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GAMETE TRAITS INFLUENCE THE VARIANCE IN REPRODUCTIVE SUCCESS, THE INTENSITY OF SEXUAL SELECTION, AND THE OUTCOME OF SEXUAL CONFLICT AMONG CONGENERIC SEA URCHINS

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Sea-urchin species differ in susceptibility to sperm limitation and polyspermy, but the influences of gamete traits on reproductive variance, sexual selection, and sexual conflict are unknown. I compared male and female reproductive success of two congeners at natural densities in the sea. The eggs of the species occurring at higher densities, *Strongylocentrotus purpuratus*, require higher sperm concentrations for fertilization but are more resistant to polyspermy compared to *S. franciscanus*. Both species show high variance in male fertilization success at all densities and high variance in female success at low densities, but they differ in female variance at high densities, where only *S. franciscanus* shows high female variance. The intensity of sexual selection based on Bateman gradients is high in males of both species, variable in *S. franciscanus* females, and low in *S. purpuratus* females. *Strongylocentrotus franciscanus* females experience sexual selection at low densities and sexual conflict at high densities. *Strongylocentrotus purpuratus* may rarely experience sperm limitation and may have evolved to ameliorate sexual conflict. This reduces the variance in female fertilization, providing females with more control over fertilization. Sperm availability influences sexual selection directly by determining sperm–egg encounter probabilities and indirectly through selection on gamete traits that alter reproductive variances.

KEY WORDS: **Bateman gradient, density-dependent selection, echinoid, fertilization success, paternity, polyspermy, reproductive variance.**

In external fertilizers, sperm availability influences the probability of fertilization and successful development. Low sperm density results in low levels of fertilization and high levels of reproductive variance in both males and females (Levitan 2004, 2005a). The result can be sexual selection acting on both sexes to increase reproductive success. High sperm density can also result in low average (Franke et al. 2002; Levitan 2004) and high variance (Levitan 2004, 2005a) in reproductive success caused by polyspermy. These can in turn lead to sexual conflict as males compete for fertilizations and are selected for fast fertilization whereas females are selected to avoid polyspermy by reducing fertilization rate (Styan 1998; Franke et al. 2002; Levitan 2004; Levitan and Ferrell 2006; Levitan et al. 2007).

The sexual conflict over fertilization rate occurs when matings are polygamous and males compete for fertilizations. Under these circumstances males are always selected to produce sperm that are the first to find and fertilize an egg. Even if sperm selected to fertilize eggs rapidly often kill zygotes by causing polyspermy, the males producing those sperm will sire a greater proportion of eggs not killed. When sperm from two or more males compete, producing sperm that are slow to fertilize eggs never pays.

This conflict over fertilization rate has been thought to drive the rapid evolution of gamete-recognition proteins (Palumbi 1999; Haygood 2004; Levitan and Ferrell 2006), but these proteins evolve at different rates in different taxa (see, e.g., Metz and Palumbi 1996; Biermann 1998; Palumbi 1999; Debenham et al. 2000; McCartney and Lessios 2004; Zigler et al. 2005), and this variation may reflect different selective regimes across taxa or constraints that result in response to this selection by different traits. These other targets of selection include features of adult spawning behavior (e.g., the timing and pattern of gamete release; reviewed by Levitan 1998a) and gametes (e.g., egg target size and sperm swimming ability; reviewed by Levitan 2006) that can increase or decrease the rate of collisions, the probability of fertilization given a collision, and the ability of the egg to resist polyspermy.

Species and individuals within species vary in their abilities to be fertilized and to block polyspermy. Laboratory studies indicate that both among and within congeneric sea-urchin species, females that produce eggs that require more sperm to achieve fertilization are also more resistant to polyspermy. Reciprocally, the cost of producing easy-to-fertilize eggs under conditions of sperm limitation is that they suffer an increased risk of polyspermy at high sperm concentrations (Levitan et al. 2007).

Eggs able to resist polyspermy by means of a more efficient block may be able to alleviate the costs of sexual conflict in both sexes. Efficient blocks to polyspermy reduce the cost resulting from competition among males to produce sperm swiftest at fertilizing eggs, because only secondary sperm collisions, which are destined to lose anyway, are blocked, and they are thereby prevented from killing the egg. Increased egg resistance might also result in selection for increased sperm fusion rates, setting up an escalating “arms race” between egg and sperm traits (as noted in adult morphological traits by, e.g., Arnqvist and Rowe 1995).

Although we know that species differ in their ease of fertilization and their resistance to polyspermy, no comparative data are available on how this variation influences reproductive success in males and females and patterns of sexual selection under natural conditions. To date, only data on the variance in reproductive success and the intensity of sexual selection in a single species of broadcast spawning invertebrate have been collected (Levitan 2004, 2005a). Here I present data on reproductive success, variance in reproductive success, and the relationship between mating success and reproductive success (Bateman gradients, Arnold and Duvall 1994) in two congeneric sea urchins. The results indicate that species-specific gamete traits, and conditions of sperm availability, lead to different patterns of average and variance in reproductive success and the nature of sexual selection.

Methods

FIELD EXPERIMENT

Subtidal experiments were conducted in the springs of 2003 to 2005 off the west coast of Vancouver Island in the Deer Island Group within Barkley Sound, British Columbia. The purple sea urchin, *Strongylocentrotus purpuratus*, was induced to spawn over a range of natural population densities. On each experimental day, two divers would locate a natural aggregation of sea urchins. The aggregation was generally associated with a crevice or depression in the rocky bottom and was usually within a 1-m^2 area and at a depth of 2–5 m. These aggregations were generally just below the macroalga-dominated intertidal and shallow subtidal and were on a substratum of coralline algae. *Strongylocentrotus franciscanus* is also found at this depth, and deeper, at variable densities that generally keep the macroalgae from dominating these subtidal sites.

An InterOcean S4 current meter (InterOcean Systems, San Diego, CA) was placed within 5 m of the aggregation at a height of 0.5 m off the bottom. The current meter was programmed to record depth and flow in the north–south direction every 0.5 sec. From these data, the average surge velocity (mean velocity calculated from the absolute values of the 0.5-sec values) and the average advection (total linear distance moved divided by the total time) were calculated. The first value provides an estimate of the degree of turbulence and mixing, and the second provides an estimate of the time the gametes remain over the spawning sea urchins.

Each of the *S. purpuratus* individuals that made up the aggregation was picked up from its natural position, injected with approximately 1 mL of 0.55 M KCl, tagged with a numbered band of surgical tubing stretched over its test, and replaced in the same location. Injected sea urchins initiated spawning within approximately 1 min and continue spawning for approximately 1 h.

Egg samples were collected above each female between 20 and 30 min after the initiation of spawning. These sea urchins live in a generally high-surge environment (see Results), and eggs were collected in the water column, with an underwater plankton pump, after they had been advected off the female and were lifted approximately 15–20 cm above or to the side of each spawning female. The time of each collection was noted, and a 1-min rinse of the plankton pump with upstream seawater removed any residual sperm. The pump was then switched to the next filter chamber for collection of eggs from the next female (see Levitan 1998b for control data and Levitan 2004 for a parallel investigation of *S. franciscanus*). The plankton pump had 12 filter chambers, so on the one occasion on which 13 females spawned, one haphazardly chosen female was left unsampled. Occasionally females reduced their level of spawning before collection, and the sampling was therefore unsuccessful.

During egg collection, the positions and sexes of all individuals in the aggregation were mapped. From these maps the distances between all males and females were measured, and the densities of males and females were calculated on the basis of a circle of a diameter equal to the distance between the two most widely separated individuals. This estimate of density, unlike one based on an arbitrary quadrat size, provides a distinction between the number of individuals in an aggregation and the density within that aggregation (Levitan 2002a, 2004).

After all females were sampled, all the adults in the spawning event were collected and their test diameters measured. Tube-foot tissue samples were taken and placed in 95% EtOH for microsatellite genotyping. Sea urchins were then returned to the site. This experiment was replicated 15 times; each replicate used different sea urchins at a different location.

POTENTIAL ARTIFACTS OF FIELD SAMPLING

The mechanics of spawning are that eggs and sperm released from individuals reside on the aboral surface of the spawning individual for several seconds to a few minutes depending on water movement, and then lift off the individual and disperse as a plume (D. R. Levitan, pers. obs.). Although turbulence makes predictions of instantaneous gamete concentrations difficult (Crimaldi et al. 2006), on average the plume structure produces a gradient of gamete concentrations that decrease with distance from each individual and distance from the spawning aggregation (Denny and Shibata 1989; Levitan and Young 1995). As eggs lift off the female's surface they drift out of this collective sperm plume at a rate determined by water flow. Thus, during spawning intensity remains relatively constant, parcels of eggs released at different times experience the same average sperm concentration gradient. Tests of collecting eggs from four females at two intervals between 10 and 40 min after the initiation of spawning indicated no significant difference in fertilization as function of collection time ($P = 0.97$); whereas success among females ranged from 40 to 80%, samples within females averaged a difference of $< 2\%$ across times.

The limitation of this method is that once eggs are collected, and if they are not fertilized, the possibility of being fertilized at some later time is eliminated. However, in the relatively high flow environments and steep bottom topography of the outer coast of Vancouver Island, eggs rapidly drift out of this gamete plume and dilute into a large body of water. The highest likelihood of fertilization is at the time of spawning and then decreases rapidly (Levitan 2002a). Studies of *S. droebachiensis* in a flume indicate that most fertilization occurs while the eggs are on the surface of the female, and that little additional fertilization occurs after the eggs are advected off the sea urchin (Yund and Meidel 2003). This suggests that this method provides a reasonable measure of actual levels of fertilization.

Patterns of fertilization of these induced spawning experiments have been compared to natural spawning events. Although rarely observed, I have collected fertilization data from natural spawning events using the same methods as described here with induced events. The pattern of aggregation and fertilization during natural spawns was no different from that during experimental studies. Fertilization could accurately be predicted by the distribution of sea urchins regardless of whether the urchins spawned naturally or were induced to spawn (Levitan 2002a). These natural observations indicate that sea urchin spatial distribution and movement, gamete release, and gamete quality are not detectably different during natural and induced spawning events.

ESTIMATES OF FERTILIZATION AND POLYSPERMY

Three hours after collection, I inspected a sample of approximately 200 eggs for the presence of early development to determine the fraction fertilized. In 2004, when the bulk of the experiment was conducted (12 of 15 spawning events), I cultured this sample of inspected eggs in 200 mL of filtered seawater and then inspected it again after 48 h to determine the number of swimming embryos. This second inspection was designed to reveal whether embryo survivorship varied as a function of either the percentage of eggs fertilized or the density of sea urchins, as a test for evidence of polyspermy at high sperm concentrations.

For all spawning dates, the remaining collected eggs were cultured for three days without food before 30–50 larvae were individually transferred to ultrapure water, and each was frozen in a total volume of 1 μl for genetic analysis.

PARENTAGE ASSIGNMENTS

All adults and 20 larvae per female were genotyped with microsatellite loci (seven loci from Cameron et al. 1999, five loci from Addison and Hart 2002). DNA was extracted from 5–7 tube feet from each adult. Tube feet were taken from their EtOH storage, air dried on a Kimwipe (Kimberly-Clark, Dallas, TX) for 1 min and placed in a 1.5-mL microcentrifuge tube containing 500 μl of CTAB (2% hexadecyltrimethyl ammonium-bromide, 1.4 M NaCl, 0.2% 2-mercaptoethanol, 20 mM EDTA, and 100 mM Tris (pH 8.0)) and 10 μl of Proteinase K (25 mg/mL). The tube was mixed and heated in a 65°C water bath overnight. Then 50 μl of the digested sample was extracted with 10 μl Sprintprep Activator (Magnetic Beads) (Agencourt Bioscience Corporation, Beverly, MA) and 80 μl of 100% isopropanol. After mixing, the sample was placed on a magnetic plate for 10 min and then drained and rinsed five times with 70% EtOH. After a 1-h drying, the sample was resuspended in 50 μl of 1× TE buffer; DNA concentration was measured and the sample was diluted to a concentration of 5 ng/μl.

I extracted larval tissue by adding 7.9 μl sterile ddH$_2$ O, 1 μl GenAmp 10× PCR Buffer, 1 μl proteinase K (25 mg/mL), and 0.1 μl Tween to the 0.5-mL microcentrifuge tube containing the individual larva. The tube was transferred to a thermal cycler and heated at 65°C for 1 h, raised to 95°C for 15 min, and then cooled to 4°C. DNA concentration was then measured and the sample diluted to a concentration of 5 ng/μl.

Diluted DNA (from either adult tissue or whole larvae) was added to a standard PCR cocktail (5.9 μl autoclaved ddH$_2$0, 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 sec, 55°C for 30 sec, 72°C for 1 min; then 75°C for 20 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 ABI Prism's 3100 automated sequencer equipped with Genescan software (Applied Biosystems, Foster City, CA). The data were further analyzed with Genotyper software (Applied Biosystems) that, by applying a series of filters to remove anomalies such as stutter bands, determines DNA fragment length within one nucleotide. First, adults were screened for diagnostic loci, and the larvae were then tested with those loci. The procedure for assigning parentage was first to confirm the presence of one of the focal female's alleles and then to use enough loci to exclude all but one male and to confirm the presence of that male's alleles with at least two loci (Levitan 2004). Of the 1420 genotyped embryos, 21 (1.5%) could not be assigned to the focal female. When an embryo could not be assigned, another was genotyped to fill out the design.

COMPARISONS BETWEEN SPECIES

A parallel investigation, using the same methods, was conducted earlier in Barkley Sound with the congener *S. franciscanus* (Levitan 2004, 2005a). In that study, 35 independent spawning events were induced by injecting 6–32 sea urchins with KCl and placing them within a 5 × 5 m area over a range of densities (Levitan 2004) that reflected the natural range noted from field surveys (Levitan 2002a). Because the current results differ qualitatively from those of this early study, I present those data for comparison. At these study sites in Barkley Sound, *S. purpuratus* has a higher tendency to aggregate, and within occupied sites densities can be one to two orders of magnitude higher than those of *S. franciscanus* (Levitan 2002a). Surveys from other locations along these species' range indicate similar differences in abundances between them (Schroeter 1978).

CALCULATION OF REPRODUCTIVE SUCCESS

Reproductive success can be examined either as the total male or female success or as pairwise reproductive success. Total reproductive success is the success of an individual summed across all mating partners. For example if a male fertilized 15, 10, and 2% of all eggs produced by three females in a spawning event, he would have a total reproductive success of 0.45 (45%), a total success equivalent to that of an individual female who had 45% of her whose eggs fertilized by sperm from one or more males in the population. Total reproductive success is bounded by 1.0 for females (100% of eggs fertilized) but bounded by the number of females for males (a male that fertilized all the eggs of five females would have a total reproductive success of 5.0). Pairwise success is the proportion of one female's eggs fertilized by one male; if a specific male fertilized 7% of a specific female's eggs, their success would be 0.07. Pairwise success is bounded by a value of 1.0 for both sexes. These measurements do not consider variation in the numbers of eggs released by females. Although this variation is important to reproductive success and fitness, it is generally considered to be independent of sexual selection (Arnold 1994).

CALCULATING BATEMAN GRADIENTS

Sexual selection has been defined as "selection that arises from differences in mating success," where mating success is the "number of mates that bear or sire progeny over some standardized time interval" (Arnold 1994). On the basis of this definition, a Bateman gradient (the slope of the number of offspring as a function of the number of mates) can be calculated (Arnold and Duvall 1994; Jones et al. 2000). The intensity of sexual selection is then estimated from the steepness of the slope; the sex whose offspring production shows greater gains with increasing number of mates is considered to be under more intense sexual selection (e.g., by Jones et al. 2005).

Each spawning event can be viewed as a parental table of male and female reproductive success where each pairwise success is an element in the table or matrix and the total male and female success can be calculated as the sum of an individual's pairwise successes as row or column totals (Arnold and Duvall 1994). I used the number of nonzero elements in a particular column (for males) or rows (for females) as the number of mates for that individual. Total reproductive success was plotted as a function of number of mates, generating the Bateman gradient for that particular spawning event. These gradients were estimated independently for each spawning event and plotted as a function of mate density for each sex.

Statistical analyses were conducted using a general linear model (SAS, Cary, NC). Multiple regression was used when only continuous variable were considered and analysis of covariance (ANCOVA) was used when additional main effects (e.g., sampling date) were included in the model. Interactions were considered and if they were not significant they were excluded from the analyses. Type III sums of squares were used for significance testing.

Results

AVERAGE, INDIVIDUAL, AND PAIRWISE REPRODUCTIVE SUCCESS

Spawning densities in *S. purpuratus* ranged from slightly less than 1/m^2 in both sexes up to 170/m^2 in males and 102/m^2 in females. Average surge velocity ranged from 5.7 to 17.8 cm/sec, and advection ranged from 0.1 to 4.0 cm/sec. Average female fertilization success reached an asymptote of over 90% fertilization at male densities greater than 10/m^2 (Fig. 1A).

Polyspermy was rarely noted in these experiments. In one experimental day, at the second highest male density (143 males/m^2), a small fraction of eggs (10%) failed to develop past the raising of the fertilization envelope at the 3-h census. Cleavage in fertilized eggs was noted in all other replicates. Overall, no pattern of differential developmental success at 48 h was apparent as a function of either male density ($P = 0.12, R^2 = 0.037$) or the percentage of eggs fertilized ($P = 0.31, R^2 = 0.015$). Polyspermy did not have a major influence on reproductive success under these flow and demographic conditions in this species.

Spawning events were compared with respect to the average total reproductive success for individuals within a spawning event, with a multiple regression that considered the density (log transformed) of mates and competitors, surge velocity, and

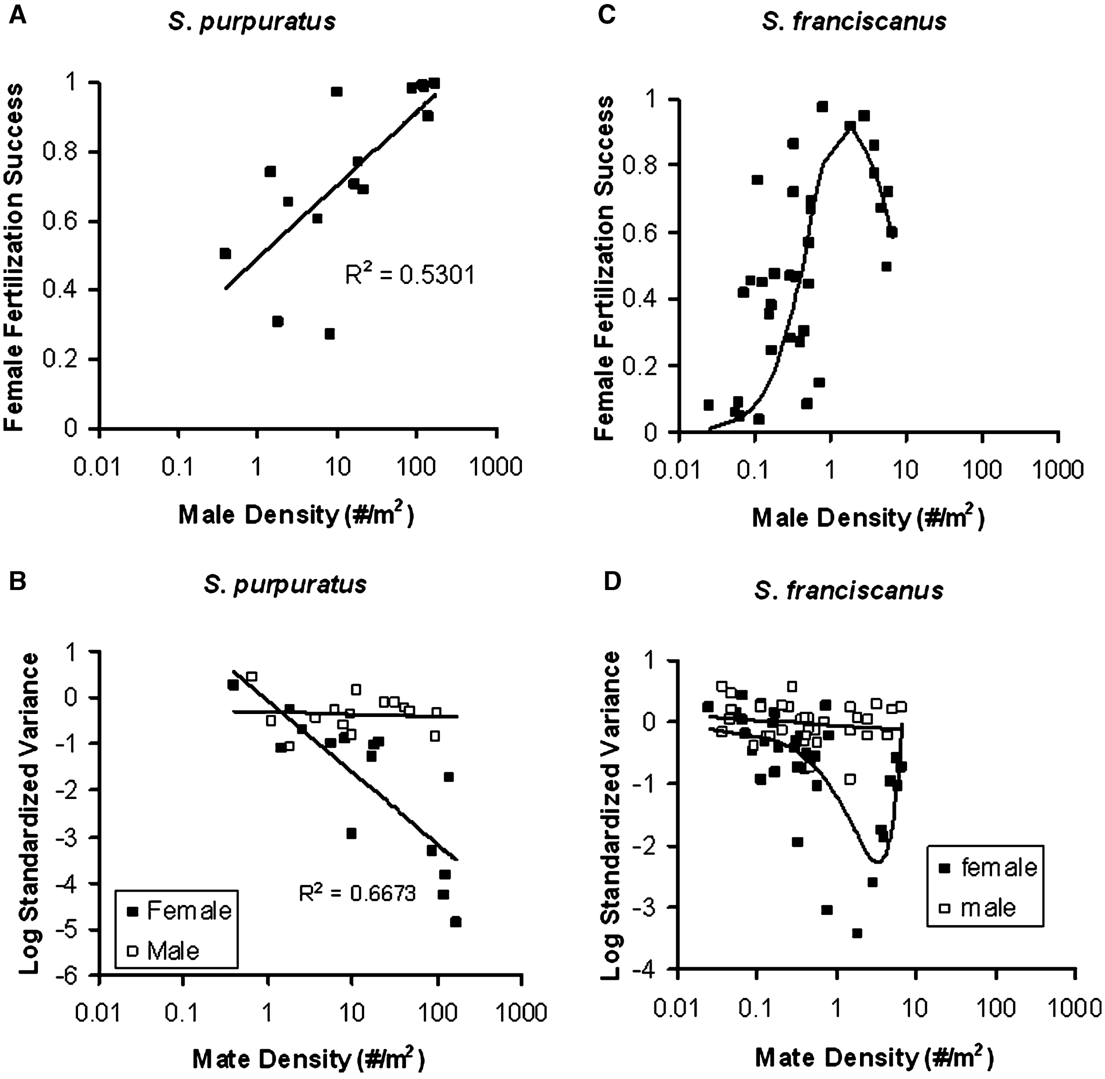

Figure 1. Average (A,C) and standardized variance (B,D) in fertilization success in *Strongylocentrotus purpuratus* (A,B) and *S. franciscanus* (C,D) as a function of mate density. *Strongylocentrotus purpuratus* is resistant to polyspermy; average female fertilization success rises asymptotically with increasing male density, and female variance decreases with increasing male density. *Strongylocentrotus franciscanus* is susceptible to polyspermy at much lower densities, and both female fertilization success and variance show curvilinear relationships with increasing male density. In both species, male variance in fertilization success species is relatively high across female spawning densities. For details of *S. franciscanus* data collection see Levitan (2004).

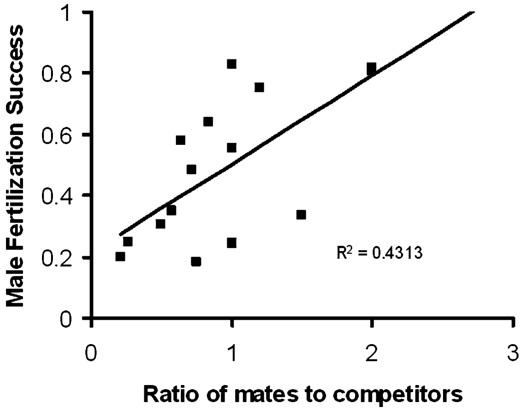

Figure 2. Average male fertilization success of *S. purpuratus* in each induced spawning event as a function of the ratio of the number of mates to the number of competing males. Male success increases with increasing numbers of mates and decreasing numbers of competitors.

advection. For females, average total reproductive success was significantly positively related to male density ($P < 0.01$) and marginally nonsignificantly negatively related to advection ($P = 0.07$, full model $R^2 = 0.65$). For males, success was significantly positively related to female density ($P < 0.001$), significantly negatively related to density of competing males ($P < 0.001$), and nonsignificantly negatively related to advection ($P = 0.22$, full model $R^2 = 0.72$). The opposing influence of mates and competitors is illustrated by the relationship between the ratio of mate to competitor density and male reproductive success (Fig. 2). As the spawning density becomes more female-biased, males have, on average, higher reproductive success.

Individual reproductive success of all individuals within and across spawning events was also subjected to a multiple regression. In addition to the same factors considered above (density of mates and competitors, surge velocity, and advection), the individual traits of the distance to the closest mate, individual body size, and when the egg samples were collected (for females) were considered. For females, fertilization success was negatively related to distance to the nearest male ($P < 0.001$, Fig. 3A) and advection ($P < 0.05$) and positively related to male density ($P < 0.05$). Time of collection was marginally nonsignificant ($P = 0.07$). This negative trend was caused by the four samples collected at the greatest time (> 45 min) following KCl injection (average time = 20 min, SD = 10 min). When those samples were removed the trend vanished ($P = 0.61$). This last result suggests that spawning

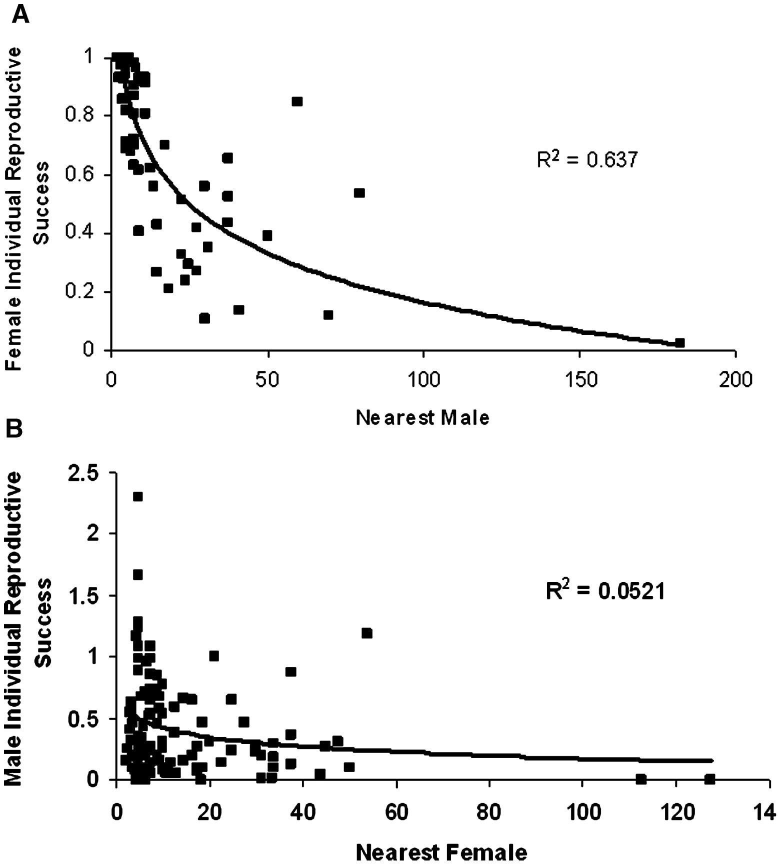

Figure 3. Individual fertilization success of *S. purpuratus* within and across spawning events plotted as a function of mate distance in females (A) and males (B). For both sexes, fertilization success decreases with nearest mate distance.

intensity was relatively constant for at least 45 min following KCl injection. For males, fertilization success was negatively related to the distance to the nearest female ($P < 0.0001$, Fig. 3B) and the density of competing males ($P < 0.0001$) and positively related to the density of mates ($P < 0.0001$) and male size ($P < 0.05$). Advection showed a marginally nonsignificant negative relation with fertilization success ($P = 0.09$).

Pairwise reproductive success was investigated testing the covariates of the distance between the pair, the number of competing males closer to the focal female than the focal male, the effect of sampling date, and the effects of male and female identity nested within sampling date. Pairwise success was negatively influenced by distance between mates ($P < 0.0001$) and the number of intervening competing males ($P < 0.0001$), and there was a significant effect of date ($P < 0.0001$), male identity ($P < 0.0001$), and a marginally nonsignificant effect of female identity ($P = 0.064$).

VARIANCE IN REPRODUCTIVE SUCCESS IN MALES AND FEMALES

The standardized variance (variance in reproductive success divided by the average squared reproductive success; Arnold and Wade 1980) among individuals within a spawning event was plotted as a function of mate density. For females, the standardized variance was negatively associated with mate density. For males, mate density and reproductive variance showed a slight, but nonsignificant, trend toward negative association. At densities lower than 10 mates/m^2, when sperm were slightly limiting, male and female variances overlapped. As the density of mates increased, and females became more uniformly saturated with sperm, the difference between male and female reproductive variance increased to approximately two orders of magnitude (Fig. 1B).

For females, independent of mate density, as the number of mates increased, the standardized variance decreased. For males, the standardized variance was not affected by mate density, mate number, or the ratio of mates to competitors. The degree of mate competition had no effect on the variance in male reproductive success, but it did affect the relative difference between male and female reproductive variances as eggs became more uniformly saturated with sperm.

THE DEGREE OF MULTIPLE MATING

Almost all males and females had multiple mates. Only one female produced offspring with a single male; the rest produced offspring from multiple males within a spawning event (99% multiple paternity). Four males sired offspring with a single female, the rest sired offspring with multiple females within a single spawning event (98% multiple maternity). No females suffered total reproductive failure (zero eggs fertilized), whereas three males (2%) were estimated to do so. The ability to detect reproductive failure in the two sexes was approximately equal. Although approximately 200 eggs were inspected for fertilization and only 20 embryos were genotyped for male success for each female, multiple females were genotyped (range: 3–12 per event), so similar numbers of eggs and embryos were investigated for maternal (ca. 200 eggs) and paternal (60 to 240 embryos) success. Regardless of any potential sampling bias across sexes, the degree of multiple parentage, viewed from either sex, was nearly complete.

The majority of individuals mated with the majority of available mates within a spawning event. Overall, individuals mated with 76% of available mates during a single spawning event. This proportion of mating was regressed on the number of spawning males and the result explained 41% of the variation in the proportion of available mates mated (Fig. 4A). When fewer males spawned, almost all individuals mated with every potential mate in the spawning aggregation.

Another way to view the relative success of males is to calculate average paternal success (average pairwise success) of the most successful male in a spawning event. This index provides a measure of how even reproductive success is across spawning events and is not sensitive to the decreasing power of detecting a particular male as the number of spawning males increases. The most successful male sired an average of 30% of offspring and was negatively influenced by the number of spawning males (Fig. 4B). Overall, single males did not dominate in paternal success and exerted decreasing dominance as the number of competitors increased.

COMPARISONS ACROSS SPECIES

Strongylocentrotus purpuratus and *S. franciscanus* differed qualitatively in both the mean and variance in female reproductive success (Fig. 1). Overall, *S. franciscanus* was successfully fertilized at much lower densities than was *S. purpuratus* (compare Fig. 1A with 1C). At densities near 1 male/m^2, *S. franciscanus* had average fertilization success near 100%, whereas this level of fertilization was not reached until *S. purpuratus* male density approached 50 males/m^2. *Strongylocentrotus franciscanus* was susceptible to polyspermy, producing a convex relationship between mate density and female fertilization success (Fig. 1C; see Levitan 2004 for significance test of model fit to data). This pattern resulted in a concave relationship between the standardized reproductive variance and mate density (Fig 1D; see Levitan 2004 for significance test of polynomials). In *S. franciscanus*, female reproductive variance was similar to male variance at low and high densities but was two orders of magnitude lower than male variance at the intermediate densities, where sperm were abundant enough to fertilize but not to cause excessive polyspermy. This nonlinear pattern of reproductive success and the variance in reproductive success caused by polyspermy was not evident in the current study of *S. purpuratus* (Fig. 1B, D), even though this current study was conducted at mate densities that exceeded

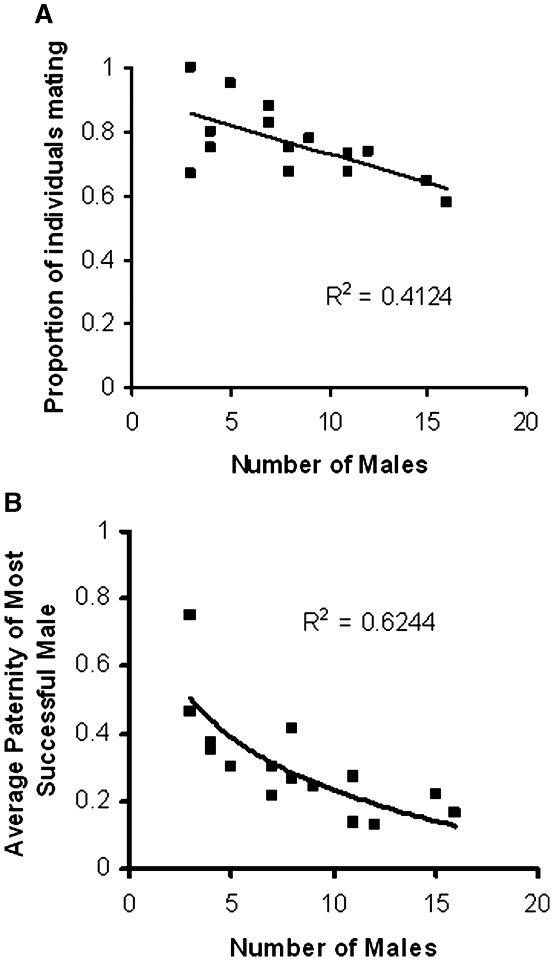

Figure 4. The degree of multiple mating in *S. purpuratus*. (A) The proportion of possible mates that actually produced offspring plotted as a function of the number of males in a spawning event. Most individuals mate with most available mates in a spawning event, but the number that do so decreases with the number of spawning males. (B) The average paternal share of the most successful male plotted as a function of the number of spawning males. The male that garners the greater number of fertilizations in a spawning event still fertilizes less than a majority of eggs fertilized, and the ability to dominate spawning events decreases with the number of spawning males.

those of *S. franciscanus* by an order of magnitude (maximum mate density ~ 6/m^2 for *S. franciscanus* and ~ 160/m^2 for *S. purpuratus*).

These species did not differ in the relationship between the variance in male reproductive success and female density. An ANCOVA testing for differences in the log standardized variance in fertilization success with a main effect of species and the covariate of log female density detected no species effect ($P = 0.13$) or covariate effect ($P = 0.36$), but when the two species were pooled, the log standardized variance weakly, but significantly, decreased with log female density ($P < 0.01$, $R^2 = 0.15$).

MATING SUCCESS AND REPRODUCTIVE SUCCESS

For males, Bateman's gradients were positive but did not differ as a function of either species or as mate density (ANCOVA, $P = 0.64$ for main effect of species and $P = 0.90$ for covariate of log female density). *Strongylocentrotus purpuratus* averaged a gradient of 0.25 (SE: 0.06, Student's $t = 4.03$, $P < 0.01$ for mean = 0), and *S. franciscanus* averaged a gradient of 0.32 (SE: 0.05, Student's $t = 6.52$, $P < 0.001$ for mean = 0). For females, a similar ANCOVA analysis indicated a significant interaction between species and log male density ($P < 0.01$). For *S. purpuratus*, Bateman gradients were independent of log mate density ($P = 0.11$) and no different from 0 (Student's $t = 0.80$, $P > 0.5$), but for female *S. franciscanus*, Bateman gradients decreased significantly with increases in log male density ($P < 0.05$, Fig. 5). Females demonstrated a positive slope under conditions of sperm limitation but a negative slope under conditions of high density, when polyspermy occurred. The slope crossed the zero value for Bateman's gradient at the density at which sperm were saturating but not oversaturating (high fertilization but low polyspermy; compare with Fig. 1C, D).

Discussion

Natural densities of *S. franciscanus* range from < 1 to 30 individuals/m^2 in British Columbia, Canada (Bernard and Miller 1973; Rumrill 1987; Bureau 1996; Levitan 2002a). In Barkley Sound male density averages 0.9 males/m^2 (Levitan 2002a) and average nearest neighbor distance is 19 cm (Levitan 1998b) for this species. These densities result in periodic exposure to both conditions of sperm limitation and conditions promoting polyspermy. In contrast, *S. purpuratus* is found at densities ranging from 4 to > 300/m^2, with an average density of 6.0 males/m^2, and average nearest neighbor distance of 19 cm (Thomas 1990; Levitan 1998b, Levitan 2002a; present study). Because *S. purpuratus* has a tendency to aggregate in cracks and crevices in high-surge environments, even where the absolute abundance of this species may be less than that of *S. franciscanus*, it may experience a temporally and spatially more consistent level of high sperm availability than the more dispersed *S. franciscanus* (Levitan 1998b, 2002a). These different conditions of sperm availability not only influence fertilization success but might also impose density-dependent selection on gamete traits for conditions of either sperm limitation or sperm oversaturation (Levitan 1998b, 2002a,b; Levitan and Ferrell 2006).

Strongylocentrotus purpuratus, unlike its congener *S. franciscanus*, is resistant to polyspermy even at extremely high spawning densities. *Strongylocentrotus franciscanus* reached an asymptote of near 100% fertilization at densities of around 1/m^2 and then exhibited increasingly deleterious levels of polyspermy at greater densities. In contrast, *S. purpuratus* reached an asymptote near

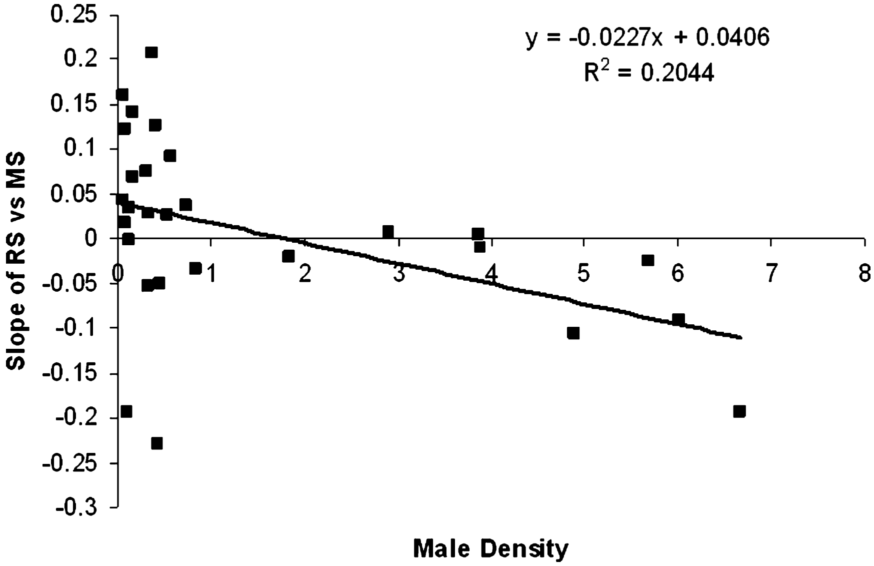

Figure 5. The intensity of sexual selection on female *S. franciscanus* as a function of male density as estimated by Bateman gradient (slope of reproductive success, RS, as a function of mating success, MS). Positive gradients indicate increased reproductive success with increasing mate number, and negative gradients decreased reproductive success, caused by polyspermy, with increasing mate number. Females of *S. purpuratus* had Bateman gradients no different from zero and unrelated to mate density. Males of both species had Bateman gradients that were positive but unrelated to mate density.

50 males/m^2 and demonstrated resistance to polyspermy through spawning densities exceeding 150 males/m^2. Although body size in *S. franciscanus* is larger than that in *S. purpuratus*, the rate of sperm release in these species is similar (3.8×10^7 vs. 3.2×10^7 sperm/s, during induced laboratory spawning; D. R. Levitan, unpubl. data) and cannot explain these large species differences in fertilization dynamics. Laboratory and field studies with controlled sperm concentrations confirm these species differences in how eggs respond to sperm availability. *Strongylocentrotus franciscanus* requires less sperm to achieve fertilization (Levitan 1993, 1998b) and is more susceptible to polyspermy at lower sperm concentrations (Levitan et al. 2007) than is *S. purpuratus*.

These two species and another congener, *S. droebachiensis*, which is the least abundant of these three species on the West Coast of North America, exhibit a continuum of gamete traits consistent with density-dependent selection (Levitan 1993, 1998b, 2002a,b; Levitan et al. 2007). Across these three species ranging from least to most dense, eggs exhibit decreasing ease of fertilization and increasing resistance to polyspermy, whereas sperm exhibit decreasing longevity and increasing velocity. The rarest species has gamete traits suited to conditions of sperm limitation, whereas the most clumped has gamete traits suited to conditions of sperm competition.

Experiments with *S. franciscanus* indicate that gamete recognition proteins may also exhibit a range in selective responses in response to spawning density. At low spawning densities, where sperm are limiting, mates that matched at the sperm bindin locus have higher reproductive success, whereas at high densities, when polyspermy is common, mismatched mates have higher reproductive success (Levitan and Ferrell 2006). Examination of how sperm bindin genotypes influence reproductive success in *S. purpuratus* and how its influence might be reflected in the pattern of molecular evolution will be interesting. One prediction is that selection at high densities generates sexual conflict that has resulted in highly polymorphic recognition loci that mismatch and allow eggs to avoid polyspermy. The other is that the intrinsic block to polyspermy is more powerful in this species generally found at high densities and that the result is reduced sexual conflict and reduced selection for diversifying selection on gamete recognition loci. What is clear, in these species, is that individual variation in ease of fertilization is related to susceptibility to polyspermy, so that selection on traits that do well under conditions of sperm limitation trades off with success under conditions promoting polyspermy (but see Styan and Butler 2000 for two scallop species in which one has a narrow and the other has a wide range of optimal sperm concentrations). Selection associated with sperm availability has cascading effects on a spectrum of interrelated morphological, behavioral, and compatibility traits.

PATTERNS OF VARIANCE IN REPRODUCTIVE SUCCESS AND THE INTENSITY OF SEXUAL SELECTION

In *S. purpuratus*, fertilization success depends on mate availability in both sexes, but only males are influenced by the presence of mating competitors. Male success is positively related to mate density but negatively related to competitor density. Female success was also related to mate density and the distance to the nearest male but was not influenced by competition with other females. Pairwise reproductive success was also dependent on these factors, but in addition individual identity was highly significant; even in the face of variation induced by distribution and abundance, some individuals did better than others.

In both species, the variance in reproductive success is relatively high in males and decreased only slightly with increasing mate density, but the nature of male–male competition changes with increasing density. At low densities, eggs are sperm limited and males are more likely to have low reproductive success because sperm fail to find eggs. Under these conditions, male competition is indirect; one male sires more offspring than another male but not at the expense of the other male. As densities increase and more males compete and successfully fertilize the same pool of eggs, competition is direct, and one male's success robs other males of siring opportunities. The linear relationship between male reproductive variance and spawning density indicates a seamless transition between direct and indirect competition for mating and fertilization success. This transition might be viewed as one from hard (selection for absolute success) to soft (selection for relative success) sexual selection (e.g., Wallace 1968; Wade 1985 for natural selection). Although male variance may remain high across this transition, the optimal trait values may switch from long-lived sperm when collisions are rare to rapidly swimming sperm when direct competition is intense (Levitan 1993, 2000).

The pattern of variance in reproductive success is more complex in females and depends on spawning density and species. *Strongylocentrotus franciscanus* females exhibit variance as high as that of males at both low and high mate densities. At low densities the variance is caused by sperm limitation, whereas at high densities it is caused by polyspermy. Variance is low in females only at the intermediate densities where sperm were neither under- or oversaturating and most or all eggs were fertilized by single spermatozoa (Fig. 1B, D). In contrast, *S. purpuratus* females demonstrate variance as high as that of males at low densities, but then, because eggs of these females can resist polyspermy, demonstrate a decrease in variance with higher mate densities as eggs become more uniformly saturated with sperm (Fig. 1A, C).

Estimates of Bateman gradients were positive for males of both *S. franciscanus* and *S. purpuratus*. In neither species was the intensity of sexual selection, as estimated by the Bateman gradient, related to mate density. Consistent with the patterns of reproductive variance, the transition between direct and indirect competition is also seamless, as reflected in these Bateman gradients. Like the patterns of reproductive variance, Bateman gradients in females were more variable across species and densities. *Strongylocentrotus franciscanus* females demonstrated a positive gradient between mating success and reproductive success at low densities, where sperm were limiting, and a negative gradient at high densities, where polyspermy was common. This pattern suggests selection for greater mating success in females at low densities, where sperm are limiting, and sexual conflict at high densities, where higher mate numbers and sperm availability result in polyspermy. The gradients were estimated to be zero at male densities at which sperm were neither under- nor oversaturating (compare transitions in Figs. 1C, 1D, and 5). At these densities, where sperm concentrations are "just right" for females, no directional selection for mating success takes place. In contrast, *S. purpuratus* females demonstrated no significant Bateman gradients across densities. This result is not surprising because, over these broad spawning densities, sperm were generally neither limiting nor oversaturating, given the generally higher densities of this species and its eggs' ability to resist polyspermy. In this species sperm concentrations are "just right" for females over a broader range of conditions, and no directional selection for mating success takes place.

The targets of selection for increasing mating success appear to be spawning behavior and gamete traits. Males are more likely to spawn and initiate spawning before females during spawning events (Levitan 2005b). As nearest mate distances increase, males that initiate spawning earlier are more competitive as they can spread their sperm cloud over a greater spatial area and potentially a greater number of mates (Levitan 2005b). Females vary in the receptiveness of the eggs they produce. Females that produce eggs easy to fertilize at low sperm concentrations are also more easily fertilized by heterospecific sperm (Levitan 2002b) and are more susceptible to polyspermy at higher sperm concentrations (Levitan et al. 2007). As sperm availability increases it might alter selection for egg receptivity, through either physical egg attributes like egg size or jelly coat thickness (reviewed in Levitan 2006). Selection may also act on gamete recognition proteins for common or rare types depending on whether matched (favored under sperm limitation) or mismatched (favored under polyspermy) proteins are favored.

Measures of reproductive success and reproductive variances at any one time are not equivalent to lifetime measures. Typically these measures and Bateman gradients have been based on one event or season. Bateman gradients provide an estimate of mate competition and the potential for sexual selection (Jones et al. 2004, 2005). However, if the covariance in success among seasons or events is negative, then this potential may not be realized because summed over all reproductive events, any particular trait value might not have a selective advantage (A. Jones, pers. comm.). The present study has replicate measures of the intensity of sexual selection. The results indicate strong conditional differences in the intensity of mate competition among sexes and species dependent on mate density and that the potential for sexual selection is very different for these two species. It does not have replicated measures for single individuals across spawning events. However, there is good reason to expect a positive covariance of reproductive success across spawning events, because egg and sperm traits, both within and across species influence reproductive success, in the sea, in a predictable fashion (Levitan 1996, 1998a, 2002a). For instance, even in the face of variation induced by the distribution and abundance of mates and competitors and variation in water flow, *S. franciscanus* males with common sperm bindin genotypes had four times the reproductive success as males with rare genotypes (Levitan and Ferrell 2006). In the present study, some individuals did significantly better than others, independent of density, body size, distance and water flow effects. This variation can at least partially be explained by variation in sperm bindin genotype (D. R. Levitan, unpubl. ms.). Such findings suggest that who wins and who loses in the competition for mates, particularly when summed across all the lifetime of an individual, is not random and the traits mediating success are subject to sexual selection.

External fertilization is common, widespread, and the likely ancestral mating strategy (Giese and Kanatani 1987). Patterns of sexual selection in these taxa are broad, because in the ocean, sperm and eggs can mix in the ocean, sperm and eggs can mix over a wide range of concentrations. This makes the direction and intensity of sexual selection dependent on variation in local gamete concentrations (Levitan 2004). The current study demonstrates how Bateman gradients can be used to directly measure the intensity of not only sexual selection but also sexual conflict, by examining how the relationship between mating success and reproductive success varies with mating conditions. The current study also reveals how species or populations living at high levels of sperm availability might adapt to polyspermic conditions at a cost of being susceptible to sperm limitation. A consequence of this adaptation is a reduction in not only sexual conflict but also reproductive variance.

This reduction in female reproductive variance is further refined during the evolutionary transition to internal fertilization. This transition provided females with increased control over paternity and also a mechanism to more carefully titrate sperm to maximize fertilization and control for polyspermy. The harbinger of this transition can be found in sperm-casting species (noted in some sponges, corals, bryozoans, bivalves, ascidians) in which females filter released sperm out of the water column for storage and fertilization even when males are rare (Pemberton et al. 2003; Phillipi et al. 2004). This control over sperm availability, like efficient blocks to polyspermy, reduces the variance in female fertilization success and reduces sexual conflict over fertilization rate. The shift of organisms from group spawning to pair spawning and then internal fertilization coincides with a shift in the targets of selection from the gamete traits and spawning behaviors that mediate gamete interactions to the adult traits that mediate courtship and copulatory success (Levitan 1998a, 2005b). This also may be true for sexual conflict. As females evolve to gain control over the deleterious effects of abundant sperm, conflict over mating rate often shifts to the deleterious effects of aggressive male behavior (e.g., McKinney et al. 1983; Mitani 1985; Magurran and Seghers 1994; Arnqvist and Rowe 1995; McLain and Pratt 1999; Bateman et al. 2006). Shifts in sperm availability, like shifts in mating strategies, influence the pattern of sexual selection and which traits are under selection.

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