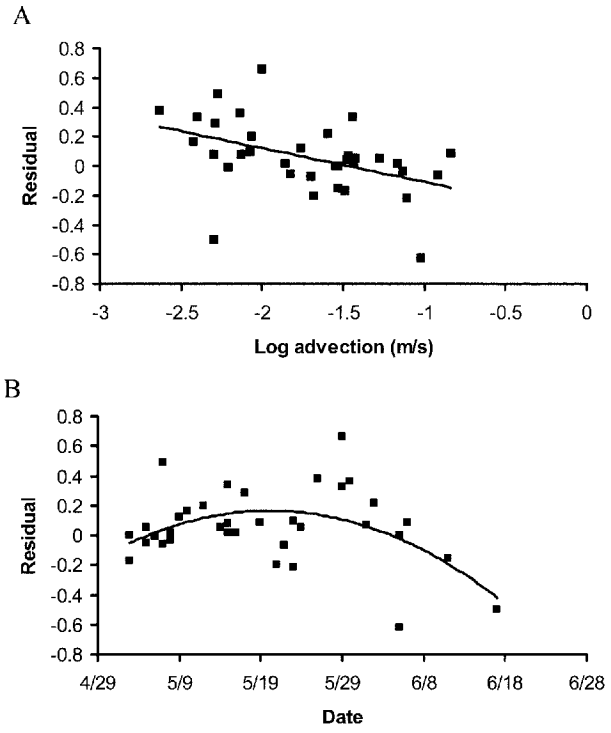

Figure 3: Residuals of the relationship of average female fertilization success as a function of male density. *A*, Residuals as a function of the natural log of advection ($P = .0014$). *B*, Residuals as a function of the day of the year ($P = .0028$ and .0017 for linear and polynomial components).

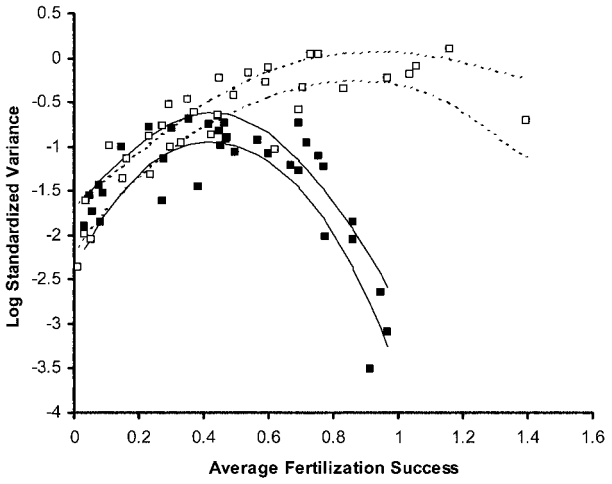

Figure 4: Standardized variance in fertilization success as a function of average fertilization success. The variances in fertilization success for males (*open squares*) and females (*filled squares*) differ only at high average female fertilization success. Lines represent the upper and lower 95% confidence intervals of the mean for males (*dashed lines*) and females (*solid lines*) generated from polynomial regressions (table A2 in the online edition of the *American Naturalist*).

of the *American Naturalist*). Overall, the residuals represented 21% of the unexplained variance in fertilization success, so these factors had a relatively minor influence on fertilization success in these experiments.

No significant relationship was detected between the day of the year and the density of males induced to spawn ($P = .69$ for linear and $P = .90$ for polynomial tests), and the five dates with the highest male densities and lower average fertilization successes were not conducted at significantly different times of year compared with the rest of the trials ($t = 0.418$, $P = .68$). Male density and advection were not significantly related ($P = .43$), so the density effect is not an artifact of flow differences.

Variance in Male and Female Fertilization Success

Adults ($N = 428$) and 20 larvae per female ($n = 3{,}425$) were genotyped for microsatellite loci. For two of the 35 replicates, not enough larvae could be cultured, and these dates were not included in analysis of male fertilization success (neither of these dates had extreme values of density or number of mates). The standardized variance in fertilization success depended on the average fertilization success in each spawning event for both females and males (polynomial regression: $P = .0001$, $R^2 = 0.77$ and $P = .0001$, $R^2 = 0.80$, respectively; table A2 in the online edition of the *American Naturalist*). At lower levels of fertilization success, males and females had similar levels of variance. Male and female variances started to diverge as the average proportion of eggs fertilized exceeded 50% and female variance decreased (fig. 4). Low female variance is expected when all females experience near total fertilization success.

Because no first-principle model is available with which to test for the effects of density on the standardized variance in fertilization success, I tested this variance against the polynomial components of density (table A3 in the online edition of the *American Naturalist*). For females, the relationship between variance and density was a complex curve related to the pattern of mean fertilization success and density ($P = .0001$, .1503, .0001, and .001 for the first- through fourth-order polynomial components). For males, the pattern was an increasing but curvilinear function of density ($P = .2801$ and .0288 for first- and second-order polynomial components). Males and females did not differ significantly in fertilization variances at mate densities below 0.5/m^2 (fig. 5). Fertilization variances of males and females diverged when mate density ranged from 0.5 to 5 mates/m^2. At these intermediate densities, female variance dipped below that of males to low levels as female fertilization success neared 100%. At mate densities exceeding 5/m^2, female variance was higher and did not differ significantly from male variance in relative fertilization success.

The residuals of this density relationship were regressed on the number of mates, the number of competing individuals, the density of competing individuals, the level

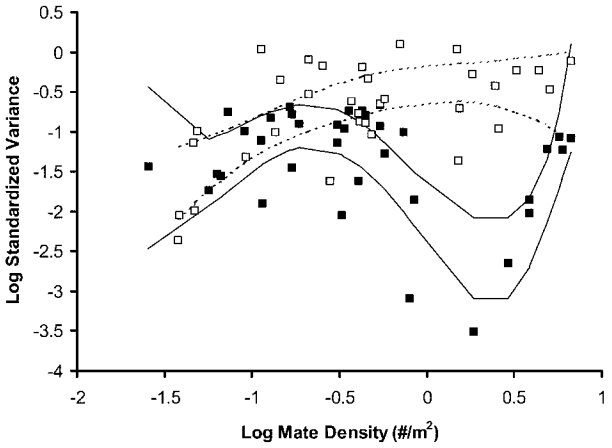

Figure 5: Standardized variance in fertilization success for males (*open squares*) and females (*filled squares*) as a function of mate density. The variances in fertilization success for males and females differ only at intermediate mate densities. Lines represent the upper and lower 95% confidence intervals of the mean for males (*dashed lines*) and females (*solid lines*) generated from polynomial regressions (table A3 in the online edition of the *American Naturalist*).

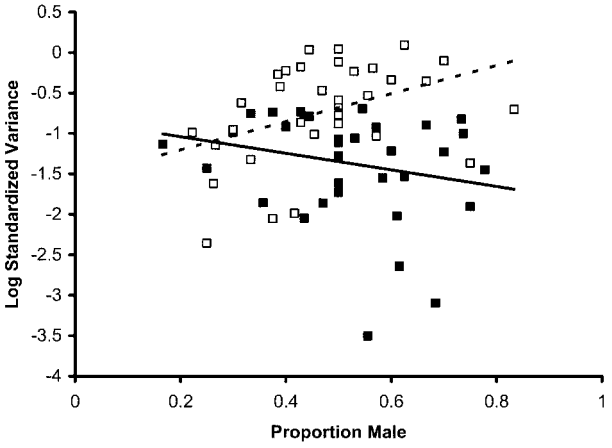

Figure 6: Standardized variance in fertilization success of males (*open squares, dashed line*) and females (*filled squares, solid line*) as a function of sex ratio. Male variance in fertilization success increased with the male bias in sex ratio. Female variance in fertilization success decreased with the male bias in sex ratio (slopes significantly different by ANCOVA; see text).

of advection, and the polynomial components of day of the year (table A4 in the online edition of the *American Naturalist*). For females, no factors were significant. For males, the level of advection ($P = .0248$) and day of the year (.0392 and .0340 for polynomial components) were significant.

Variance in Fertilization Success and Sex Ratio

The overall sex ratio across all spawning events was not significantly different from 1 : 1 (53% male; χ^2 $P > .1$), and overall, there were no significant differences in the number of males and females that spawned (Student's *t*-test, $P = .35$). Sex ratio in individual spawning events ranged from 17% to 78% male. Sex ratio and the density of mates for either males or females were not significantly related ($P = .852$ for males and .297 for females). An ANCOVA testing male and female standardized variance in fertilization success with the proportion of males as the covariate indicated a significant interaction ($P = .0116$), indicating significantly different responses of the sexes to changes in sex ratio. Male standardized variance in fertilization success increased with the male bias in sex ratio. Female standardized variance in fertilization success decreased with the male bias in sex ratio (fig. 6).

Multiple Paternity

Of the 178 females induced to spawn, 169 successfully produced larvae, and of those, 166 (98%) produced larvae sired by more than one male. This estimate is conservative because, if more than 20 larvae had been subjected to paternity analysis, additional sires might have been detected. The most successful male, for each female, sired an average of 58% of the larvae. The ability of the most successful male to monopolize a female's offspring production decreased as a function of the number and density of spawning males (fig. 7).

Relationship between Density and Nearest Mate Distances

There was a correlation between male density and the average nearest male distance to each female across spawning events (−0.567). A potential bias to this experiment is that a strong correlation between these measures might make it unlikely to observe rare but important close encounters between mates at low densities. However, a plot of nearest male-female distance as a function of density indicates that these close pairings were observed across all densities in these experiments, and the correlation is largely driven by the absence of more distant pairings at

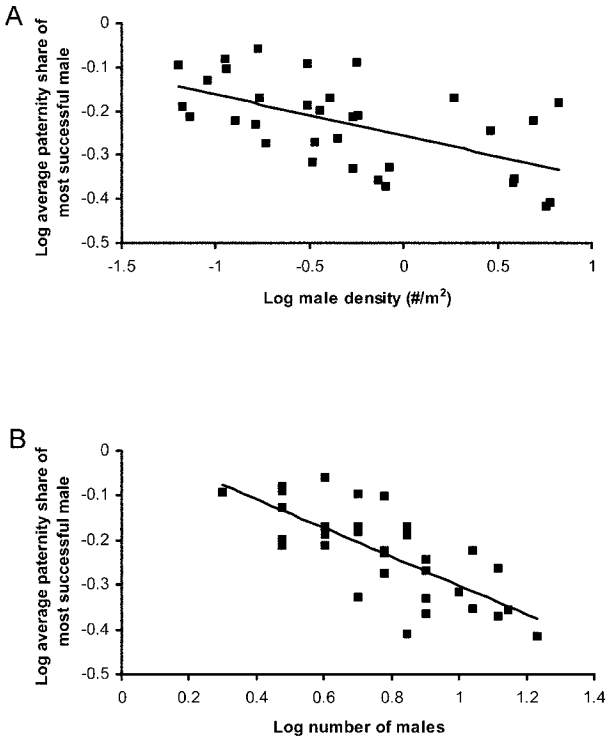

Figure 7: Average paternity share of the most successful male for each female in a spawning event as a function of the density (*A*) and number (*B*) of males. Multiple regression indicates that both the number and density of males in a spawning event significantly influenced average paternity share ($P = .0001$ for number, $P = .0024$ for density, $R^2 = 0.67$).

high density rather than an absence of close pairings at low densities (fig. 8).

Discussion

The Symmetry of Sexual Selection

The sexes differ in the pattern of fertilization variance but only under moderately high population densities. At lower or higher densities, the sexes are surprisingly similar in this variance (fig. 3). This result differs qualitatively from that predicted by previous studies of sexual selection, which suggest that female variance should be much lower than male variance, especially as mating opportunities increase (Arnold 1994*a*).

These results suggest that the magnitude and nature of sexual selection in this broadcast-spawning sea urchin are density dependent. At low densities, both males and females are limited by fertilization success, and sexual selection for greater fertilization success is equally intense for males and females. At these low densities, individuals of both sexes are competing, mostly indirectly, with same-sex individuals for increased mating success. Although at these low densities competition for mates may not reduce the absolute fertilization success of an individual, an individual that has traits that result in increased fertilization success compared with other individuals will be selected.

At intermediate densities, females are more likely to be saturated with sperm, and males would compete directly for fertilizations. This is the more typically studied condition noted in internally fertilizing taxa, in which the offspring production may remain constant but the proportions of offspring sired by particular males vary. This intermediate density is the zone in which Bateman's principle—about the way gender differences in fertilization variances can drive asymmetrical sexual selection—might apply.

At higher densities, females may become oversaturated with sperm, and male and female variances again become similar, but this similarity in fertilization variance does not imply similar selective pressures, as suggested at low density. In contrast, at these high densities, sexual conflict may result in sexual selection for females to avoid polyspermy (Styan 1998; Franke et al. 2002). Males may also suffer a cost from polyspermy, but as long as the optimal mating rate is higher for males than for females, sexual conflict will persist (Holland and Rice 1998). It is also possible that there is little cost of polyspermy to late-arriving sperm from a second male, if those sperm have little chance of disengaging from that egg and finding a second egg to fertilize. Such sperm might even benefit the male by killing potentially unrelated and competing embryos. If there is a high degree of intermale competition for eggs, there would be no benefit for males to produce slow-fertilizing sperm that are more easily blocked to prevent polyspermy. Such sperm would lose in competition to faster-fertilizing sperm from other males.

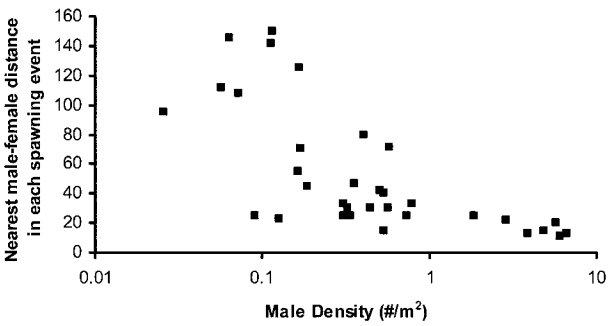

Figure 8: Relationship between the nearest male-female distance and the density of spawning males. Close pairings between males and females were noted, at least occasionally, across all densities.

If sperm outnumber eggs and some degree of multiple paternity occurs, then males should be selected to have a higher mating rate than females. The high degree of multiple paternity noted in this study suggests that sexual conflict occurs at high spawning densities. Almost all females released eggs that were fertilized by more than one male (98%). As population size and density increased, the most successful male, on average, failed to get the majority share of even a single female's eggs (fig. 7). Given the importance of male-female distance in mediating female fertilization success in previous studies that did not measure male success (reviewed in Levitan 1995), a potential outcome of this experiment was that group-spawning events might have resulted in a series of pair-spawning events dictated by nearest neighbor distances. If that were the case, the cost of polyspermy would be more similar for males and females. In free-spawning invertebrates, males and females may have little control over whose gametes come in contact, setting the stage for intense gamete competition and sexual conflict.

These density-dependent selective regimes form a continuum, and the transitions between them are gradual. Only one replicate mass spawning resulted in an average female fertilization success over 95% of eggs fertilized (fig. 1). Although fertilization dynamics will have a decreasing influence on zygote production at optimal sperm concentrations, in virtually every case examined, variation in sperm availability influenced female offspring production through either sperm limitation or polyspermy. For the majority of demographic conditions, sexual selection should be applying pressure on both males and females to increase the proportion of successful gametes.

Lab and field studies of sea urchins have noted that

polyspermy becomes apparent at sperm concentrations below complete sperm saturation (Styan 1998; Franke et al. 2002), probably as a result of random variation in sperm encounters. This variation would be greatly increased by turbulent sea conditions that result in heterogeneous eddy structure (Vincent and Meneguzzi 1991) that might produce spatial and temporal variation in gamete concentrations. Males release wisps of sperm that partially or completely break up with water movement (D. R. Levitan, personal observation). Even different eggs from a single female spawn may encounter a wisp and be faced with too many sperm or miss the wisp entirely and never encounter sperm (Levitan 1998*b*). It is therefore not surprising that single eggs show adaptations that both increase encounter rates with sperm (e.g., larger egg cell and jelly coat size; Levitan 1993, 1996, 1998*b*; Farley and Levitan 2001; Levitan and Irvine 2001; Podolsky 2001) and decrease polyspermy (fast and slow blocks to polyspermy; Styan 1998; Franke et al. 2002). These combinations of traits increase the number of collisions while preventing developmental failure caused by excess sperm (Levitan 1998*b*).

Patterns across Related Species

The conflict between attracting rare sperm and repelling abundant sperm produces a window of sperm concentrations that result in optimal fertilization. Species differ in the location of this window across a sperm-availability gradient. Along the outer west coast of North America, the rank order of density in *Strongylocentrotus franciscanus* and two congeners are related to the amount of sperm needed to produce fertilization (Levitan 2002*a*). At low sperm concentrations, the species found at the lowest densities (*Strongylocentrotus droebachiensis*) produces the highest fertilization rates, the species at intermediate densities (*S. franciscanus*) produces intermediate fertilization rates, and the most clumped species (*Strongylocentrotus purpuratus*) produces the lowest fertilization rates (Levitan 1993, 1998*a*, 2002*a*). When the most clumped species was experimentally thinned to the lower densities typically found in the other two species, it suffered drastic reductions in fertilization success compared with the other species (Levitan 2002*a*). These species differences in fertilization rate are a function of egg and sperm traits (Levitan 1993, 1996, 1998*a*, 2000, 2002*a*; Levitan and Irvine 2001). Large, sperm-permeable eggs and long-lived but slow sperm are noted in the species found at lowest densities (*S. droebachiensis*) and small, less permeable eggs and short-lived but fast sperm are noted in species found at the highest densities (*S. purpuratus*). *Strongylocentrotus franciscanus* is found at intermediate densities and has intermediate values for these traits (Levitan 1993).

It is yet to be determined how these species differ in their susceptibility to polyspermy and whether traits that increase fertilization success also increase the likelihood of polyspermy. It is interesting to note that *S. purpuratus*, which is found naturally at densities much higher than *S. franciscanus*, showed no indication of polyspermy at densities far exceeding those that resulted in polyspermy in the current study with *S. franciscanus* (Levitan 2002*a*). This result suggests that species typically living at higher densities are better able to resist polyspermy.

Together, these patterns are consistent with the notion that as the population density and sperm availability varies among these species, sexual selection targets different gamete trait values (Levitan 2002*a*). Future work will examine how these gamete traits vary within species among isolated populations that differ in density (D. R. Levitan, unpublished data).

The Resolution of Sexual Conflict

Molecular data are accumulating that suggest that gamete recognition proteins are evolving in many taxa, including some sea urchin species, faster than predicted by neutral models (reviewed in Swanson and Vacquier 2002). Several hypotheses purport to explain this positive selection (Vacquier et al. 1997), but one of the most popular is that sexual conflict selects females that produce eggs that are more difficult to fertilize under conditions of sperm competition (Palumbi 1999). At least one laboratory study suggests that under conditions of sperm competition, males whose gamete-recognition-protein alleles match those of a female garner a greater share of paternity than do males with unmatched alleles (Palumbi 1999). It is not yet known whether individuals with matched alleles are also more likely to result in polyspermic interactions. Strong support for the notion that sexual conflict is the driving mechanism of positive selection on gamete-recognition proteins requires further field studies designed to establish whether rare female genotypes are more successful at producing healthy embryos under conditions of sperm competition than are females with common genotypes.

While the rapid evolution of gamete recognition proteins has received most of the attention in regard to sexual conflict in external fertilizers, it is by no means the only possible outcome. Physical and electrical blocks to polyspermy can prevent developmental failure, and the rapidity at which these processes operate may be under selection. In addition, male and female spawning behavior can mediate the abundance of gametes in the water, and females may be reluctant to release eggs if sperm concentration is too high (or too low). It may be fruitful to investigate gender differences in the spawning strategies of broadcast-

spawning species (Levitan 1998*b*; D. R. Levitan, unpublished data). Both gamete and adult behavioral traits may be under selection to reduce the consequences caused by the twin constraints of sperm limitation and sexual conflict.

Sexual Selection in External Fertilizers

The distinction between natural selection and sexual selection can at times be subtle (e.g., Arnold 1994*b*). However, it can be instructive to wrestle with these distinctions, particularly in external fertilizers, because they represent the ancestral mating strategy from which mating strategies with a clearer distinction between these kinds of selection are derived (Levitan 1998*b*). I use Arnold's (1994*b*, p. S9) definition of sexual selection as "selection that arises from differences in mating success." Thus, while different degrees of sperm abundance might result in different selective pressures, all of these pressures are a function of the relationship between reproductive success (production of offspring) and mating success (successful encounters between eggs and sperm). The target of selection varies somewhat from taxa with internal fertilization, but this should not be surprising given the differences in the mating tactics. Externally fertilizing marine invertebrates generally do not physically battle with other adults, mate guard, or use visual cues of vigor or readiness, so morphological traits that give advantages with those interactions do not differ between the sexes (Levitan 1998*b*). Adults do control the timing and nature of gamete release, and the gametes themselves interact in the water column; these are the traits that seem to respond to sexual selection in external fertilizers (Levitan 1998*b*).

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