Selection on Gamete Recognition Proteins Depends on Sex, Density, and Genotype Frequency

Don R. Levitan* and David L. Ferrell*

Gamete recognition proteins can evolve at astonishing rates and lie at the heart of reproductive isolation and speciation in diverse taxa. However, the source of selection driving this evolution remains unknown. We report on how the sperm bindin genotype influences reproductive success under natural conditions. An interaction between genotype frequency and spawning density determines how sperm bindin genotype influences reproductive success. Common genotypes are selected under sperm-limited conditions, whereas rare genotypes are selected under conditions of intense sperm competition and sexual conflict. Variation in the evolutionary rates of bindin may reflect historic differences in sperm availability.

Gamete recognition proteins determine whether sperm and eggs are compatible at fertilization and often evolve at an exceptionally rapid pace. This is manifested as positive selection, an excess of nonsynonymous nucleotide substitutions, and can be associated with divergent proteins across species, polymorphic proteins within species, or both (*1*). Theory suggests that sexual selection might drive the rapid divergence in these proteins across species and that sexual conflict over the rate of fertilization might result in selection for polymorphic genotypes within species (*1*–*7*). Within species, high sperm concentration may select for eggs with rare alleles that slightly mismatch with sperm, thereby reducing gamete affinities and preventing egg death by polyspermy. The conflict is that sperm compete to be the first sperm to fertilize an egg and "chase" the evolution of these rare alleles. Despite the popularity of these ideas, (i) alternate hypotheses exist for why these proteins evolve quickly, (ii) it is unclear why rates of evolution in these proteins vary across species and, most important, (iii) no data examine how these proteins influence reproductive success in nature (*1*). Measuring the success of different genotypes under natural conditions is the only way to determine how selection influences the evolution of these proteins. We show how sperm bindin genotypes influence sea urchin reproductive success in the sea.

Our expectation is that under sperm-limited conditions, binding rates between eggs and sperm should be selected to be fast (high affinity) for both males and females (*8*). Under sperm-saturating conditions, sexual conflict over fusion rates might result in selection for slow binding rates (low affinity) in females but high binding rates in males. Polyspermy is costly to both sexes, but it never pays to be the second sperm to reach an egg. Thus, males are selected to have fast binding rates, assuming multiple males are competing for fertilizations. Consequently, we investigated the effect of spawning density and bindin genotype frequency on reproductive success and specifically the interaction between frequency and density.

First, we explored the pattern of genotype frequency and reproductive success. Then, because laboratory experiments suggest that matched genotypes have an increased chance of fertilization (*4*), we examined how the degree of matching between male and female genotypes influences reproductive success. Experiments were conducted in the ocean in natural populations of the sea urchin *Strongylocentrotus franciscanus*. Independent groups of sea urchins were induced to spawn at 35 population densities along the outer west coast of British Columbia (*9*). Reproductive success was determined by capturing eggs from each spawning female in the water column, measuring the fraction of eggs developing, and freezing the produced larvae for parentage analysis (*8*). For each spawning event, adult locations were mapped and water movement was recorded (*8*). Female reproductive success first increased and then decreased with male spawning density because of sperm limitation and polyspermy, respectively (Fig. 1) (*8*). Multiple paternity was rampant within females, setting the conditions for sexual conflict (*9*). The 127 adults that spawned in nine of these events (range 8 to 29 adults) were sequenced for a 273–base pair variable portion of the bindin locus (*8*); four events were under sperm-limited conditions at low density, and five events were under sperm-saturated and polyspermic conditions at high density (Fig. 1).

The bindin locus was polymorphic, and genotype frequencies did not differ from Hardy-Weinberg expectations. There was 1 common allele (0.58), 2 alleles at moderate frequency (0.15, 0.14), and 12 rare alleles. The sequences of 3 of the most common alleles and 2 of the rare alleles were identical to and not detectably different in frequency from alleles obtained from 134 individuals spread from Baja, Mexico, to Alaska, USA (*10*). This geographically broader sample, our population and the combined data, showed no evidence of positive selection in this variable domain of bindin (*8*). Because we sequenced only a subset of the bindin gene, we assumed that all substitutions, both synonymous and nonsynonymous, could be used as a genetic marker for unique phenotypes. Possible recombination with linked substitutions outside the sequenced region makes this a conservative test for detecting effects of density and frequency.

Despite the large variation in distance to the nearest mate (0.07 to 11.25 m) and average water flow (0.002 to 0.077 m/s) that largely determine reproductive success (*11*, *12*), there was a significant relation between bindin genotype frequency and male reproductive success. The overall variation explained by a male's bindin genotype frequency was small, but the average effect was large (Fig. 2A) (table S1). Males with common genotypes had an average of four times the reproductive success of males with rare genotypes. This runs counter to the expectation of rapid or even neutral evolution of diverse genotypes. Strong selection against rare genotypes, combined with common genotypes being widespread geographically, should constrain evolutionary diversification. What maintains variation in the face of this selection?

Females exhibited the opposite pattern; females with those same common genotypes had

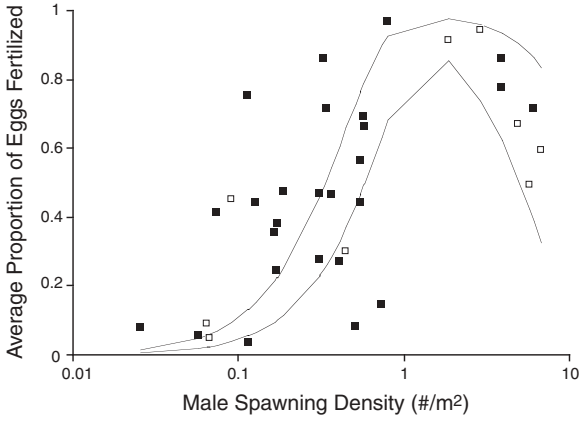

Fig. 1. Average female success and male density. Solid lines indicate the 95% confidence intervals from a fertilization kinetics model fit to these data. Reduced fertilization at low densities is caused by sperm limitation and at high densities by polyspermy. The four spawning events at low density (< 1 m²) and five at high density (> 1 m²) examined in the present study are highlighted as open symbols.

Department of Biological Science, Florida State University, Tallahassee, Florida 32306–1100, USA.

*E-mail: levitan@bio.fsu.edu (D.R.L.); ferrell@bio.fsu.edu (D.L.F.)

half the reproductive success of females with rare genotypes (Fig. 2B and table S2). As a result of the divergent patterns of male and female success, there was no pooled effect of genotype frequency on reproductive success ($P > 0.5$).

We examined how genotype frequency influenced reproductive success across densities, but because frequencies vary among small discrete spawning events, we measured the reproductive success of an individual as a function of how common his or her genotype was among potential mates for each spawning event. A positive relationship indicates that individuals matching genotypes with a high proportion of mates will have increased success. A negative relationship indicates the reciprocal. For each event, this slope was plotted as a function of male spawning density (*8*). There was a significant frequency by density interaction for both sexes (Fig. 3). At low density, individuals that shared their genotype with a high frequency of mates had high reproductive success, whereas at high density the reciprocal was evident. This suggests that the direction and intensity of frequency-dependent selection depends on density. In addition, the relationship passes through zero (no frequency dependence) at the approximate spawning density where the transition from sperm limitation to polyspermy and sexual conflict occurs.

The significance of female bindin genotype (Fig. 2B and Fig. 3) suggests that patterns of matching between male and female genotypes might explain pairwise reproductive success. We examined pairwise reproductive success as a function of spawning density, bindin genotype matching, male and female genotype frequency, mate distance, number of competing males, and water flow ($N = 514$ matings). As predicted (*9*, *11*, *12*), increases in mate distance, competitors, and water flow often decreased reproductive success. Rising out of the variance of these demographic and environmental effects was a complex pattern of interactions between male genotype frequency, genotype matching, and spawning density (tables S3 to S5) (*8*). As noted above, males with common genotypes had higher overall levels of reproductive success compared to males with rare genotypes. However, the effect of female genotype frequency was not significant; female success was a function of genotype matching with the male. The critical result was a significant density by matching interaction. At low density, nonmatched mates had the lowest reproductive success, whereas at high density, fully matched mates had the lowest reproductive success (Fig. 4A). The most common male genotype (AA) had poor reproductive success with non-A females at low density but had the highest reproductive success with these females at high density (Fig. 4B). Rare males did better at high density compared to low density with either matched or unmatched mates, because they were closer to females but not likely to cause polyspermy (rare matches are not likely close neighbors). Rare females are successful because they have high success with partial matches at all densities (Fig. 4A) and do particularly well with common, but unmatched, AA males at high density (Fig. 4B).

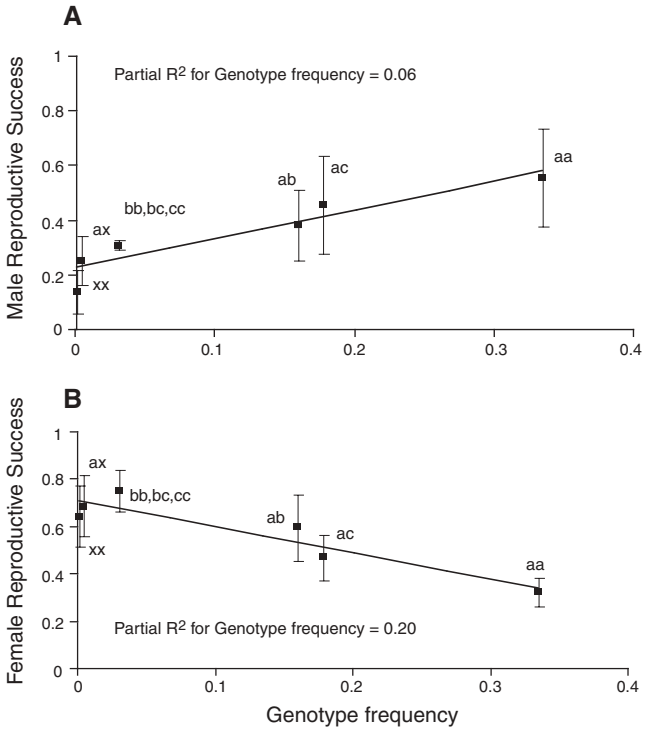

Fig. 2. Bindin genotype frequency (standard error) and total reproductive success. Allele x refers to the pool of rare alleles, and xx refers to any combination of rare genotypes not specifically listed. (**A**) Common genotypes have higher success in males, and (**B**) rare genotypes have higher success in females.

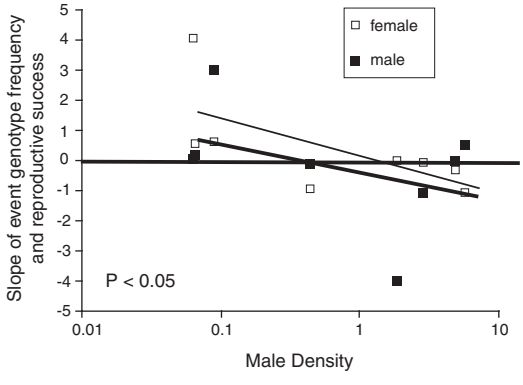

Fig. 3. Relationship between frequency-dependent reproductive success and male spawning density. Positive values indicate an advantage to commonly matching mates, negative values an advantage to rarely matching mates.

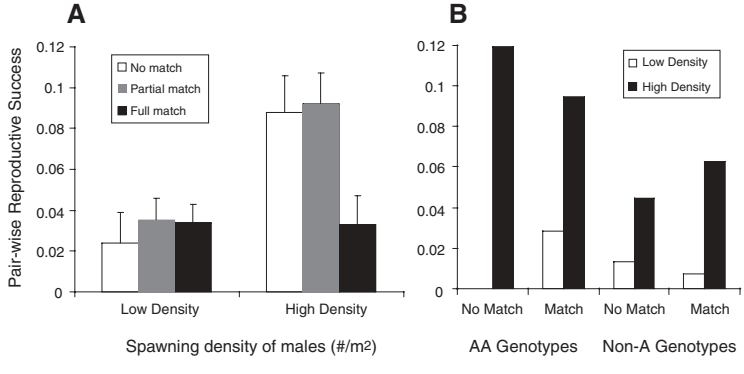

Fig. 4. Pairwise reproductive success as a function of spawning density and matching rules. (**A**) There was a significant interaction between density and matching rules and a significant effect of matching within each density. (**B**) Pairwise reproductive success of AA males and non-A males with females that match at some allele or no alleles at high and low densities.

There are at least three possible mechanisms for the association of female success with their bindin genotype [also noted in (*4*)]. First, although bindin transcription has yet to be detected in eggs or ovaries (*13*), bindin might be pleiotropic and expressed in females. Second, models (*14*, *15*) indicate that female preferences and male traits can become associated by linkage disequilibrium through assortative mating. Third, there may be a tight physical linkage between sperm bindin and a female receptor locus. A female receptor locus has been found in this species (*16*), but it has not been mapped. There may be a cascade of different recognition proteins involved in fertilization (*17*), and linkage disequilibrium at any of these loci might result in this association.

Regardless of the mechanisms that explain why the female bindin genotype influences fitness, these results provide an explanation for the maintenance of rare bindin alleles and the prevention of selective sweeps from common alleles. Rare females are favored because, as the degree of sperm competition increases, matings between unmatched genotypes become increasingly successful. Although common males do best with unmatched females at high density, they also outcompete rare males for the matched eggs that survive at these high sperm concentrations. These results are consistent with sexual conflict driving diversifying selection. Some mutations might simply result in inefficient binding, which would reduce polyspermy. Other mutations might match a complementary rare allele in the other sex. These novel matched proteins would increase in frequency under sperm competitive conditions, because matches are rare and less likely to cause polyspermy (e.g., non-A males in Fig. 4B). As their frequency increased, these alleles would be less advantageous under sperm-competitive conditions but increasingly advantageous under sperm-limited conditions (e.g., AA males in Fig. 4B). The initial process of mutation to an egg that slows binding might facilitate the second by allowing the persistence of rare alleles until the emergence of a complementary mutation. These processes set the stage for not only diversifying selection but also divergent selection, because different, but matched, loci are potentially selected allopatrically or even sympatrically, as males chase the evolution of different female receptors (*6*, *7*).

The interaction between spawning density and genotype frequency provides insight into why different species evolve at different rates. About half the echinoid species sequenced for bindin, including *S. franciscanus*, do not exhibit positive selection (*4*). However, as shown here, bindin genotypes are not selectively neutral and have a dramatic influence on reproductive success. While mate distance and water flow explain the majority of the variation in reproductive success, bindin has a large average effect, particularly given the dozens to hundreds of spawning events an individual may experience (*18*). The genetic signature of bindin in this species does not differ from neutral expectations because of counteracting, density-dependent selective forces in this species that experiences a wide range of spawning densities (*9*, *12*). Species found at either end of the continuum of sperm availability are more likely to show a consistent pattern of selection.

Data on bindin evolution from two congeners suggest stronger evidence of positive selection in *S. purpuratus* compared with *S. droebachiensis* (*8*, *19*). The species with the strongest evidence lives at higher densities than *S. franciscanus*, and the species without supporting evidence lives at lower densities along the Pacific Coast. These species also show patterns of gamete traits that match expectations of sperm limitation and competition across this gradient of sperm availability (*12*, *20*, *21*). Interestingly, *S. droebachiensis* has higher bindin allelic diversity in the Atlantic (*19*), where populations are often at higher densities (*9*). This is consistent with increasing sperm competition resulting in selection for polymorphic genotypes. Similarly, in the genus *Echinometra*, the highest level of positive selection was noted in the species most likely to experience high levels of sperm competition (*22*). Overall, these results demonstrate the powerful force of density-dependent selection on gametes and show how the interaction of density- and frequency-dependent selection can lead to a spectrum of evolutionary rates in recognition proteins.

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Supporting Online Material

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Materials and Methods
SOM Text
Tables S1 to S5
References

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CTCF Mediates Interchromosomal Colocalization Between *Igf2/H19* and *Wsb1/Nf1*

Jian Qun Ling,[1] Tao Li,[1] Ji Fan Hu,[1] Thanh H. Vu,[1] Hui Ling Chen,[1] Xin Wen Qiu,[1] Athena M. Cherry,[2] Andrew R. Hoffman[1]*

Gene transcription may be regulated by remote enhancer or insulator regions through chromosome looping. Using a modification of chromosome conformation capture (3C) and fluorescence in situ hybridization, we found that one allele of the insulin-like growth factor 2 (*Igf2*)/*H19* imprinting control region (ICR) on chromosome 7 colocalized with one allele of *Wsb1/Nf1* on chromosome 11. Omission of CCCTC-binding factor (CTCF) or deletion of the maternal ICR abrogated this association and altered *Wsb1/Nf1* gene expression. These findings demonstrate that CTCF mediates an interchromosomal association, perhaps by directing distant DNA segments to a common transcription factory, and the data provide a model for long-range allele-specific associations between gene regions on different chromosomes that suggest a framework for DNA recombination and RNA trans-splicing.

Igf2 and *H19* are coordinately regulated adjacent imprinted genes located ~80 kb apart on mouse chromosome 7 (*1*). An ICR located between the genes contains four binding regions for CTCF (*2*–*5*), a zinc finger–binding protein that binds to a variety