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CONTEMPORARY EVOLUTION OF SEA URCHIN GAMETE-RECOGNITION PROTEINS: EXPERIMENTAL EVIDENCE OF DENSITY-DEPENDENT GAMETE PERFORMANCE PREDICTS SHIFTS IN ALLELE FREQUENCIES OVER TIME

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Species whose reproductive strategies evolved at one density regime might be poorly adapted to other regimes. Field and laboratory experiments on the sea urchin *Strongylocentrotus franciscanus* examined the influences of the two most common sperm-bindin alleles, which differ at two amino acid sites, on fertilization success. In the field experiment, the arginine/glycine (RG) genotype performed best at low densities and the glycine/arginine (GR) genotype at high densities. In the laboratory experiment, the RG genotype had a higher affinity with available eggs, whereas the GR genotype was less likely to induce polyspermy. These sea urchins can reach 200 years of age. The RG allele dominates in larger/old sea urchins, whereas smaller/younger sea urchins have near-equal RG and GR allele frequencies. A latitudinal cline in RG and GR genotypes is consistent with longer survival of sea urchins in the north and with predominance of RG genotypes in older individuals. The largest/oldest sea urchins were likely conceived at low densities, before sea-urchin predators, such as sea otters, were overharvested and sea-urchin densities exploded off the west coast of North America. Contemporary evolution of gamete-recognition proteins might allow species to adapt to shifts in abundances and reduces the risk of reproductive failure in altered populations.

KEY WORDS: **Density-dependent selection, fertilization success, frequency-dependent selection, polyspermy, sexual conflict, sperm bindin.**

Rapid shifts in abundance caused by human activities or natural events might put populations or species at risk of reproductive failure if individuals have traits that do not perform adequately in novel environments. This problem is often viewed in the context of Allee effects, in which reduced abundance can result in mate or sperm limitation and decreased per capita reproductive success (Levitan et al. 1992; Tegner et al. 1996; MacDiarmid and Butler 1999; Stoner and Ray-Culp 2000), but rapid shifts to high abundances might also lead to decreases in fitness. High densities increase not only resource competition but also mate competition and the likelihood of sexual conflict, which can reduce reproductive success (Warner et al. 1995; Styan 1998; Franke et al. 2002; Levitan 2004). The ability to adapt in pace with either increases or decreases in abundance could alleviate density-mediated changes

in fitness and perhaps rescue populations facing Allee effects from reproductive failure. Here, I report how sea-urchin gametes have evolved in a direction predicted by performance assays and increase in abundance suggested by the overharvesting of predatory sea otters.

For broadcast-spawning organisms such as sea urchins, reproductive failure is caused at low densities by sperm limitation and at high densities by polyspermy (Styan 1998; Franke et al. 2002; Levitan 2004). Gametes with high fertilization rates allow for fertilization at low sperm concentrations when mates are scarce, but these same gamete traits result in increased polyspermy at high sperm concentrations (Levitan et al. 2007; Levitan 2008). For any given level of sperm availability, a particular optimal level of gamete affinity maximizes female fertilization success (Levitan et al. 2007). Depending on the degree of sperm competition, males can be in sexual conflict over this affinity. If sperm from two or more males compete for the same egg, then the optimal affinity from the male perspective will be higher than that for females. In the absence of this competition, male and female interests are more aligned and lower gamete affinity would be advantageous for both sexes (Levitan 2010; Tomaiuolo and Levitan 2010).

Traits that determine fertilization rate include physical traits, such as egg size and sperm swimming ability (Levitan 1993, 1996, 2000; Kupriyanova and Havenhand 2002; Marshall et al. 2002), but also include gamete-recognition proteins that mediate sperm–egg compatibility (Palumbi 1999; Levitan and Ferrell 2006; Levitan and Stapper 2010). In sea urchins, the sperm-bindin protein is expressed on the tip of the sperm (Vacquier and Moy 1977) and binds to the egg receptor (EBR1; Kamei and Glabe 2003). Sperm bindin, like many gamete-recognition proteins, can be under positive selection (Swanson and Vacquier 2002). Some, but not all, species show higher degrees of divergence among species (Biermann 1998; Zigler et al. 2005) and polymorphism within species (Metz and Palumbi 1996; Geyer and Palumbi 2003) than expected from neutral models. Field experiments indicate that density- and frequency-dependent selection on sperm bindin can lead to purifying selection when sperm are limiting and to balancing selection when sperm are overabundant (Levitan and Ferrell 2006; Levitan and Stapper 2010). The degree of gamete compatibility can be controlled by single point substitutions (Levitan and Stapper 2010), and the frequency of these alternate forms is predicted to reach an equilibrium dependent on the level of sperm availability and the degree of sperm competition (Tomaiuolo and Levitan 2010).

Although an optimal set of gamete traits can be predicted for a specific level of sperm availability, spawning densities, and water-flow conditions that dictate sperm dispersal and dilution can vary greatly spatially and temporally (Pennington 1985; Peterson et al. 1992; Levitan 2002). In addition, broadcast-spawning species such as sea urchins often produce larvae that typically spend long intervals dispersing in the water column, producing gene flow (Moberg and Burton 2000; Flowers et al. 2002) and reducing opportunities for local adaptation. Therefore, although good evidence indicates how selection might operate at the level of a local spawning event (Levitan 1996, 2004; Levitan and Ferrell 2006), little evidence supports local adaptation because the offspring produced from any event are not likely to recruit back to that local site (but see Luttikhuizen et al. 2011 for an example of egg-size variation associated with local density in clams). Supporting evidence for how sperm availability might influence the evolution of gamete traits generally comes from comparative analysis across species; among congeneric sea-urchin species, common species have gamete traits that do best under conditions of sperm overabundance, whereas rare species have gamete traits that perform best under conditions of sperm limitation (Levitan 1993, 1998, 2002). Long-term differences in species' average levels of sperm availability are therefore probably needed to produce predictable shifts in the evolution of gamete traits.

Major increases in sea-urchin abundances, caused by human removal of sea-urchin predators (Jackson et al. 2001), have been noted in a variety of species worldwide, for example, in the Caribbean (Hay 1984), the Mediterranean (Sala et al. 1998), East Africa (McClanahan and Muthiga 1989), and the east (Estes et al. 2010) and west coasts (Estes and Palmisano 1974) of North America. Although the identity of the predator differs in different locations and has been disputed within locations, ample evidence indicates that sea urchins can and often do dominate subtidal habitats as a result of human exploitation of sea-urchin predators over the past few hundred years (Jackson et al. 2001; Estes et al. 2010). One of the best studied of these interactions is the dramatic influence of human-induced shifts in sea-otter abundance on abundance of sea urchins along the west coast of North America (Estes et al. 2010).

Currently sea urchins often dominate shallow subtidal communities along the North American west coast, reaching densities of tens per square meter (Schroeter 1978; Rogers-Bennett et al. 1995; Levitan 2002), reportedly as a result of the removal of predatory sea otters and perhaps other predators. Although common in historic times from Baja California to Alaska, sea otters were hunted to near extinction from 1741 until 1911, when the population size was estimated to be only 1000–2000 individuals (Estes and Duggins 1995). Although quantifying historic predator–prey interactions is problematic (Foster and Schiel 2010), if current estimates of the influence of sea otters on sea-urchin abundances can be extrapolated to times past, mature sea urchins are likely to have been at low densities (e.g., $<0.1/m^2$, Estes and Duggins 1995; Watson and Estes 2011) along much of the west coast of North America before the human exploitation of sea otters and other predators (e.g., fish, Cowen 1983; lobsters, Tegner and Levin 1983).

The common subtidal sea urchin of this region, *Strongylocentrotus franciscanus*, has a potential life span of over 200 years (Ebert et al. 1999; Ebert and Southon 2003). Individuals 17 cm in test diameter are estimated to be approximately 200 years old and were therefore conceived before sea otters were largely eliminated from the Pacific coast. This long life span allows direct comparisons of frequencies of genes coding for gamete-recognition proteins produced when sea otters were common and sea urchins were presumably at low densities with those of more recent times, when sea urchins are abundant. I examined the performances of these alternate forms of sperm-bindin proteins in the field under a variety of population densities and in the laboratory under choice and no-choice fertilization assays. These performance assays were matched with patterns of genotype frequencies as a function of sea-urchin size (and estimated age) and also compared with latitudinal gradients in body size and bindin genotype frequencies from the literature. The results suggest that the protein that does best under conditions of sperm limitation is very common in older sea urchins presumably conceived at lower densities, whereas younger sea urchins, conceived under higher densities, commonly have a protein less likely to cause polyspermy.

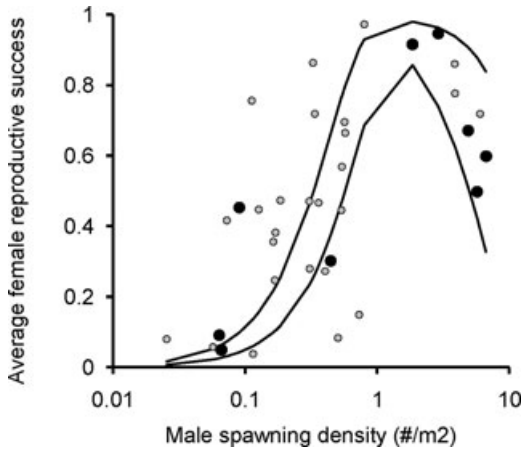

Figure 1. Average female reproductive success as a function of the density of spawning males (redrawn from Levitan and Ferrell 2006). Each datum represents the average proportion of developing eggs for all females that spawned in the event. Large solid symbols are the trials chosen for sequencing of the adults for sperm bindin. Sperm were limiting at low densities, whereas polyspermy reduced reproductive success at high densities.

Methods

FIELD EXPERIMENT

Details of the field experiment were described by Levitan (2004) and Levitan and Ferrell (2006), who studied patterns of sexual selection (Levitan 2004) and reproductive success of rare and common sperm-bindin proteins (Levitan and Ferrell 2006). Those analyses indicated (1) that males with rare proteins did poorly; (2) that female success could be predicted by their sperm-binding protein (probably through linkage disequilibrium with the egg receptor); and (3) that at low densities, males and females matched at the sperm-bindin locus had higher reproductive success than mismatched pairs, whereas at high densities, the reciprocal was true (mismatches reduce the likelihood of polyspermy). Here, I present a new analysis of how males carrying the different common proteins differ in success as a function of male spawning density.

Briefly, between 2000 and 2002 in Barkley Sound, British Columbia, at 35 sites that ranged in sea-urchin density from 0.1 to 10/m^2 (Fig. 1), a 5 × 5 m grid was established within which all sea urchins were induced to spawn with injections of KCl. Individuals were each tagged with a latex band and then scattered over the 5 × 5 m plot. Thirty minutes later, the position of each urchin was mapped, and a sample of eggs was collected in the water column above each spawning female. Depth and water flow were measured with an S4 current meter. Adults were brought to the laboratory for tissue sampling (tube-foot collection) and measurement of body size (test diameter). Egg samples were also transported to the laboratory, where the percentage of eggs developing was determined after 3 h, and the embryos were reared for three days before being frozen for paternity analysis by means of microsatellite loci (for details see Levitan 2004). In this experiment, 20 larvae per female were genotyped. Pairwise reproductive success is the fraction of eggs fertilized by a male with a particular female and is calculated as the product of the paternity share (proportion of a female's embryos sired by a male) and the female fertilization success (proportion of eggs successfully developing). Total male success is the sum of pairwise success across all females in a spawning event. Polyspermy at high densities was confirmed visually with a confocal microscope (Levitan 2004). Controls for KCl induction of spawning used the same experimental protocol but with sea urchins serendipitously found to be spawning naturally. These controls indicate that the levels of aggregation and fertilization did not differ from the predictions based on population density from the experimental trials (Levitan 2002).

From these 35 experimental trials, nine trials were chosen (four under conditions of sperm limitation and five under conditions promoting polyspermy; Fig. 1), and the sperm-bindin genotypes of all adults (124) were determined (Levitan and Ferrell 2006).

LABORATORY EXPERIMENT

In 2009, *S. franciscanus* was collected and induced to spawn by KCl injection in the laboratory (Bamfield Marine Sciences Centre, Barkley Sound, British Columbia). On independent days, with independent sea urchins, four replicate matrices were established for examination of fertilization between a set of males and

females (matrix sizes of 6 males × 6 females, 9 × 7, 10 × 7, and 8 × 8). Dry (undiluted) sperm were kept on ice until use, when it was diluted through five serial 10-fold dilutions, and then 1 mL of the diluted sperm suspension was added to a vial containing 1 mL of an egg suspension (ca. 5000 eggs) and 8 mL of filtered seawater (no-choice fertilization assay). Then sperm from all males were pooled and added to another set of vials each containing the eggs of a female (choice assay). These experiments were conducted under conditions of near sperm saturation to oversaturation (concentrations promoting polyspermy) established on the basis of previous work (Levitan et al. 2007). Three hours after fertilization, the fraction of eggs showing signs of development (raised fertilization membrane or cleavage) was recorded for each experimental vial. In this species, polyspermic eggs often fail to raise a fertilization membrane (Levitan 2004; Levitan et al. 2007). One milliliter of the sperm suspension was fixed in formalin for later sperm counts (eight replicate counts with a hemocytometer). For the choice assays, embryos were cultured for three days before being frozen for determination of paternity. Tube-foot samples were collected from each adult for sequencing of the sperm-bindin genotype and for amplification of microsatellite loci for parentage analysis (described below). In this experiment, 40 larvae per female were also genotyped for microsatellite loci. Larvae from three matrices were genotyped for the choice experiment because the forth matrix did not have informative bindin genotypes. Paternity was assigned by CERVIS (Kalinowski et al. 2007). Because maternity was known and the number of potential sires was low, only three loci were generally needed to assign paternity unambiguously. Additional loci were used when needed to confirm paternity (see McCartney et al. 2004 for description of loci).

CHANGE OVER SIZE CLASSES

Sperm-bindin genotype was investigated as a function of test diameter from three field collections. The first was based on the field experiment described above, and the second on collections for another field study, made in 2011. These two samples represent a random sample of adult sea urchins from Barkley Sound. Because small urchins are less abundant and large urchins are generally rare, a search was conducted for small (<10 cm test diameter) and large (>16 cm) *S. franciscanus*, which were collected in the summer of 2010. Ebert (2008) has estimated the annual survival probability of large (>14 cm) urchins to be 0.98 and stable at larger (older) stages; no evidence of senescence was reported by that author. This survivorship estimate suggests that, in a population of 1000 individuals at age x, 18 surviving individuals would be at age $x + 200$ years. Old individuals might therefore be reasonably expected to occur occasionally among the abundant urchins in Barkley Sound.

ESTIMATING SEA-URCHIN AGE

Ebert and colleagues (1999) tagged populations of *S. franciscanus* with tetracycline at 18 locations from southeast Alaska through southern California, collected samples a year later, and found that a Tanaka function best fit the relationship between the size of the demipyramid (tooth in Aristotle's lantern) at time $t + 1$ and its size at time t. These age estimates were later confirmed by radiocarbon data that revealed the signal of nuclear testing initiated in the 1950s (Ebert and Southon 2003). For each site, Ebert et al. (1999) also determined the allometric relationship between demipyramid length and test diameter to estimate the relationship between test diameter and age for each site. Their analysis indicated a latitudinal gradient in survivorship but not in growth rate. They also found no difference in growth rates between fished and unfished sites. I used the average parameter values for the Tanaka function to estimate the relationship between size and age (as in Ebert et al. 1999). I also used the individual parameter values for 17 of the populations (the intertidal site was excluded) along the west coast to estimate the variation in age estimates of a sea urchin ranging in size from 5 to 18 cm test diameter.

SEQUENCING SPERM BINDIN

Tube feet were digested in either CTAB and proteinase K (field experiment) or Sarc Urea (matrix laboratory experiment and sample of small and large individuals) in a 65°C water bath for 12 h. DNA was extracted with a SprintPrep DNA purification kit and stored at –20°C. A 431-bp region was amplified with the primers developed by Debenham et al. (2000a): FNbindin5′ (5′-AGTCGACGTTCGACAGACGAC-3′) and FNbindin3′ (5′-TTACATGGTCCATTATAGTATGCC-3′). The polymerase chain reaction (PCR) cocktail consisted of 45 μl Platinum PCR SuperMix High Fidelity, 2.5 μl 10 μM bovine serum albumin, 1.2 μl 10 μM FNbindin5′ primer, 1.2 μl 10 μM FNbindin3′ primer, and 1.5 μl DNA (5 ng/μl). The PCR program was as follows: 5 min at 95°C; 32 cycles of 1 min at 94°C, 1 min at 57°C, and 2 min at 72°C; and 10 min at 72°C. An internal sequencing primer, KTseq3′ (5′-ATACACACGATGGTCAAG-3′), was used to sequence a 273-bp variable region sperm bindin (bp 944–1217 in Minor et al. 1991). For the field experiment of 124 individuals, all heterozygous individuals with more than one polymorphism were cloned, and at least four clones were sequenced for determination of haplotype structure (details in Levitan and Ferrell 2006). All homozygous individuals and individuals with only one polymorphism were sequenced in the reverse direction for confirmation with the internal primer KTseq5′ (GGAGCGCGTAAGAAGCGTTAT) or KTseqn5′ (ACGTTCGACAGACGACGAC).

The program PHASE (Stephans et al. 2001; Stephens and Scheet 2005) was used to predict haplotype structure from diplotype data from the field experiment of 124 individuals (all haplotypes known through cloning). PHASE correctly assigned

Table 1. Nonsynonymous amino acid code for the 273-bp variable region of *Strongylocentrotus franciscanus* sperm bindin. The table includes haplotypes from the present study and from Debenham et al. (2000a,b, identified with asterisks). A total of 24 haplotypes comprise 13 nonsynonymous alleles (Fig. 1). The four most common haplotypes form three nonsynonymous alleles (A, B + C, and D) and sum to a frequency of 0.95. The most common haplotype (A, frequency 0.58), six rare synonymous haplotypes (F, I, M, O, R, and S), and three rare nonsynonymous haplotypes (E, I, and N) all have arginine (R) at amino acid site 13 and glycine (G) at site 35 (RG allele) The second (B, frequency 0.14) and third (C, frequency 0.15) most common haplotypes, four rare synonymous haplotypes (J, U, V, and X), and one rare nonsynonymous haplotype (H) all have glycine at site 13 and arginine at site 35 (GR allele). The fourth most common haplotype (D, frequency 0.08), one rare synonymous haplotype (W), and five rare nonsynonymous haplotypes (G, K, L, Q, and T) all have glycine at both site 13 and site 35 (GG allele). The lone haplotype P has arginine at both sites (RR allele). Each letter in the table represents all synonymous haplotypes with similar amino acid sequences. Amino sites 13 and 35 are noted on the top row.

Haplotype	13	35
A	QGNYPQAMNPQSRGVNYGQPAQQGYGAQGMGGAFGGGQGMGGAVRGGQGMGGAVGGGQFGAFSPGEAEADNADYDEYSDSLDEGDTTISAA
B	. . . . . G . . . . . R . . . . .
D	. . . . . G . . . . . R . . . . .
E*	. . . . . T . . . . .
G	. . . . . G . . . . .
H*	. . . . . G . . . . . R . . . . . P . . . . .
I*	. . . . . Y . . . . .
K*	. . . . . G . . . . . R . . . . .
L*	. . . . . G . . . . . R . . . . . R . . . . .
N*	. . . . . T . . . . .
P	. . . . . R . . . . .
Q	. . . . . R . . . . . G . . . . .
T	. . . . . R G . . . . .

haplotype structure in all but two of 124 individuals. The highest probability of assignment of the misassigned individuals was 49%. With this error rate in mind, I applied PHASE to reconstruct haplotype structure with the additional sample of individuals from the 2009 matrix laboratory experiment and the 2010 and 2011 field collections of sea urchins. For these individuals, the sequencing protocol was the same, but haplotype structure of heterozygous individuals was first estimated with PHASE. PHASE assigned haplotype structure to all but six individuals, with a probability of 0.995 or greater. These six individuals with a lower probability of assignment were cloned for haplotype structure. A haplotype network was estimated by means of TCS 1.21 (Clement et al. 2000) on the initial field collection of 124 individuals.

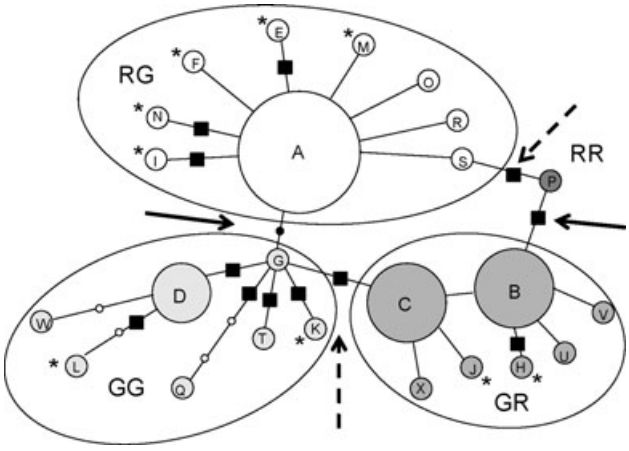

Figure 2. Sperm-bindin haplotype network for *Strongylocentrotus franciscanus.* Symbol size indicates the frequency of each haplotype (all but A, B, C, D were found only once), block squares indicate nonsynonymous substitutions, and open dots indicate an additional (synonymous) substitution between known haplotypes. Large circles and shading indicate the RG, GR, GG, and lone RR haplotypes. The solid arrow indicates the transition between arginine and glycine at amino acid site 13; dashed arrows indicate the glycine to arginine transition at site 35 (Table 1). Asterisks indicate haplotypes noted by Debenham et al. (2000a,b) but not in the present study. Letters indicating haplotypes are same as those indicated by Debenham et al. (2000a,b).

Results

FIELD EXPERIMENT

Sequence. analysis of the first exon of the sperm-bindin protein in *S. franciscanus* from Barkley Sound along the western coast of Vancouver Island reveals four relatively common haplotypes at frequencies ranging from 58% to 8% (Table 1). The most common haplotype (A) is distinguished by having arginine (R) at amino acid site 13 and glycine (G) at amino acid site 35 (termed the RG allele, Fig. 2). The second and third most common haplotypes (B and C) are distinguished by a single synonymous substitution, produce the same functional protein, and show reciprocal placement of the two amino acids distinguishing haplotype A (glycine at site 13 and arginine at site 35, termed the GR allele). The least common of these four haplotypes (D) has glycine at both these amino acid sites (the GG allele). The RR allele was rare (haplotype P) and found in a single male in the heterozygous form.

Overall the homozygous and heterozygous RG and GR genotypes have higher total male reproductive success than homozygous or heterozygous individuals with the less common GG allele (Fig. 3). The rare haplotypes were only found in the heterozygous form, including the single male heterozygous for the RR allele, and these males had low reproductive success (Levitan and Ferrell 2006). An ANCOVA was used to examine male pairwise success as a function of three main effects—male genotype, female genotype in that pair, and male density (either sperm limiting or sperm oversaturating, Fig. 1.)—four covariates (average distance to the

Table 2. ANCOVA of field experiment. Main effects of male genotype, female genotype, and density (sperm limited or polyspermic) with the covariates of advection (log transformed), distance between male and female (log transformed), average distance between males and closest female in a spawning event, and the number of male competitors in a spawning event. Response variable is pairwise reproductive success for each male. In this analysis, all nonsynonymous genotypes were included. A significant three-way interaction was apparent among density, male genotype, and female genotype. Males and females that matched in genotype did well at low density, whereas mismatched genotypes did well at high density (Fig. 4). There is also a significant two-way interaction between density and male genotype. F = female, M = Male, gt = genotype, M. comp = no. of male competitiors, SS = Sum of squares, MS = Mean squares.

Source	df	Type III SS	MS	*F*	*P*
Male genotype	4	0.115	0.029	2.278	0.0607
Female genotype	5	0.055	0.011	0.86	0.5059
Density	1	0.003	0.003	0.26	0.6073
Advection	1	0.142	0.142	11.11	0.0009
Distance	1	0.064	0.064	5.02	0.0255
Ave. nearest female	1	0.062	0.062	4.85	0.0282
Male competitors	1	0.074	0.074	5.80	0.0164
F. gt×density	4	0.097	0.024	1.91	0.1085
M. gt×F. gt	24	0.349	0.015	1.14	0.2928
M. comp. ×M. gt	4	0.113	0.028	2.22	0.0662
M. gt×density	4	0.218	0.054	4.28	0.0021
M. gt×F. gt×density	9	0.232	0.026	2.02	0.0352
Error	451	5.749	0.013		
Total	513	8.150			

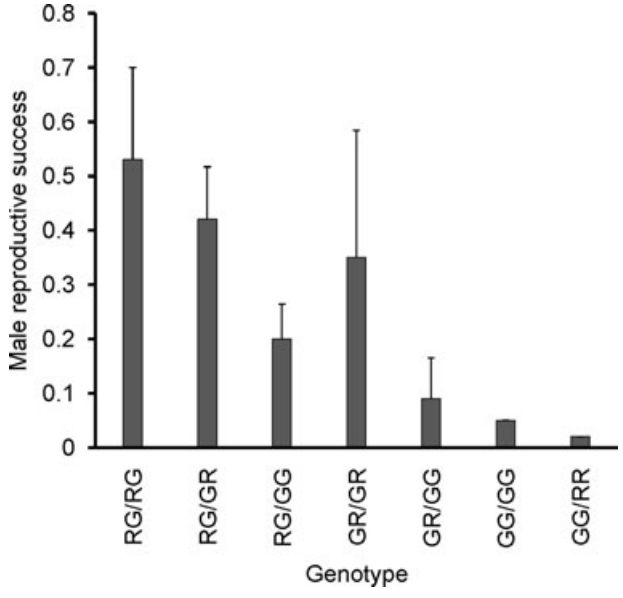

Figure 3. Total male success in the field experiment as a function of sperm-bindin genotype. Mean (SE) success for homozygous and heterozygous individuals at amino acid sites 13 and 35.

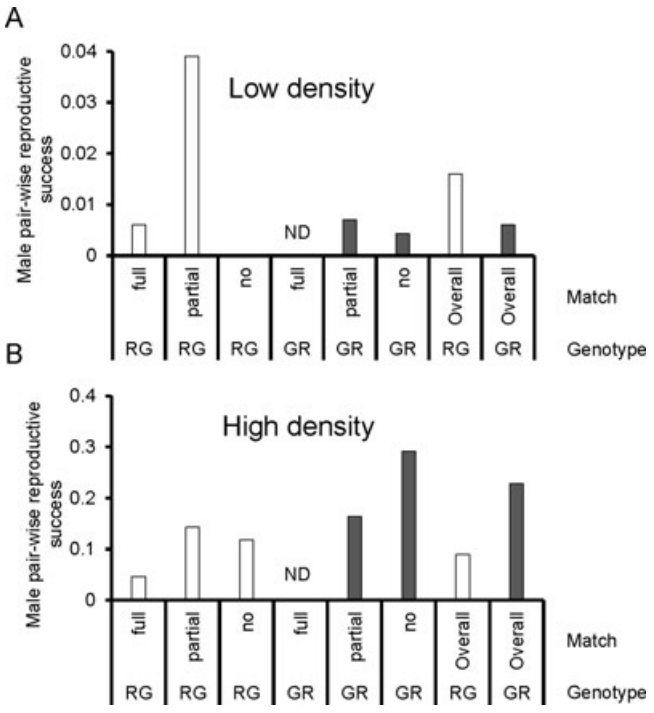

Figure 4. Male pairwise success in the field experiment as a function of male genotype (RG open, GR gray; only homozygous males plotted), matching with the female genotype (full, partial, or no matching alleles), and male density (Panel A, densities lower than 1 male/m^2; Panel B, densities greater than 1 male/m^2; see Fig. 1). A significant three-way interaction was evident among male genotype, matching, and density (Table 2). In both panels, the last set of bars illustrates the significant two-way interaction (Table 2) between male genotype and male density; RG genotypes outperformed GR genotypes at low density, but the opposite was true at high density. ND indicates no data for GR males matching with GR females.

nearest female in a spawning event; distance between the male and female in that pair, log transformed); number of competing males; and level of advection—water flow averaged over the full experimental time period, log transformed), and significant interactions of these terms (Table 2). The result included a significant three-way interaction among male density, male genotype, and female genotype; at low-density (Fig. 4A), matched male and female genotypes did better than mismatched genotypes, whereas at high densities (Fig. 4B), mismatched genotypes did better than matched genotypes (at both densities partial matches did best). There was also a significant two-way interaction between male density and male genotype (Table 2). To specifically test the density by male genotype interaction of males homozygous for the A or B/C haplotypes (RG or GR males), I conducted a simplified two-way ANOVA testing pairwise reproductive success as a function of density (low or high) and male genotype (RG or GR) and their interaction. The interaction ($P = 0.006$) and the two main effects (density $P < 0.0001$, genotype $P = 0.016$, $N = 195$) were

Table 3. ANCOVA of laboratory experiment. On independent days, with independent sea urchins, four replicate matrices were established for examination of fertilization between a set of males and females (matrix sizes of 6 males × 6 females, 9 × 7, 10 × 7, and 8 × 8). In the no-choice ANCOVA, male genotype was the main effect (only males homozygous for RG or GR considered); sperm concentration was the covariate. An interaction was significant; RG males had a negative slope (lower success with increasing sperm concentrations) associated with a susceptibility to polyspermy, GR males had a positive slope indicating a higher resistance to polyspermy. In the choice ANCOVA, male genotype was the main effect (only males homozygous for RG or GR were considered); success in the no-choice experiment was used as a covariate (adjusted by sperm concentration). The response variable was choice success (adjusted by sperm concentration and number of competing males). An interaction was significant; RG males that caused polyspermy in no-choice assays were highly competitive in choice assays, whereas GR males that did well in no-choice experiments also did relatively well in choice experiments. Overall, RG males outcompeted GR males when sperm from all males where well mixed.

Source	df	Type III SS	MS	*F*	*P*
No-choice results (for all four matrices)					
Sperm	1	0.0598	0.0598	2.20	0.142
Genotype	1	0.0698	0.0698	2.57	0.113
Sperm × genotype	1	0.1876	0.1876	6.90	0.010
Error	85	2.311	0.02718		
Total	88	2.830			
Choice results (for the three matrices with the most informative genotypes)					
No-choice success	1	68.784	68.783	2.13	0.151
Genotype	1	223.432	223.423	6.91	0.012
No-choice × genotype	1	161.362	161.362	4.99	0.030
Error	47	1520.367	32.348		
Total	51	1893.153			

all significant. Males at high density did better overall than males at low density because they are closer to females, and within a density, RG males had 2.7 times the pairwise reproductive success of GR males at low densities, whereas GR males had 2.6 times the pairwise reproductive success of RG males at higher densities (Fig. 4).

NO-CHOICE AND CHOICE LABORATORY EXPERIMENT

The laboratory experiments were conducted at sperm concentrations that can cause polyspermy (Levitan et al. 2007). Males and/or females homozygous for RG and GR were present in the matrices, but no matrix had both a male and a female homozygous for GR (the less common allele). In the no-choice experiments, an ANCOVA testing male reproductive success as a function of male genotype (only homozygote individuals included) and sperm concentration indicated a significant interaction ($P = 0.01$, Table 3). Independent regressions indicate that males with the RG genotype had a significantly negative slope with sperm concentration ($P = 0.009$), suggesting they produced sperm that induced higher levels of polyspermy with increasing sperm concentrations. In contrast, males with GR genotypes had a nonsignificant positive slope ($P = 0.25$), indicating that these males produced sperm that were less likely to induce polyspermy at these same sperm concentrations (Fig. 5A). These results suggest that RG male produce sperm with a higher affinity for a random sample of eggs than do GR males. Because the results of the ANCOVA might be sensitive to the two replicates conduced at the lowest sperm concentrations, these data were removed for additional analyses. The results indicate a nonsignificant interaction and covariate (sperm concentration). This reduced the model to a one-way ANOVA that revealed a highly significant main effect; RG genotypes had lower success than GR genotypes at the replicates constrained to the higher sperm concentrations ($P = 0.0006$). This result is also consistent with RG males producing high-affinity sperm more likely to cause polyspermy, confirmation of this hypothesis is found in the choice experiment.

To examine if lower reproductive success at higher densities is caused by an RG sperm having a high affinity with eggs leading to increased tendency of RG males to induce polyspermy, the success of RG and GR males were examined when they directly competed for eggs. When in direct competition, males with a higher fertilization rate (affinity) should garner a higher proportion of fertilizations, even if high affinity causes some degree of polyspermy. Thus, if the poor success of RG males in no-choice experiments (as noted above) is caused by high affinity and polyspermy, then RG males with low reproductive success in no-choice experiments should outcompete other males in direct competition. To test this prediction, I used an ANCOVA to examine male reproductive success in choice experiments (adjusted by sperm concentration and the number of males competing) as a function of male genotype and success in no-choice assays (adjusted by sperm concentration). The results indicated a significant interaction ($P = 0.03$, Table 3, Fig. 5B). Independent regressions

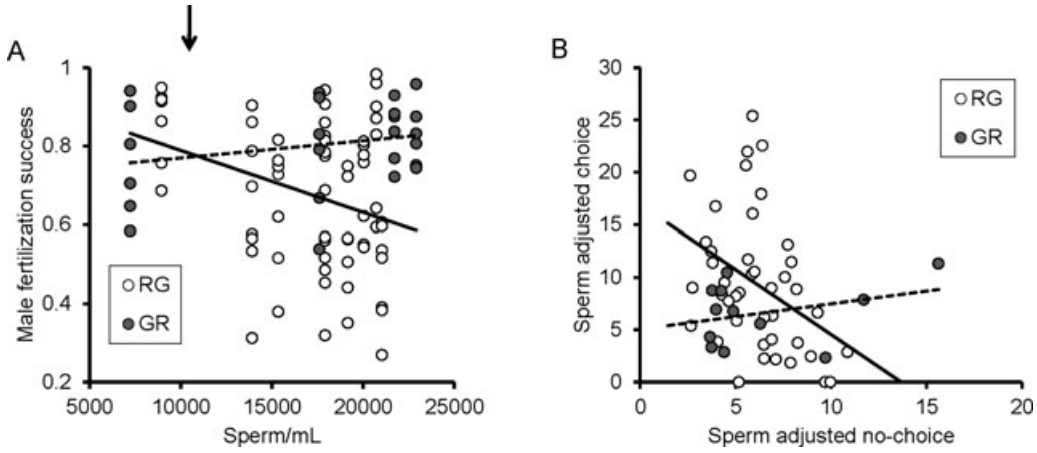

Figure 5. **Laboratory experiment conducted at sperm concentrations that can produce polyspermy. Polyspermy becomes evident at concentrations above 10,000 sperm/mL (arrow; based on Levitan et al. 2007). Open symbols for RG males, solid symbols for GR males. (A) No-choice experiment in which sperm and eggs for all pairwise crosses were tested independently. Male success is plotted as a function of sperm concentration. An ANCOVA indicates that the slopes of RG and GR males differ significantly. Independent regressions indicate a significant negative slope in RG males (solid line) and a nonsignificant positive slope in GR males (dashed line); increasing sperm concentrations decrease RG but not GR success. (B) Choice experiment in which the sperm from all males in the matrix were pooled and crossed with all females. Choice success (adjusted by sperm concentration of that male relative to other males, and by the number of males) is plotted as a function of no-choice success (adjusted by sperm concentration). An ANCOVA indicates that the slopes of RG and GR males differed significantly. Independent regressions indicate a significant negative slope in RG males (solid line) and a nonsignificant positive slope in GR males (dashed line); RG males that caused excessive polyspermy in no-choice assays were particularly successful in competition with other males. The ANCOVA also revealed a significant main effect; RG males outcompeted GR males.**

indicate that for RG males, lower reproductive success in no-choice assays (presumably caused by polyspermy) was associated with higher success in competition with other males ($P = 0.02$). Males with the GR genotype did not show this inverse relationship ($P = 0.32$), supporting the notion that GR males were less likely to induce polyspermy at these sperm concentrations. Importantly, the main effect of male genotype was significant (Table 3) with RG males outcompeting GR males when in direct competition, indicating that RG sperm have a higher affinity than GR sperm with the available eggs (Fig. 5B). Whether GR males would have a higher affinity with GR females remains undetermined, but both RG and GR males were tested against heterozygous females, and RG males had 35% higher success than GR males (choice success of RG = 10.18, SE = 1.56; GR = 6.60, SE = 0.86). In nature, RG males are successful under conditions of sperm limitation in part because their sperm have a higher affinity with eggs produced by heterozygous females and in part because RG females are common and produce eggs that have a high affinity with RG sperm.

CHANGE OVER SIZE CLASSES

Figure 6A shows the size distribution of individual sea urchins used to estimate the size–age frequency distribution of sperm bindin. The relationship between test diameter and age estimated using Ebert et al.'s (1999) parameter values from 17 subtidal sites (Fig. 6B) suggests that the largest sea urchins found in Barkley Sound (one individual at 18 cm) approach 300 years of age. The slightly more common large size of 17 cm is estimated to be 223 years old, with a standard error of 43 years.

The largest size class of sea urchins (>17.0 cm test diameter), estimated to be over 200 years old, has an RG allele frequency of 75% and a GR frequency of 10%. The smallest size class, (estimated to be <10 years of age), has an RG frequency of 48% and a GR frequency of 42% (Fig. 7). Regression analysis indicates a significant increase in the RG allele ($R^2 = 0.70$, $P = 0.01$) and decrease in the GR allele ($R^2 = 0.86$, $P = 0.0009$) with increasing size. The GG allele (haplotype D) has remained relatively constant at a frequency of 9% (regression, $P = 0.73$). Converting test diameter to age estimates (Figs. 6B, 7) indicates that there has been an accelerating increase in the GR allele and decrease in the RG allele over the past hundred or so years. The significance of the increasing GR allele is not sensitive to the extreme values of the youngest or oldest sea urchins. When these two size classes were sequentially removed from the regression, the slope remained significant ($P < 0.05$ for both cases); the change in allele frequencies is not driven by a single cohort of recruits.

Tests of departure from Hardy–Weinberg equilibrium of these different age classes indicate no significant departure, but the youngest age class shows a marginally nonsignificant excess of heterozygous individuals ($P = 0.064$, $FIS = -0.171$, Robertson and Hill estimate with GENEPOP 4.1 testing for

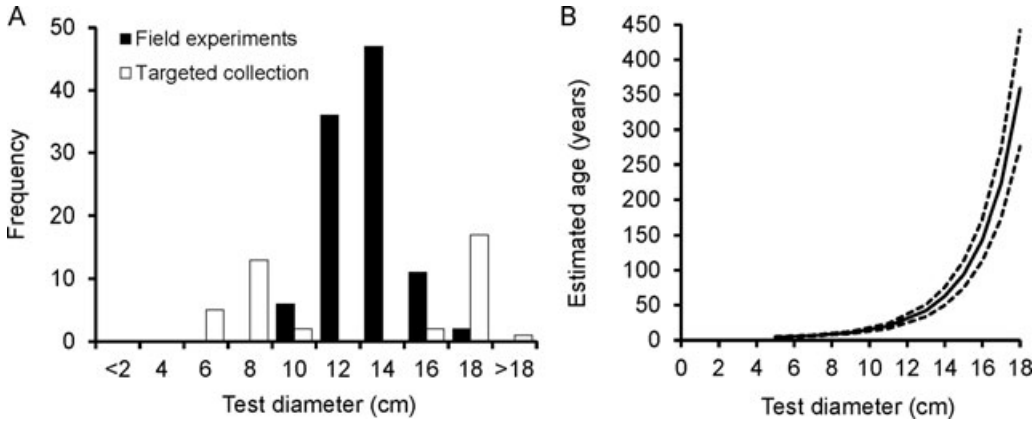

Figure 6. Panel A. The size-frequency distribution of *S. franciscanus*. Data are from random sampling of adults used in field experiments (solid symbol) and a search for particularly small and particularly large individuals (open symbol). Panel B. Age as a function of test diameter in *S. franciscanus*. Parameter estimates are from Ebert et al. (1999), whose empirical data were based on tetracycline tagging of 18 populations along the west coast of North America from southern California to Alaska. The solid line is the estimate from the mean parameter values for all sites (as in Ebert et al. 1999). The dotted lines are the standard errors, based on the estimated age of each size class from the independent parameter values for each of the 17 subtidal populations.

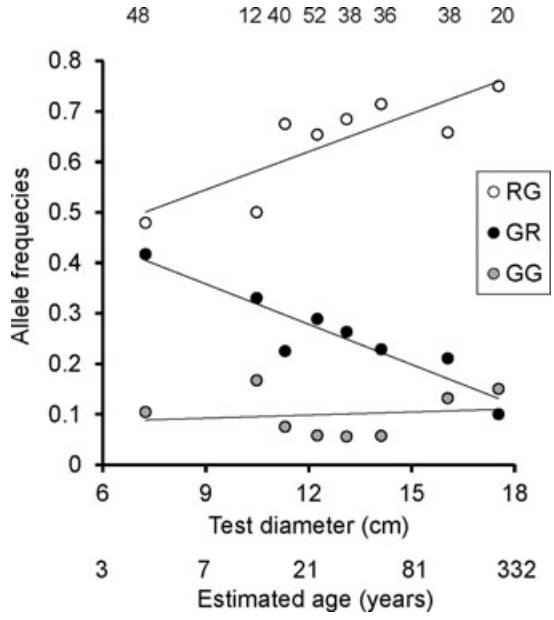

Figure 7. Sperm-bindin allele frequencies as a function of size class and estimated age. Ages were estimated from parameter values illustrated in Figure 6B, based on Ebert et al. (1999). Numbers at the top represent numbers of alleles in each age class. The RG allele significantly decreased and the GR allele significantly increased from largest to smallest size classes. Individuals estimated to be older individuals had a high frequency of the allele that confers high reproductive success when sperm are limiting; smaller individuals estimated to be younger have a high frequency of the allele that reduces the risk of polyspermy when sperm are overly abundant.

heterozygous excess, Rousset 2008). All other age classes have probabilities greater than 0.25 for departures from heterozygous excess or deficit. Heterozygous excess is the expected outcome in these younger sea urchins at higher densities, because recent conditions of higher densities favor mismatched mates (negative assortative mating). For example, at high densities, pairwise success of male RG homozygotes with matched female RG homozygotes have less than half the success of same males with mismatched GR female homozygotes (Fig. 4B); the former crosses produce all homozygous offspring, whereas the latter produces all heterozygous offspring at this locus.

Genetic structure was examined between the smallest (5–10 cm) and largest (>16 cm) size classes from the targeted search of sea urchins using the sperm-bindin locus along with the five most commonly used microsatellite loci from the paternity analysis (Table 4). Microsatellite loci were examined using MICRO-CHECKER (Oosterhout et al. 2004). There was no evidence of scoring errors or large allele drop out, but two loci (GAO20, GAO16) showed consistent evidence for null alleles. The results suggest that only sperm bindin demonstrated evidence of significant structure ($F_{ST} = 0.0418$—SPAGeDi 1.2, Hardy and Vekemans 2002). The five presumably neutral microsatellite loci show no evidence of structure between the smallest and largest sea urchins. The uniform lack of structure across all microsatellite loci suggests that issues with null alleles did not have a large skew on the results. Unreported estimates of G''_{ST} and D_est (GenoDive—Meirmans and Hendrick 2011) provided the same pattern of structure and significance.

LATITUDINAL PATTERNS OF SPERM-BINDIN ALLELE FREQUENCY AND BODY SIZE FROM THE LITERATURE

Debenham et al. (2000b) examined the frequency distribution of *S. franciscanus* sperm-bindin alleles across the range of this species from Mexico to Alaska. They found no evidence of genetic

Table 4. Tests of genetic subdivision of the sperm-bindin locus and five microsatellite loci between 20 small (5–10 cm) and 20 large (> 16 cm) sea urchins from the targeted field collection. Microsatellite loci described in McCartney et al. (2004). Estimates for individual loci and a global estimate of the microsatellite loci. Only sperm bindin shows significant structure ($P < 0.05$) using a one-tailed permutation test of observed F_{ST} being greater than permuted value (10,000 permutations—SPAGeDi 1.2, Hardy and Vekemans 2002).

Locus	No. of alleles	H(obs)	H(exp)	F_{ST}	P (obs.>exp.)
Bindin	5	0.741	0.670	0.0418	0.0381
MS-GAO20	35	0.730	0.977	−0.0022	0.5944
MS-GAP1	14	0.658	0.907	−0.0201	0.9627
MS-GAO16	16	0.675	0.888	−0.0178	0.9403
MS-GAO11	17	0.795	0.915	0.0000	0.4205
MS-GTM2	20	0.863	0.929	−0.0060	0.6652
All microsats		0.744	0.923	−0.0090	0.9483

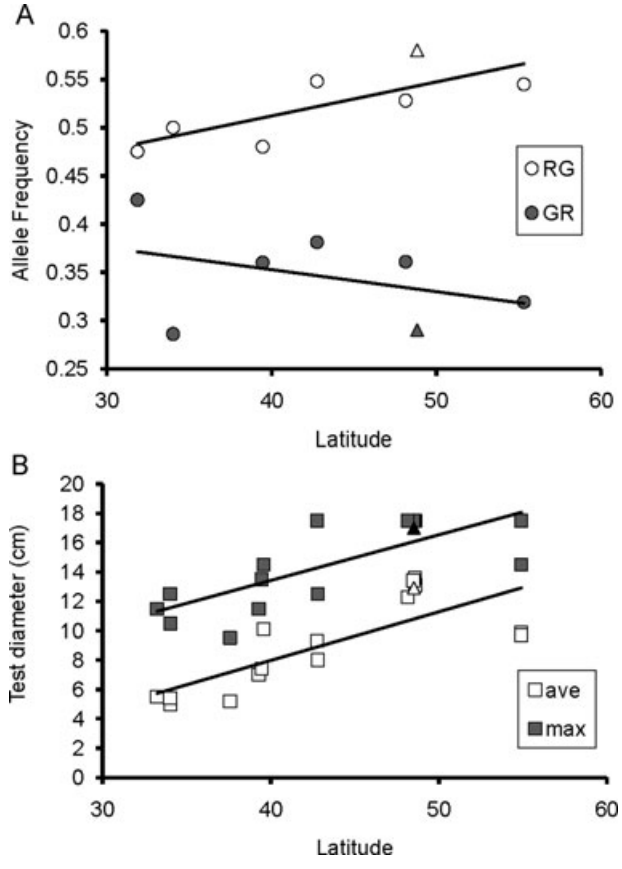

Figure 8. Latitudinal gradient in allele frequencies and body size. (A) Latitudinal gradient in the RG (haplotype A) and GR (haplotypes B and C) alleles. Data are from Debeham et al. (2000b) (circle symbols) and the present study (triangle symbols). (B) Latitudinal gradient in average and maximum test diameter. Data are from Ebert et al. 1999 (square symbols) and the present study (triangle symbols).

subdivision in sperm bindin and concluded that panmixia prevails. The most common haplotype (A = RG) ranged from 0.475 to 0.548, the sum of the second and third most common haplotypes (B and C = GR) ranged from 0.286 to 0.425. Plotting the frequency of the RG and GR alleles reported by Debeham et al. (2000b) along with the present data from Barkley Sound indicates a significant latitudinal gradient of increasing RG and decreasing GR alleles with latitude ($R^2 = 0.59$, $P < 0.05$, Fig. 8A). Debenham et al. did not record the sizes of individuals in their study but did note that all sequenced individuals were adults at least 8 cm in test diameter.

Ebert et al. (1999) examined body size of *S. franciscanus* along a similar geographic range from southern California to Alaska. They reported the average size of sea urchins and a histogram of sizes from each of 17 subtidal locations. Both reported average size ($R^2 = 0.60$, $P < 0.001$) and the maximum size ($R^2 = 0.59$, $P < 0.001$) noted in each histogram indicate a significant increase in test diameter with latitude (Fig. 8B). The average and maximum sizes from the randomly collected sea urchins from the present study (Fig. 6) have values consistent with those of Ebert et al. (1999) on the basis of latitude (triangles in Fig. 8B). Ebert et al.'s analysis indicated no difference in growth rate as a function of latitude but did find survivorship differences unrelated to fishing pressure. They concluded that latitudinal differences in water temperature are responsible, either directly or indirectly through promotion of disease, for the survivorship differences. The paucity of *S. franciscanus* individuals larger than 12 cm (and older than 20 years) in southern California and Mexico coincides with decrease in the RG allele to less than 0.5 at these lower latitudes; differential age structure provides a simple explanation for this cline.

Discussion

SPERM-BINDIN GENOTYPES

Arginine is a basic and polar amino acid, whereas glycine is neutral and nonpolar. In *Strongylocentrotus purpuratus*, replacement of a single polar amino acid (serine) with a nonpolar one (proline) also changes sperm–egg compatibility (Levitan and Stapper 2010). That reciprocal forms of the bindin protein (RG and GR) are more common (sum of >90%; Fig. 2) and have higher

reproductive success (Fig. 3) than either of the rarer proteins with arginine or glycine at both amino acid sites is interesting. The protein structure of sperm bindin and the egg receptor (EBR1) are not known, but shifts in the polarity of these proteins appear to play a key role in these structures and patterns of compatibility. Inserts and deletions were not observed in the first exon of sperm bindin in this species, whereas they were relatively common in *S. purpuratus* (Levitan and Stapper 2010). Analysis of the second exon of *S. franciscanus* (unpubl. data) indicates that it is essentially monomorphic (rare single incidences of polymorphisms). Simple amino acid substitutions in the first exon of sperm bindin appear to have dramatic influences on intraspecific compatibility in this species.

Patterns of expression in sperm bindin have been investigated in males and females, ovaries and testes, and various adult and larval tissues. Results indicate that sperm bindin was only expressed in testes (Gao et al. 1986). Further work on expression noted that sperm bindin accumulates during the transition from late spermatocytes to early spermatids (Cameron et al. 1990). The sperm-bindin protein has been experimentally shown to adhere in a species-specific manner to a complementary egg receptor (Glade and Vacquier 1978, Kamei and Glabe 2003). These results suggest that sperm bindin is only active during the process of fertilization and is not likely to directly influence fitness after fertilization.

DENSITY- AND FREQUENCY-DEPENDENT SELECTION

Field and laboratory experiments indicate that low densities or low levels of sperm availability males with the RG genotype are favored, whereas high densities or conditions of overabundant sperm males with the GR genotype are favored. The laboratory experiment indicates that RG males produce sperm that have a higher affinity with the eggs tested, but this also induces polyspermy at lower sperm concentrations than GR males (Fig. 5). This result matches a parallel study that examined intraspecific variation in fertilizability of eggs in this species (and two congeners); females producing eggs that require higher concentrations of sperm for fertilization are also more resistant to polyspermy (Levitan et al. 2007).

The success of RG males at low density appears to be a function of two factors noted in both the field and laboratory studies. First, RG males have a higher affinity than GR males with heterozygous (laboratory) or partially matching (field) females. Second, RG is the most common form of the sperm-bindin protein, and was particularly so in times past. Males with the RG genotype are more likely to encounter RG (than GR) females, and matching genotypes do better than mismatched genotypes when sperm are limiting. Although GR male and female matches might be successful, these encounters would be rare at low densities given the observed frequency of 10% in the largest and oldest sea urchins. Even if both GR males and females were in the same local spawning group, they would unlikely to be nearest neighbors (the primary determinant of pairwise reproductive success—Levitan 2004; Levitan and Ferrell 2006) given the high frequency of RG individuals. The success of GR males at higher density appears to be caused by a lower affinity with available mates, which reduces the risk of polyspermy. Even if GR males occasionally lose in competition to RG males when sperm from both types arrive near simultaneously at an egg (choice result), in nature, variable nearest neighbor distances, heterogeneity of water flow, and unequal sperm release would all favor the male whose sperm arrived first or in higher concentrations. If the sperm from other males arrives even seconds later, or at lower concentrations, fertilization has probably already occurred (Levitan 2005). Under these conditions, GR males, less likely to induce polyspermy, would be favored.

Theory suggests that when sperm are overabundant, these two sperm-bindin alleles should reach an equilibrium frequency (for a given level of sperm availability), at which the more compatible sperm–egg matching ligand (sperm bindin) and egg receptor (EBR1) would be less common than the less compatible match (Tomaiuolo and Levitan 2010). The rationale for this prediction is that the more compatible matches would suffer a higher level of polyspermy for a given encounter frequency. This would decrease the frequency of a highly compatible egg receptor, which would dictate the frequency of the matching sperm-bindin ligand. Current allele frequencies give no indication of being at equilibrium, but the trajectory of the less compatible GR allele is consistent with this theory. Although data on the egg receptor is lacking, evidence for linkage disequilibrium has been noted between sperm and egg recognition proteins in abalone (Clark et al. 2009) and in *S. purpuratus*, a congener of the *S. franciscanus* (A. Stapper, P. Beerli, and D. Levitan, unpubl. data).

CHANGES OVER TIME

Genetic structure in *S. franciscanus* (Moberg and Burton 2000), like that of the cooccurring *S. purpuratus* (Flowers et al. 2002), suggests that individual cohorts of recruits can differ genetically at neutral loci, but these cohorts tend to accumulate over time, reducing or eliminating spatial structure in older adult populations. The evidence for structure in recruits is at small spatial scales, there is no evidence for regional or clinal genetic structure (Moberg and Burton 2000). The accumulation of distinct cohorts from different spawning populations would tend to minimize or eliminate local adaptation for gamete-recognition proteins. A specific spawning event might favor one or another sperm-bindin allele (Calderon and Turon 2010), but these offspring might settle into other or a variety of sites that differ in sperm availability. This process would tend to homogenize spatial differences in selection toward the species average.

However, longer term and geographically widespread shifts in spawning densities would provide more consistent selection, perhaps resulting in temporal shifts in gene frequencies. This scenario is consistent with two observations. First, there is a consistent change of sperm-bindin allele frequencies in Barkley Sound, British Columbia across all size/age classes (Fig. 7). Although there is variability in the relationship between size and age across geographic locations, the consistent pattern within each location (see Ebert et al. 1999) and restricted variation among sites (Fig. 6B) suggest that it is reasonable to assume that small sea urchins are younger than large sea urchins and that the small sea urchins in this study are several years old and the largest are in the neighborhood of 100–300 years old. In Barkley Sound, larger/older sea urchins are dominated by the RG allele (75%) and the GR allele is uncommon (10%), whereas smaller/younger sea urchins have near-equal GR and RG genotypes. This shift results in significant F_{ST} values between the youngest and oldest sea urchins examined at the sperm-bindin locus, but not at the putative neutral microsatellite loci (Table 4). Because this shift is not detected in the putative neutral loci, it suggests that selection, rather than genetic drift (e.g., sweepstakes), has caused a continuous shift in sperm-bindin allele frequencies over time.

The second observation consistent with temporal shifts in allele frequencies is that sperm bindin does show a geographic cline in sperm-bindin allele frequencies, but this cline is consistent with differences in age structure driven by survivorship differences (Ebert et al. 1999), rather than geographic genetic subdivision in sperm bindin (Debenham et al. 2000b) or neutral loci (Moberg and Burton 2000). *Strongylocentrotus franciscanus* in southern latitudes experience similar growth rates, but higher mortality than in the northern latitudes, likely driven by temperature-induced stress or disease (Ebert et al. 1999). Older sea urchins and the RG alleles they carry are less likely to be present in lower latitude populations.

An unexplored alternate hypothesis that might explain temporal shifts in allele frequencies in sperm bindin is that selection is acting on some unknown closely linked loci. If these loci were expressed in adult individuals, the shift in allele frequencies could be a result of differential survivorship rather than fertilization.

The shift in bindin allele frequencies from RG to GR is predicted by the field and laboratory experiments in conjunction with circumstantial evidence of increases in sea-urchin abundance. There is evidence that sea-urchin predators, most notably sea otters (Estes and Duggins 1995; Estes et al. 2010; Watson and Estes 2011), but also fish (Cowen 1983) and lobsters (Tegner and Levin 1983), were common along the west coast of North America and were removed by human fishing pressure. There is also evidence that sea otters have a large effect on sea-urchin densities, particularly the adult size classes (Breen et al. 1982; Estes and Duggins 1995; Kvitek et al. 1998; Watson and Estes 2011). Although, patterns of predator–prey relationships are complex and difficult to infer in times past (e.g., Foster and Schiel 2010), the weight of evidence suggests that sea-urchin densities have increased over the past few hundred years. Using the estimated ages of current sea urchins, this presumed increase in abundance coincides with a dramatic increase in the GR genotype, which is more resistant to polyspermy, from a frequency of 10% to over 40%. The dominance of the RG allele in the larger and presumed older individuals is consistent with purifying selection under sperm-limited conditions likely common in times past, whereas the present maintenance of multiple common alleles, noted in smaller and presumed younger individuals, is consistent with conditions promoting polyspermy (Tomaiuolo and Levitan 2010).

An unexplored alternative hypothesis is that factors other than sea-urchin abundance, such as shifts in water chemistry, temperature, or turbulence, differentially influence the success of the RG and GR genotype and these oceanographic factors have changed sufficiently over the past 100 years to explain the shift in sperm-bindin genotypes. These changes would presumably have to be quite large, since the spawning season of *S. franciscanus* extends from mid-winter through early summer and thus these sea urchins currently experience large fluctuations in both water temperature and flow conditions (Levitan 2002).

Using the estimated ages, there is an increase in the GR allele from >200-year-old sea urchins (10%) compared to the 100- to 200-year-old sea urchins (21%). These changes have subtly accelerated over the past 70 years. The largest shift in allele frequencies in the youngest individuals (to >40%) is accompanied by a marginally nonsignificant excess of heterozygous individuals. Presently, high sea-urchin densities favor mates with mismatched bindin alleles, and this negative assortative mating would result in heterozygous excess.

An accelerated shift in allele frequencies is expected for at least three reasons. First, matched male and female genotypes have higher affinity. Assuming sea-urchin densities have increased, at low densities in times past, GR males would rarely encounter GR females. As densities increased, GR females that avoided polyspermy by means of mismatches and lower affinity would increase in numbers. The increase in GR females would increase the encounter probability with GR males, and they would start to be more successful. Second, sea otters were common along the full coast of North America, and few source populations of sea urchins at higher densities probably existed to produce a large cohort of recruits. Although isolated removal of sea urchins can be followed by high recruitment (Scheibling 1984), when populations are sparse throughout their range, recruitment can be very low for decades. For example, the once common sea urchin *Diadema antillarum* was reduced by 99% from historic densities across its range in 1983–1984. Nearly 30 years later, densities have remained low in most locations in the Caribbean because of

an absence of recruiting larvae (Bak 1985; Lessios 2005). *Strongylocentrotus franciscanus* populations may have remained low for many decades after the removal of sea otters, and population growth and density have been accelerating. Third, sea-urchin fecundity increases with size and age (Ebert 2008), and individuals may take a decade or more to become large enough to contribute substantial numbers of offspring to the next generation. All three of these factors would result in an accelerating rate of change in the frequency of the GR allele.

In sum, there is a consistent match between theory, experimental work, and the direction of change in allele frequencies. However, no data is presented on historical sea-urchin densities or fertilization dynamics in times past; such data might not be possible to collect in this system. Further studies in other systems where densities are currently shifting would provide additional support for how gamete traits evolve with shifting demography.

Shifts in abundances of many broadcast-spawning species have been noted in the past few decades, many caused by direct human impact that decreased abundances (e.g., abalone, Tegner et al. 1996; fish, Coleman et al. 1996), indirect human impact that increased abundances by removing key predators (Jackson et al. 2001; Estes et al. 2010), or the direct and indirect effects of disease or stress that altered abundances (Scheibling 1984; Black et al. 1995; Carpenter et al. 2008). A predicted outcome of rapid decreases in abundance is an Allee effect (a decrease in population growth rate with decreasing abundance), but the evidence for Allee effects is equivocal (Levitan and McGovern 2005; Courchamp et al. 2008). Overharvested or otherwise decimated populations often recover once the source of mortality or stress has been removed (Meyers et al. 1995). The evidence that gamete traits can be adapted to different levels of sperm availability and rapidly evolve in response to changing densities might explain why species at times recover from rapid declines rather than going extinct. The likelihood that species can adapt out of these adverse situations will depend on how quickly the population shifted abundances, the strength of the selective pressure, and the extant variation in traits able to cope with alternative density regimes.

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