Modeling How Reproductive Ecology Can Drive Protein Diversification and Result in Linkage Disequilibrium between Sperm and Egg Proteins

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ABSTRACT: Gamete-recognition proteins determine whether sperm and eggs are compatible at fertilization, and they often evolve rapidly. The source of selection driving the evolution of these proteins is still debated. It has been suggested that sexual conflict can result in proliferation of genetic variation and possibly linkage disequilibrium between sperm and egg proteins. Empirical evidence suggests that both male and female reproductive success can be predicted by their sperm ligand genotype, but why female success can be predicted by a protein expressed only in males is unknown. Here we use mathematical modeling to investigate the interaction between reproductive behavior and sperm availability on the evolution of sperm ligands and egg receptors. We consider haploid and diploid expression in gametes in two possible ecological scenarios, monogamous spawning and competitive spawning. Reproductive behavior plays an important role in determining possible outcomes resulting from sexual conflict. Sperm limitation selects for common genotypes regardless of mating behavior. Under conditions of sperm abundance, competitive spawning provides conditions for the persistence of allelic variation and gametic disequilibrium. With monogamous spawning, such conditions are more restrictive.

Keywords: sexual conflict, bindin, polyspermy, gametic disequilibrium, polymorphism.

Introduction

Gamete-recognition proteins mediate fertilization and determine the compatibility between sperm and eggs both within and among species. These recognition proteins can evolve rapidly and be highly polymorphic within and across species (reviewed in Swanson and Vacquier 2002 and Palumbi 2009). This is surprising because reproduction is crucial to fitness. Why would novel proteins be better competitors than common ones? On the rare occasions when a mutant has a higher affinity for proteins that are common in its mates, it should sweep through the population. While high divergence among taxa and low variation within taxa (e.g., abalone: Metz et al. 1998) may reflect such a selective sweep, in other taxa there is evidence for high rates of evolution and intrapopulation variation (e.g., sea urchins: Metz and Palumbi 1996; Palumbi 1999; mussels: Riginos et al. 2006; oysters: Moy et al. 2008; mice: Turner and Hoekstra 2008; humans: Gasper and Swanson 2006). The source of selection driving this rapid evolution and maintaining this genetic variation is debated and largely unknown (Swanson and Vacquier 2002). However, theory (Gavrilets and Waxman 2002; Swanson and Vacquier 2002; Haygood 2004) and empirical data (Levitan and Ferrell 2006; Levitan and Stapper 2010) suggest that sexual selection and sexual conflict may play an important role.

Taxa that release unfertilized eggs into the environment for external fertilization (broadcast spawning) represent an excellent model with which to investigate the effects of sexual conflict on the evolution of gamete-recognition proteins. Broadcast spawning is the ancestral and taxonomically most widespread reproductive mode, and it is found in all animal phyla and marine algae (Giese and Kanatani 1987; Clifton 1997). Thus, it provides insight into how gametes and their associated recognition proteins evolved. This reproductive mode allows for investigations of gamete dynamics and evolution without the constraints imposed by internal fertilization and parental care (Levitan 2010). Finally, the mechanism generating sexual conflict, polyspermy, is becoming recognized as a potent selective agent in a variety of marine invertebrates and algae (Brawley 1992; Gould and Stephano 2003; Bode and Marshall 2007; Levitan et al. 2007). Polyspermy depends on sperm availability; as sperm abundance increases, so too does the risk of developmental failure caused by multiple sperm fusing with an egg (Styan 1998; Franke et al. 2002; Levitan 2004; Levitan et al. 2007). Sexual conflict arises because when

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sperm are overabundant, females may be selected to decrease the fertilization rate to allow more time to establish a successful block to polyspermy while males may be selected to increase fertilization rate to avoid being outcompeted by other males.

This conflict provides a potential mechanism for selection favoring females with rare recognition proteins. Sperm overabundance may favor eggs that have rare gamete-recognition alleles that slightly mismatch with common sperm genotypes, thereby reducing gamete affinities and preventing egg death by polyspermy. As these rare female alleles become more common, males with a novel protein that matches this female protein will be favored as they "chase" the evolution of these rare alleles (Gavrilets and Waxman 2002; Haygood 2004; Levitan and Ferrell 2006; Palumbi 2009).

Few empirical studies examine how gamete-recognition proteins influence reproductive success. Palumbi (1999) noted that eggs were fertilized at a higher rate by males with a more similar sperm-bindin genotype than dissimilar males in competitive crosses in the laboratory. Levitan and Ferrell (2006) noted that males with a more common sperm-bindin genotype had overall higher reproductive success than did rare males. The reverse was true for female sperm-bindin genotypes. Matched males and females had higher reproductive success under sperm-limited conditions, but mismatched individuals had higher success under polyspermic conditions. Finally, Levitan and Stapper (2010) noted that less common (but not rare) bindin genotypes were most successful in both males and females. What is required is a theoretical framework to explain the generation and maintenance of genetic variation and the consistent finding that female success can be predicted by a protein presumably expressed only in males.

Possible explanations for how sperm bindin predicts female success include female expression of sperm bindin or linkage disequilibrium between sperm-bindin and egg-receptor loci. There is no evidence of female expression of sperm bindin, in spite of attempts to show sperm-bindin expression in the eggs and ovaries of sea urchins (Gao et al. 1986). There is also no support for linkage disequilibrium driven by physical proximity (Sodergren et al. 2006). However, there may be reason to expect that assortative mating could drive linkage disequilibrium between loci that are not in close proximity in the genome.

Theory (Payne and Krakauer 1997; Doebeli 2005) indicates that female preferences and male traits can become associated via linkage disequilibrium through assortative mating. This hypothesis suggests that offspring are the product of successful matings and thus contain sperm- and egg-recognition proteins that were successfully combined in a specific spawning environment. Such a scenario might result in nonrandom associations between sperm and egg compatibility genes, thus predicting that demography shapes assortative mating, which in turn affects gametic disequilibrium. A recent empirical finding of linkage disequilibrium between sperm and egg proteins in abalones supports this idea (Clark et al. 2009).

We expand models introduced by Gavrilets and Waxman (2002) and Haygood (2004). We investigate the dynamics of a population of broadcast spawners and consider polyspermy as the force generating sexual conflict in two possible ecological scenarios, monogamous spawning and competitive spawning. We also consider gametes having a haploid or a diploid expression. The goal is to determine how reproductive behavior, population density, and gamete affinities influence proliferation and maintenance of allelic variation at gamete-recognition loci and linkage disequilibrium among male ligand and female receptor loci. The results shed light on how protein polymorphism can arise and be maintained under different reproductive conditions and how assortative mating can result in linkage disequilibrium between proteins expressed in males and females.

Monogamous Spawning and Competitive Spawning

Fish, aquatic invertebrates, and algal species release gametes with a single mate or in group spawns, resulting in very different evolutionary dynamics (Levitan 1998; Lotterhos and Levitan 2010). Without mate competition, polyspermy is clearly deleterious for both mates. However, when males compete, there is selection for males to have a high gamete affinity to avoid losses caused by sperm competition, whereas females are selected to have reduced affinities to avoid polyspermy; thus, sexual conflict over gamete affinities may arise.

It is important to distinguish between multiple parentage and competitive spawning. Broadcast spawning often results in females producing offspring fathered by multiple males (Neff et al. 2003; Levitan 2004, 2008). Competitive spawning in this sense requires that sperm from two or more males compete to fertilize the same egg. Thus, if a female releases eggs and one parcel of water contains a subset of eggs with sperm from a single male while a second parcel of water contains sperm only from a different male, this is a monogamous interaction in terms of gamete competition, rather than male competition. Group spawning events likely generate mixtures of monogamous and competitive interactions, depending on patterns of aggregation and water flow; here we model the two extreme conditions, effective monogamy and full mixing.

Gametes with Haploid or Diploid Expression

A gamete with haploid expression has a single recognition protein derived from its own haploid genotype (fig. 1*A*). Conversely, a gamete that expresses recognition proteins from its parental genotype is a gamete with diploid expression. Haploid- and diploid-expression gametes differ only if they derive from heterozygous parents (fig. 1*C*). In broadcast spawners, there is no conclusive evidence of diploid expression, but there are hints (Palumbi 2009). We thus explore both possibilities. We want to understand whether the expression of variants of recognition proteins on the gamete surface affects fertilization dynamics.

An egg (sperm) with diploid expression can have two different receptors on its surface, increasing its pool of potential matching ligands (receptors); depending on conditions, this can be beneficial or not. In diploid expression, the relative proportion of each protein on a gamete's surface can affect the binding process. Here we assume codominance: a heterozygote genotype produces gametes with equal proportions of different proteins on the surface. Different architectures could affect the quantitative as well as the qualitative expression of fertilization proteins on a gamete's surface. Complete dominance would cancel diploid expression. Other architectures may be possible, but they are not considered here.

Additionally, depending on the spatial arrangement of the recognition proteins on the membrane, different binding modes could be possible. If receptors are packed densely enough, a spermatozoan might hit several receptors simultaneously, and binding would always occur with the ligand-receptor pair with the highest affinity. If receptors are more sparsely packed, then a spermatozoan would bind with the first receptor with which it came in contact. In this latter case, we would expect binding between ligand-receptor pairs to be directly proportional to their frequencies. We model this last possibility; thus, if an egg expresses two different receptors and comes in contact with sperm that expresses only one ligand, we assume that

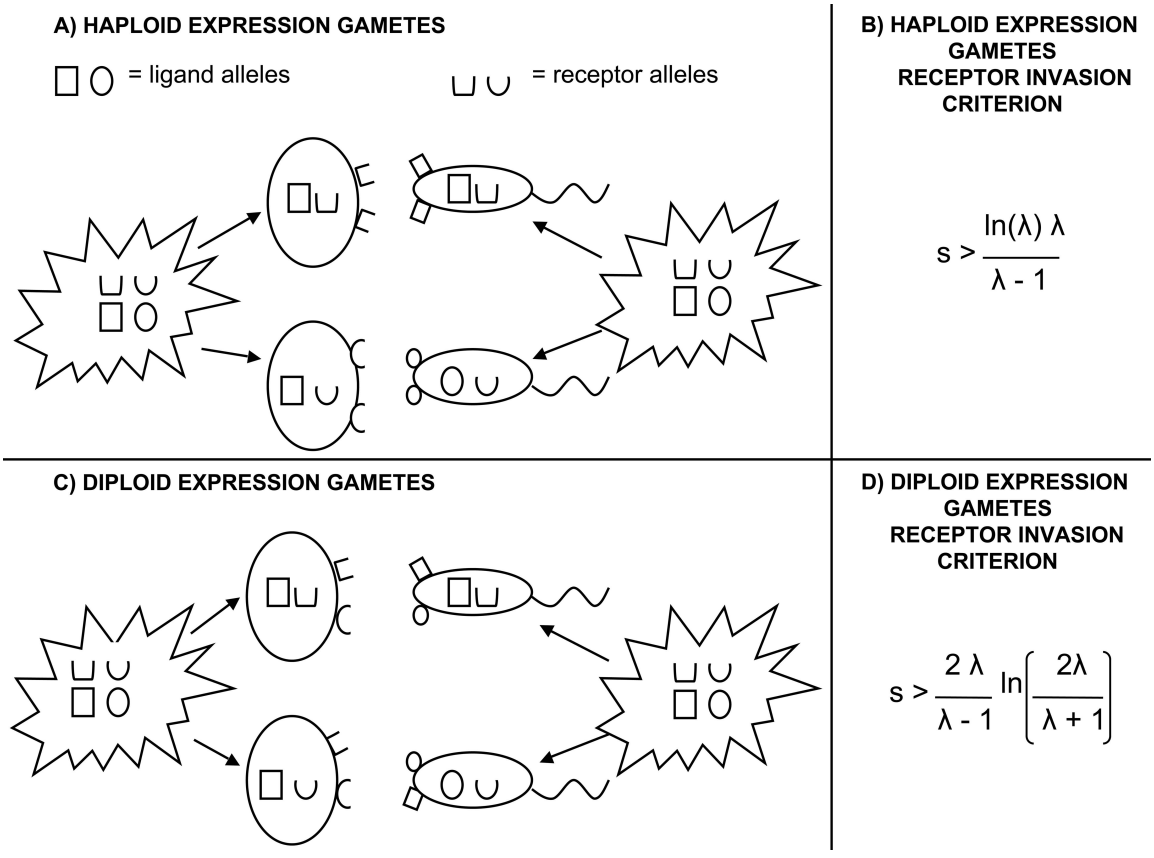

Figure 1: Model differences of haploid-expression and diploid-expression gametes. *A*, *C*, Graphic representation of potential differences between haploid- and diploid-expression gametes at fertilization. Two identical heterozygote parents produce two types of gametes that have identical genotypes but different phenotypes. *B*, *D*, Corresponding analytical invasion criterion for a receptor in a population that is monomorphic at the ligand locus.

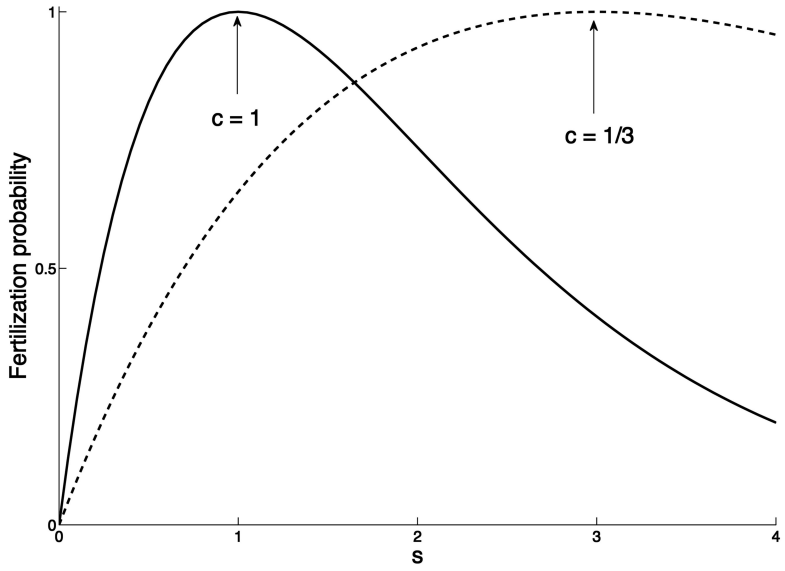

Figure 2: Fertilization probability. The *X*-axis shows the value of sperm abundance; the *Y*-axis shows the fertilization probability. The solid and dashed curves illustrate how fertilization success depends on the value of sperm abundance and that of bindin affinity between ligand-receptor pairs, *c*.

half of the sperm will bind to each kind of receptor at a rate predicted by their individual affinities.

The Model

Consider a large, constant population of N diploid broadcast spawners with an equal sex ratio. Population regulation occurs during recruitment to the adult population. We examine the interaction of one ligand protein (on the sperm) and one receptor protein (on the surface of the egg). We assume that ligands and receptors are each controlled by a single locus with two alleles. Ligands are not expressed on eggs and receptors are not expressed on sperm. Alleles at the sperm ligand locus are denoted L_j, with frequencies y_j, and alleles at the receptor locus are denoted R_i, with frequencies x_i. The bindin affinity, or compatibility, between a receptor i and a ligand j is denoted c_{ij}. Similarly, the frequency of haploid-expression gametes with receptor i and ligand j is denoted z_{ij}. Recombination rate, denoted r, is constant and free (i.e., $r = 0.5$).

Fertilization Probability

We define successful fertilization as the fusion between a single spermatozoan and an egg. We show the case for gametes with haploid expression (the diploid case is presented in appendix A in the online edition of the *American Naturalist*). The probability of fertilization of an egg expressing receptor i initially increases with increasing sperm abundance (positive density dependence), it reaches a maximum, and then it decreases as sperm becomes overabundant (negative density dependence). Each egg can come in contact with sperm from one or more males, depending on the population's spawning behavior. The effective sperm abundance contributed by a male releasing sperm that express ligand j, s_j, to an egg with receptor i is a function of its local abundance and compatibility with that female (i.e., $s_j c_{ij}$). Thus, the probability that an egg expressing receptor i (R_i) will be fertilized is a function of all the effective sperm contributions from all the males that will attempt to fertilize. As in Tomaiuolo et al. (2007), we use

$$w(R_i) = \beta\Big(\sum_j s_j c_{ij}\Big) \exp\Big[-\Big(\sum_j s_j c_{ij}\Big)\Big], \tag{1}$$

where β is a rescaling constant that sets the maximum of the fertilization probability at 1 (i.e., 100% fertilization) and s_j describes the amount of sperm released by a male on some scale. $\sum_j s_j c_{ij} < 1$ indicates sperm limitation; conversely, $\sum_j s_j c_{ij} > 1$ indicates sperm overabundance (fig. 2). More complex fertilization functions that consider polyspermy exist (Styan 1998); although these are more satisfying from a mechanistic perspective, they are also less tractable and do not substantially increase the fit to empirical data (Tomaiuolo et al. 2007).

Recursive Equations

We start by considering the probability that a sperm expressing ligand l fertilizes an egg. For simplicity, we consider that all males release the same amount of sperm (i.e., $s_i = s$). Following this assumption, the frequency of ligand k is

$$y_k = \sum_i z_{ik}.$$

We can then write the sum of all effective sperm contributions as

$$\sum_j s_j c_{ij} = s \sum_m y_m c_{im}.$$

The probability that a male gamete z_{kl}, expressing ligand l, will outcompete other sperm in a fertilization is equal to the frequency of the male gamete multiplied by its compatibility with the receptor i (c_{il}), divided by the sum of all gamete frequencies multiplied by their respective compatibilities with the same receptor:

$$w(z_{kl}|R_i) = \frac{z_{kl}c_{il}}{\sum_m y_m c_{im}}. \tag{2}$$

The new frequency of gametes z_{ij} produced by a female gamete z_{ij} fertilized by a male gamete z_{kl} will be

$$\frac{1}{2}(1 - r)z_{ij}w(R_i)w(z_{kl}|R_i)$$

$$= \frac{\beta s}{2}(1 - r)z_{ij}z_{kl}c_{il}\exp\left[-s\left(\sum_p y_p c_{ip}\right)\right].$$

By applying similar arguments to all other combinations, we can express the recursive equations for gametes in the following way:

$$z'_{ij} = \frac{1}{\overline{W}}\sum_{k,l}\left[\frac{1}{2}(1 - r)z_{ij}z_{kl}(w_{il} + w_{kj}) + \frac{1}{2}rz_{il}z_{kj}(w_{ij} + w_{kl})\right], \tag{3}$$

where

$$w_{il} = \beta s\exp\left[-s\left(\sum_m y_m c_{im}\right)\right]c_{il},$$

and where

$$\overline{W} = \sum_{i,j,k,l} z_{ij}z_{kl}w_{il}.$$

Using this notation, we recover the model introduced by Haygood (2004). The factor 1/2 appears because gametes z_{ij} and z_{kl} can combine at fertilization in two different ways, with different fertilization probabilities. The first possibility is that an egg that expresses receptor i (and carries ligand j) combines with a sperm that expresses ligand l (and carries receptor k) with fertilization probability w_{il}. The other possibility is that an egg expressing receptor k (and carrying ligand j) combines with a sperm expressing ligand j (and carrying receptor i) with fertilization probability w_{kj}. Each of these combinations will result in an individual that will release z_{ij} gametes. Numerical simulations were implemented using a program written in Java (code available from M. Tomaiuolo).

Results

Invasion Criteria

Haploid-Expression Gametes. We start with the invasion criterion for a rare mutant at the receptor locus in a monomorphic population in the competitive-spawning scenario. We define a ligand-receptor pair with the same subscript—for instance, L_1 and R_1—as "matching alleles." We define parameter λ as the ratio between the compatibility of the matching alleles (c_{ii}) and the compatibility of mismatching alleles (c_{ij}), thus, $\lambda = c_{ii}/c_{ij}$. This parameter describes the factor by which the bindin affinity between a ligand-receptor pair is different from another reference pair. We consider only the case where the resident ligand-receptor pair has a better affinity (i.e., $\lambda > 1$). We then ask how sperm abundance, s, and the relation between bindin affinities, λ, affect the invasion of a rare mutant. In appendix B in the online edition of the *American Naturalist*, we show how invasion of a rare mutant receptor occurs if the inequality shown in figure 1*B* is satisfied.

As sperm abundance increases, the reproductive output of the resident allele decreases because of negative density dependence. The poorer fit of the mutant receptor with the resident ligand allows the mutant eggs to be less affected by polyspermy, thereby resulting in higher fertilization success. The dynamics of invasion depend on sperm abundance and how different the mutant receptor is (fig. 3*A*). A mutant receptor that is similar to the resident one (i.e., λ is slightly larger than 1) will require lower levels of negative density dependence to invade compared with a mutant receptor that is very different from the resident one (i.e., $\lambda \gg 1$). On the other hand, if a population is under high levels of negative density dependence, a mu-

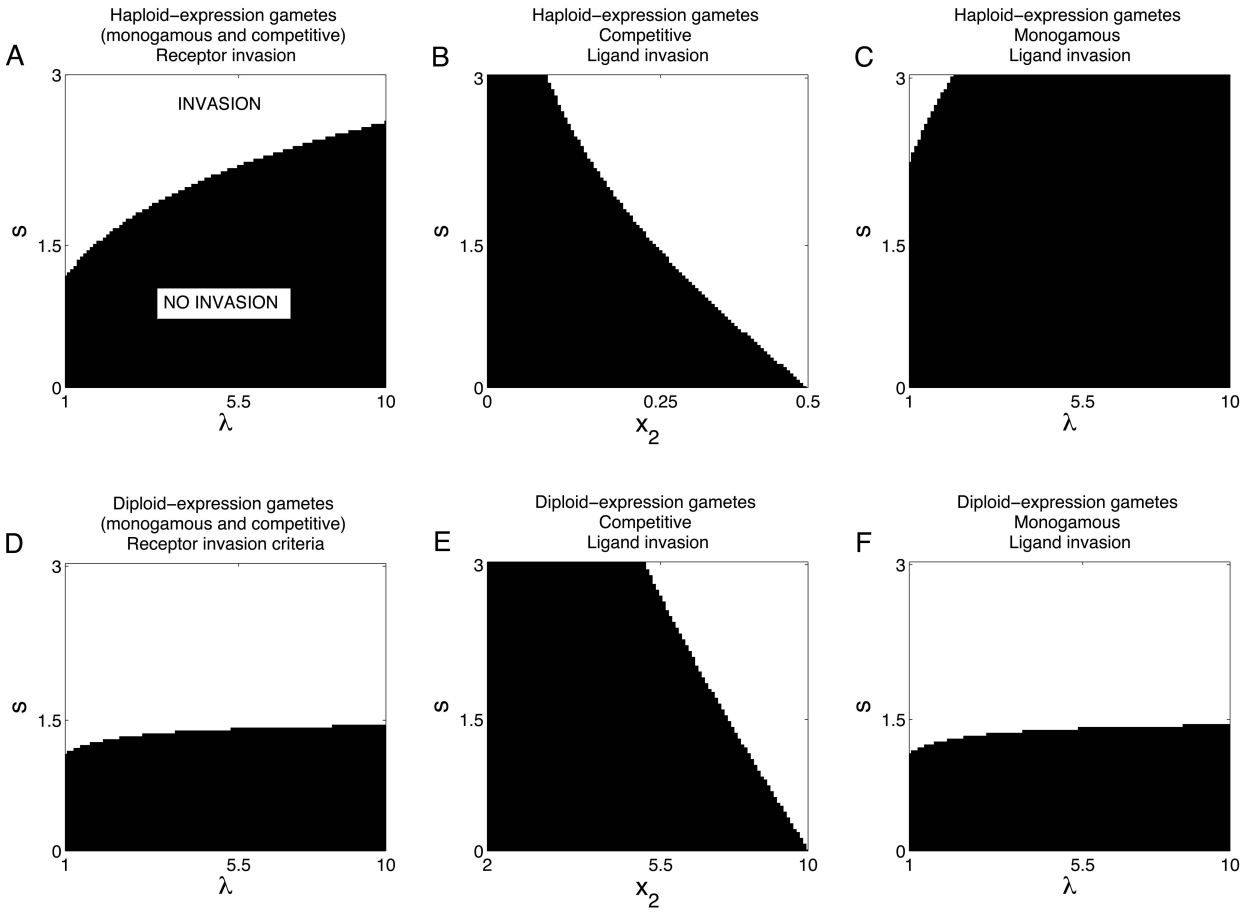

Figure 3: Invasion regions. For all panels, the black areas indicate combinations of parameters where the value of Δx_2 (i.e., the frequency of the rare mutant) after one generation is negative (white areas are positive). *A–C*, Haploid-expression gametes model. *D–F*, Diploid-expression gametes model. Parameter values are as follows: $c_{11} = c_{22} = 1$ and $c_{12} = c_{21} = 1/\lambda$. In *A*, *C*, *D*, and *F*, the *X*-axis shows increasing difference between resident and mutant bindin affinity, λ, and the *Y*-axis shows sperm abundance, *s*. In *B* and *E*, the *X*-axis shows the frequency of the receptor allele, x_2, which matches the rare mutant, and the *Y*-axis shows sperm abundance, *s*. *A*, As the difference in bindin affinity increases, so too does the level of polyspermy required for invasion to be possible. *B*, The invasion of a rare ligand allele is dependent on the frequency of the corresponding matching receptor allele on the level of negative density dependence. *C*, Under monogamous spawning, a ligand can invade without a matching receptor, but higher levels of polyspermy are required. *D*, Much lower levels of polyspermy are required for invasion to be possible; moreover, the conditions become less dependent on the difference in bindin affinity. *E*, A ligand can invade only if a corresponding receptor is present and requires higher levels of polyspermy as compared with the haploid case. *F*, The conditions for ligand invasion under monogamous spawning are identical to those of a receptor.

tation leading to a receptor that is very different from the resident one will be more favored.

We now look at the invasion dynamics of a rare mutant at the ligand locus in a population where two receptor alleles are present that have frequencies x_1 and x_2, respectively. We assume that the resident ligand is a good match with one of the receptors. A rare ligand mutant that appears can either worsen or improve the fit with one of the receptors. If the fit with either of the receptors is not improved compared with that of the resident ligand, then the mutant will not be able to invade because it would be a poor competitor. Because of this, we introduce only a ligand that has higher affinity with one of the receptors in the population. In appendix B, we show that invasion occurs if the following inequality is satisfied:

$$x_2 > \frac{1}{1 + \exp\left[s(1 - 1/\lambda)\right]}. \tag{4}$$

Equation (4) reveals that if the matching receptor is not present ($x_2 = 0$), then invasion is not possible. The invasion of a ligand mutant depends on the combination of

sperm abundance, the frequency of a matching receptor allele, and the degree of mismatch λ. Increased sperm abundance *s* reduces the frequency of the receptor allele at which invasion is possible (fig. 3*B*).

Assuming monogamous spawning, the invasion criterion for a rare mutant at the receptor locus is identical to that of the competitive case (app. B; figs. 1*B*, 3*A*). The case for a rare mutant ligand allele is different because of the absence of sperm competition (app. B). Invasion does not require a matching receptor; it only requires abundant sperm such that novel ligands, with lower binding affinities, avoid polyspermy and have greater reproductive output than the resident ligands (fig. 3*C*). In summary, spawning behavior affects the invasion conditions of a ligand but not those of a receptor.

Diploid-Expression Gametes. We use the same simplifying assumptions of the haploid case. Invasion of a rare mutant at the receptor locus, under competitive spawning, is possible if the inequality shown in fig. 1*D* is satisfied (app. A). For diploids, lower sperm abundance is required for invasion (cf. fig. 3*D* with fig. 3*A*). The biological reason for this is as follows: Assume that a rare mutant appears at some female receptor locus in a population under negative density dependence. The individual carrying the mutation will produce eggs that either carry the mutation or do not but that will express both receptors. These eggs will still be able to match well with the resident ligand, and they will be favored because the mutant receptor will decrease the effective sperm abundance and, thus, polyspermy. In gametes that have haploid expression, this twofold advantage is not possible.

The invasion conditions for a rare mutant ligand allele under competitive spawning were investigated using numerical simulations (fig. 3*E*). As was the case for haploid-expression gametes, invasion is not possible without a matching receptor allele. If such an allele is present, however, the invasion conditions are more restrictive as compared with the haploid-expression case. This happens because, with diploid expression, the effective sperm concentration is reduced for genotypes that are heterozygous at the receptor locus, thus increasing the amount of sperm abundance required for ligand invasion.

Assuming monogamous spawning (app. A), the invasion condition of a rare receptor allele is equal to the competitive-spawning case (figs. 1*D*, 3*D*). The invasion conditions for a rare mutant ligand allele are less restrictive as compared with those for the haploid-expression case (cf. fig. 3*F* with fig. 3*C*). Here the sperm from the heterozygous individual have the same phenotype, and half of them carry the mutant ligand. Under moderate conditions of sperm abundance, they all reduce the effective sperm concentration, increasing the reproductive success of the mating pair and thereby allowing invasion.

Equilibrium Allelic Frequencies

We start by assuming symmetrical compatibilities between ligand-receptor pairs (i.e., $c_{ii} = 1$, $c_{ij} = 1/\lambda$), and we investigate the effects of sperm abundance and difference in compatibilities. Because of the underlying symmetry in the model, if a polymorphism exists where all alleles are present, their frequencies will all be 0.5. All simulations started with the same initial conditions ($x_1 = y_1 = 0.999$) and were continued until frequency changes were small (i.e., $x_i(t + 1) - x_i(t) < 1 \times 10^{-9}$). In all cases considered, regions of polymorphism were found (fig. 4).

We treat the monogamous case first, for both haploid- and diploid-expression gametes (fig. 4*A* and 4*C*, respectively). If invasion is possible, then two outcomes can follow. If sperm abundance is moderate compared with the difference in compatibility (i.e., λ), then a polymorphism is possible (white area in fig. 4*A*, 4*C*). If sperm abundance is high, then the mating pair involving the mismatching homozygous individuals has the highest reproductive output, and those alleles will go to fixation (upper black area in fig. 4*A*, 4*C*). It is interesting to note that when the difference in compatibility is low (i.e., λ is slightly larger than 1) and sperm abundance is moderate, the haploid case allows for a polymorphism while the diploid case does not. This happens because, with diploid-expression gametes, higher levels of sperm abundance are required to allow for ligand invasion.

Assuming competitive spawning, both haploid and diploid cases provide conditions for a polymorphism if invasion is possible (fig. 4*B*, 4*D*). There are two main differences. First, the diploid case allows for invasion and polymorphism at lower levels of sperm abundance. Second, for moderate levels of sperm abundance, the ligand allele will not be able to invade and variation will be maintained only at the receptor locus (fig. 4*D*, *gray area*).

We now investigate equilibrium frequencies generated by different combinations of bindin affinities under competitive spawning and constant sperm overabundance. We present the results generated by the haploid-expression model, but the same conclusions apply to the diploid case.

If two alleles at each locus are present, then there are four possible bindin affinities: c_{11}, c_{12}, c_{21}, and c_{22}. We distinguish among some broad categories. For instance, there could be equal matching between corresponding alleles ($c_{11} = c_{22}$) but different matching values for their reciprocal combinations. We assume that crosses between males and females with matching alleles (i.e., L_i, R_i) always have higher affinity than do crosses between mismatching alleles (i.e., L_i, R_j). This means that the inequality $c_{ii} > c_{ij}$ is sat-

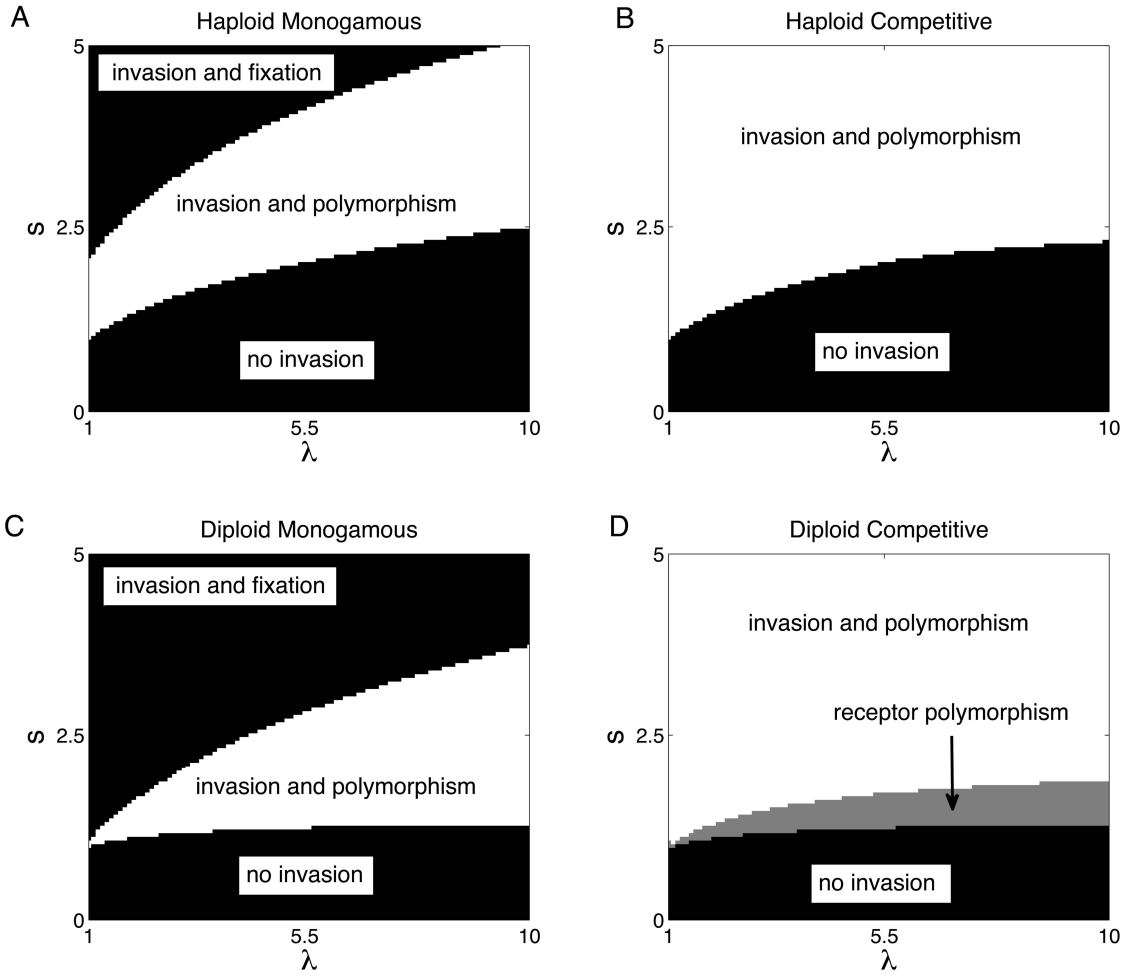

Figure 4: Polymorphism regions. For all panels, the black areas indicate combinations of parameters where two alleles are present (one per locus), gray shading indicates three alleles, and the white areas indicate four alleles. The *X*-axis shows increasing difference between resident and mutant bindin affinity, λ, and the *Y*-axis shows sperm abundance, *s*.

isfied. We used four fixed values of bindin affinities ($c_{ii} = \{0.6, 0.8\}$; $c_{ij} = \{0.2, 0.4\}$) to illustrate how the resulting allelic equilibrium frequencies can cover the full spectrum of frequency distributions (fig. 5). When $c_{11} = c_{22}$ ($c_{12} = c_{21}$), the value 0.8 (0.2) was used; using the alternate values (0.6 and 0.4, respectively) affects the results quantitatively but not qualitatively. In the top left box of figure 5, for instance, the values of the bindin affinities are $c_{22} = 0.8$, $c_{11} = 0.6$, $c_{21} = 0.4$, and $c_{12} = 0.2$. The resulting allelic frequencies at equilibrium will be symmetric for receptors but skewed for ligands. In this example, one of the ligands (L_2) has the highest affinity with its matching receptor (R_2) and the lowest affinity with its mismatching receptor (R_1). This makes L_1 more of a generalist and L_2 more of a specialist. If we reverse the mismatching relations from the previous example (i.e., $c_{12} = 0.4$, $c_{21} = 0.2$), R_1 becomes the generalist competitor. The resulting equilibrium distribution (top right box in fig. 5) is skewed for receptor alleles and symmetric for ligands. These results illustrate that if the compatibilities are asymmetric, the generalist-recognition protein (be it a receptor or a ligand) is favored if sperm are overabundant. Similar arguments can be applied to obtain the results shown in the other boxes in figure 5.

These results also suggest that matched proteins with lower affinities will be maintained at equal or higher frequencies than matched alleles with higher affinities. Depending on the affinities between mismatched alleles, this will be true for the ligand, the receptor, or both proteins. Higher-affinity matches, because they are more susceptible

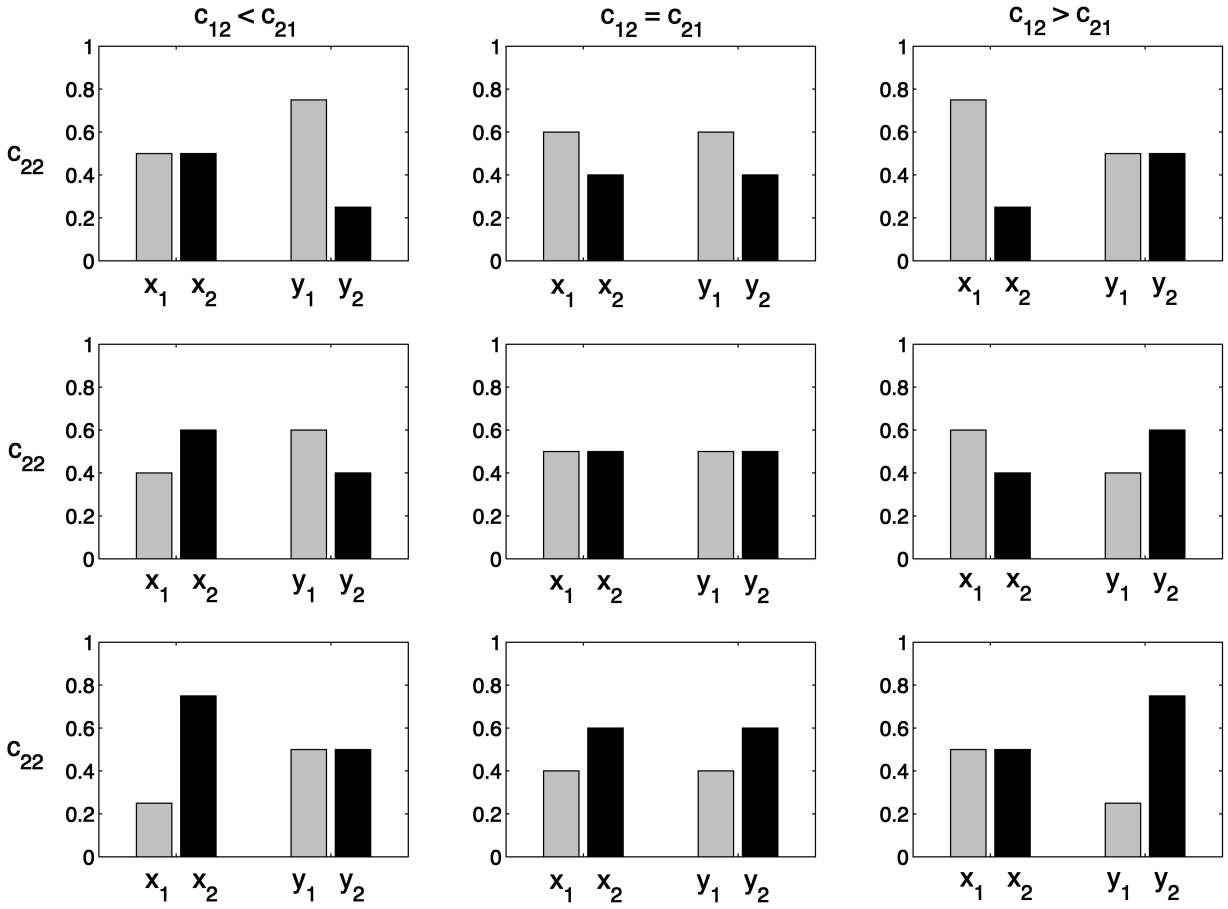

Figure 5: Equilibrium frequencies under competitive spawning. Sperm abundance is kept constant ($s = 5$). The leftmost bars in each box represent the equilibrium frequencies of the receptor alleles, denoted x_i (ligand alleles are denoted y_j). Binding affinities of matching alleles are represented by c_{11} and c_{22} (mismatching alleles are c_{12} and c_{21}). We used $c_{ii} = \{0.8, 0.6\}$ and $c_{ij} = \{0.4, 0.2\}$ to generate all the combinations. Relations among bindin affinities can generate a full range of frequency distributions, and asymmetries in bindin affinities can generate asymmetries in allelic equilibrium frequencies.

to polyspermy, are never maintained at frequencies higher than those of lower-affinity matches. This negative frequency-dependent selection sets the stage for the maintenance of polymorphisms under varying spawning conditions.

Gametic Disequilibrium

We now examine patterns of gametic disequilibrium, a measure of statistical association between loci. We present results obtained from numerical simulations of the model with symmetric compatibilities (i.e., $c_{ii} = 1$, $c_{ij} = 1/\lambda$). With this parameterization, if a polymorphism exists it will have equal allele frequencies at both loci (i.e., $x_i = y_i = 0.5$; see center box in fig. 5).

We begin with the monogamous case. We illustrate the effects of sperm abundance and the degrees of difference in affinities (fig. 6). Figure 6*A* illustrates the resulting levels of gametic disequilibrium in the region of parameter space where a polymorphism is possible. In this scenario, the level of gametic disequilibrium is always rather weak, but it attains its largest values at the lowest level of sperm abundance where a polymorphism is possible and for the largest difference in compatibility. It then decreases as sperm abundance increases or as the compatibility difference decreases, until it becomes 0 when the parameter combinations do not allow for a polymorphism to be maintained.

In monogamous spawning for diploid-expression gametes (fig. 6*C*), the corresponding level of gametic disequilibrium is very weak. In this case, however, gametic disequilibrium can be negative. In competitive spawning

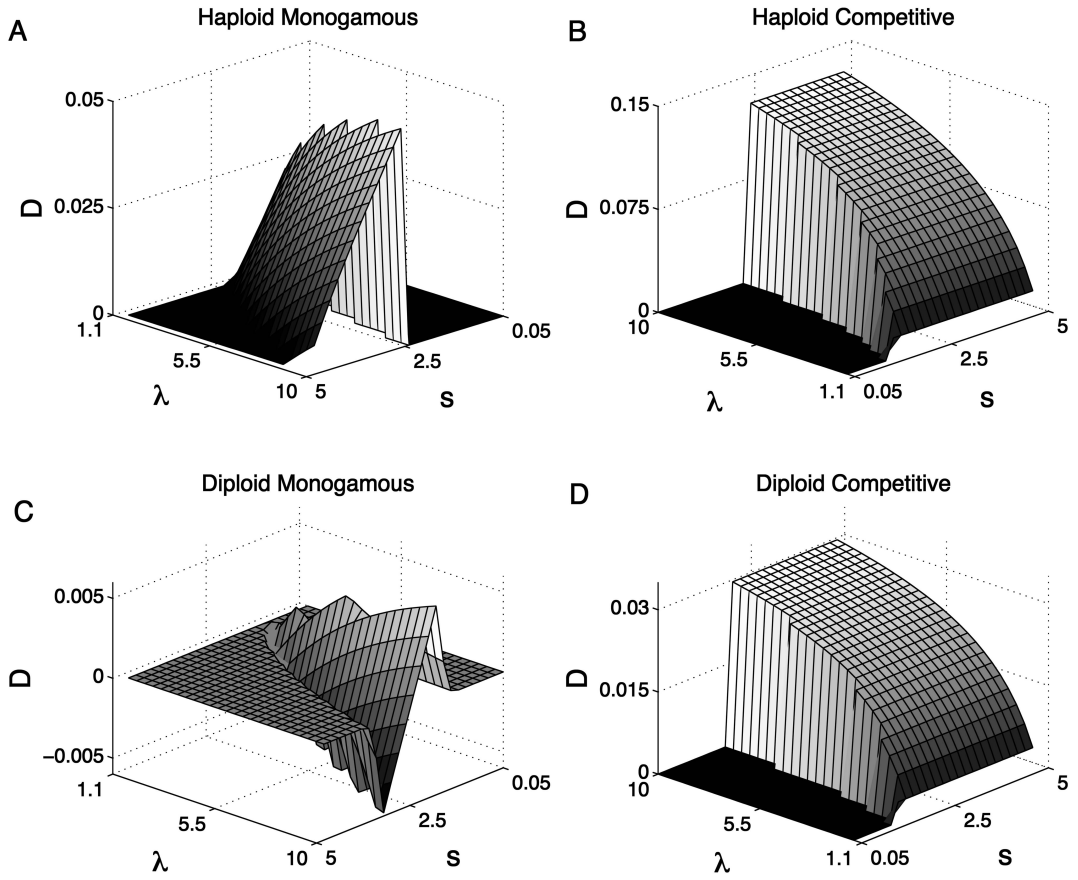

Figure 6: Patterns of gametic disequilibrium. For all panels, the *X*- and *Z*-axes show increasing difference between resident and mutant bindin affinity, λ, and sperm abundance, *s*. The *Y*-axis shows the resulting levels of gametic disequilibrium, computed as $D = z_{11}z_{22} - z_{12}z_{21}$.

with haploid-expression gametes (fig. 6*B*), the resulting degree of gametic disequilibrium depends only on the difference in bindin affinities, λ. As the difference in affinities increases, so too does the degree of gametic disequilibrium. The pattern of gametic disequilibrium produced is always positive because competition favors the association of matching ligand-receptor pairs.

Assuming competitive spawning for diploid-expression gametes, the patterns of gametic disequilibrium are qualitatively similar to the haploid case but lower in magnitude (fig. 6*D*). As for the haploid-expression model, the level of gametic disequilibrium is a function only of the difference in compatibilities. The presence of different receptors (ligands) on the surface of the egg (sperm) reduces assortative mating. Gametes produced by a heterozygote parent, for instance, can bind in more ways with their relative counterparts. This in turn weakens the association of ligand-receptor pairs and the corresponding level of gametic disequilibrium. In summary, the patterns of gametic disequilibrium can be very different, both qualitatively and quantitatively, depending on underlying biological and ecological factors.

Discussion

We considered a model to investigate possible outcomes of sexual conflict arising in broadcast-spawning taxa. Such conflict is generated by sperm availability and egg susceptibility to polyspermy. We examined the effects of reproductive behavior, sperm availability, and gamete affinities on the evolution of egg- and sperm-recognition proteins. We considered the dynamics generated assuming haploid and diploid expression in two different ecological scenarios, monogamous spawning and competitive spawning. In all cases, we found conditions that allow invasion of a rare mutant (fig. 3) and polymorphism maintenance (fig. 4). At equilibrium, allelic frequencies can vary widely, depending on the relative affinities of the different ligand

alleles with their best-matched receptor alleles and their mismatched receptor alleles. In general, under conditions of sperm overabundance, alleles with lower affinities are maintained at higher frequencies and exhibit negative frequency dependence (fig. 5). Whenever polymorphism of both is maintained, gametic disequilibrium between sperm- and egg-recognition proteins is predicted (fig. 6).

Within these general results, interesting differences emerge on the basis of reproductive behavior, sperm availability, and patterns of expression. Diploid expression requires lower sperm concentrations for invasion and maintenance of polymorphism, longer times are required to reach equilibrium (results not shown), and lower levels of linkage disequilibrium emerge when compared with haploid expression. Monogamous spawning leads to a window of sperm concentrations and gametic affinities that result in stable polymorphism bounded by conditions of low sperm availability where invasion is not possible and high levels of sperm availability where successful invaders sweep through the population. Gametic disequilibrium is stronger if gametes have haploid expression, and it is almost always positive when best-matched proteins are associated, with the exception of the combination of diploid expression and monogamous spawning, where weak positive or negative linkage disequilibrium can emerge (fig. 6). In practice, the linkage disequilibrium sign is arbitrary if the real biological matches are not known.

Populations living at sparse densities are expected to express less variation in gamete-recognition proteins. The reason can be either that females are sperm limited or that generally only one male is close to a particular female and gets the majority share of paternity (approaching monogamous spawning). Sperm overabundance can decrease a population's reproductive output because of polyspermy. Under such conditions, allelic variation would counter the effects of polyspermy because more alleles translate into fewer eggs experiencing polyspermy. Even though we explored only situations with two alleles, biological intuition would predict the number of alleles maintained by selection to be positively correlated with the levels of polyspermy. Multiple alleles or asymmetries in the compatibilities between ligands and receptors should reduce the ability to statistically detect gametic disequilibrium.

We modeled how polyspermy can be avoided indirectly via a poor ligand-receptor fit, but it can also be avoided through other mechanisms. It is possible that eggs become more resistant to polyspermy independently of recognition proteins, for example, by producing more efficient or faster blocks. Spawning time may shift to reduce the effects of polyspermy (Tomaiuolo et al. 2007). Another viable option would be to make smaller eggs (Styan 1998; Franke et al. 2002; Levitan et al. 2007). If eggs are resistant to polyspermy for reasons other than variation in gamete-recognition proteins, and if they are not sperm limited, a functional rare-mutant receptor would neither be favored nor selected against. Male gametes, on the other hand, would still compete at fertilization to increase their paternity share. This would probably produce neutral variation at the receptor locus and suppress variation at the ligand locus.

Under most conditions it appears that, for males, being common is good (with the exception of polyspermic monogamous situations). This raises the following question: is a specific ligand common because it is good (i.e., affinity relations), or is it good because it is common (i.e., positive frequency dependence)? The results we present suggest that these options are not mutually exclusive. A ligand can be common because it is favored through binding at fertilization or because the demographic history of sperm availability did not allow other ligands to be favored.

The model predictions provide some insight into empirical observations for two congeneric sea urchin species, *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. In both species, polymorphism is noted in the sperm-bindin protein and female reproductive success can be predicted by the sperm-bindin genotype (Levitan and Ferrell 2006; Levitan and Stapper 2010), which is consistent with the model predictions under sperm overabundance. The species normally found at sparser densities has fewer sperm-bindin alleles and is subject to polyspermy at lower densities and lower sperm concentrations compared with the more aggregated species. In both species, rare male genotypes have poor reproductive success, but there are a small number of common alleles with higher reproductive success that appear to be maintained by frequency-dependent selection. When the sparser species is subject to high densities, females with rare genotypes avoid polyspermy because they mismatch with the pool of common male genotypes (Levitan and Ferrell 2006). The aggregated species is more resistant to polyspermy, and two common forms of the protein appear to be maintained by negative frequency-dependent selection (Levitan and Stapper 2010). While these patterns support model predictions, a rigorous test of this model requires actual measurements of gametic disequilibrium between sperm bindin and the egg receptor, as has been noted in abalone (Clark et al. 2009). These predictions illustrate how genetic variance within loci and associations between loci can be established through subtle changes in reproductive behavior and demography.

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