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Sperm competition and female avoidance of polyspermy mediated by sperm–egg biochemistry

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ABSTRACT

Nearly simultaneous fertilization of an egg by two or more sperm (polyspermy) is a lethal condition in most organisms. Sperm competing for an egg face opposing selective pressures. The race to fertilize favours rapid penetration of the egg's outer protective layer; a close finish between two sperm leads to polyspermy and death. Under most conditions of sperm competition, selection favours maximal speed of penetration by sperm in spite of potentially significant mortality imposed on both sperm and eggs. Eggs, in response to sperm competition and polyspermy, are favoured to increase the difference in arrival times between competing sperm. I model this sperm–sperm–egg conflict to study the population genetic consequences of polyspermy. To separate sperm arrival times, selection typically favours polymorphism of egg characters that influence the rate of passage by sperm through the egg's outer protective layer. In response to diverse eggs, the population of sperm characters may be favoured to diversify in a matching way or to stabilize at a point that maximizes average penetration speed. Divergence of reproductive characters by sexual selection is frequently cited as a potentially important factor in reproductive isolation and speciation. The biochemistry of fertilization characters provides a useful model system to study these processes.

Keywords: co-evolution, fertilization, sexual selection, speciation.

INTRODUCTION

The competition among numerous sperm to fertilize relatively few eggs creates intense conflicts (Trivers, 1972). Males sometimes harm females during the competition to fertilize. For example, a newly dominant male lion often kills the cubs sired by prior males, causing the females to start their next reproductive cycle more quickly (Bertram, 1975; Packer and Pusey, 1983). Such overt male–male combat and male–female conflict have been widely documented (Darwin, 1871; Andersson, 1994).

Attention has recently turned to conflicts mediated by the biochemistry of mating fluids and gametes. In *Drosophila melanogaster*, Harshman and Prout (1994) showed that the seminal fluid of spermless males reduces the number of progeny sired by a previously

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mating male. Clark and Begun (1998), also studying *D. melanogaster*, demonstrated that female genotype influences the relative success of males in sperm competition.

Rice's (1996) experiment on *D. melanogaster* suggests that male–male competition mediated by mating products can strongly reduce female success. Rice's design effectively prevented females from evolving during 41 generations, while males evolved in response to this constant 'female environment'. The evolved males reduced female survivorship by increased toxicity of seminal fluid and by influencing the females to re-mate more frequently. Females, when allowed to co-evolve with males, appear able to evolve resistance to novel male toxicities.

These experiments indicate that selection of traits mediating male–male–female conflict may be intense and that evolutionary change in these traits may be very rapid (Rice and Holland, 1997). Co-evolution of male–male–female conflict may maintain polymorphism within species. Rapid divergence of traits influencing fertilization may also affect reproductive isolation.

I develop a model to examine the population genetics of such conflicts. The lack of detailed information about biochemical mechanisms that mediate conflict limits the scope of any modelling efforts at this time. I have chosen the problem of sperm–egg interactions over polyspermy because this system allows one to develop plausible assumptions about the mechanisms that mediate conflict (Rice and Holland, 1997).

POLYSPERMY

Nearly simultaneous fertilization of an egg by two or more sperm (polyspermy) is a lethal condition in most organisms (Gilbert, 1997). The eggs of many species deploy various blocks to polyspermy that prevent additional fertilizations. For example, sea urchin eggs express a 'fast block' within a few seconds after the first sperm penetrates the egg's inner membrane. The fast block results from a temporary switch in the polarization of the membrane; this block is effective for approximately 1 min and apparently cannot be maintained for longer. At approximately the time when the fast block decays, sea urchin eggs express a 'slow block'. This slow block is mediated by a physical separation between the egg's inner membrane and an intermediate protective layer, the vitelline envelope.

The details of slow and fast blocks appear to vary widely among groups of organisms. The fast block in ascidians has been estimated at approximately 21 s (Lambert and Lambert, 1981); in other groups, the existence of any fast blocks has been questioned (Byrd and Collins, 1975; Jaffe and Gould, 1985).

The frequency of polyspermy in natural fertilizations has received little attention. In a survey of a free-spawning brown alga, the frequency of polyspermy ranged from 1 to 9% over different locations and breeding periods (Brawley, 1992). These significant frequencies suggest that polyspermy can be a powerful selective force shaping the properties of sperm–egg interactions.

The structure of eggs and the details of sperm–egg interactions vary widely among taxa (Gilbert, 1997). For example, sea urchin eggs are covered with a thick, protective layer of jelly. The sperm, upon contact with this outer jelly layer, release special enzymes that digest a path through the jelly coat. The interaction between the sperm and the jelly layer is species-specific (Gilbert, 1997). The sperm passes through the jelly to the egg's surface (vitelline layer). Once at the vitelline layer, further sperm–egg interactions determine whether the sperm passes through to the egg's inner membrane and subsequent fertilization.

These interactions include a species-specific recognition between sperm and egg proteins (Vacquier, 1998).

Mammalian eggs differ significantly from those of sea urchins. Because fertilization is internal, the sperm must first pass through various storage and flow pathways to arrive at the egg. Hunter (1996) has argued that mammalian females use various steps to regulate the arrival of sperm at the egg and thereby reduce the risks of polyspermy. These steps include regulation of binding and release of sperm in temporary storage regions and control of sperm passage through the layer of cumulus cells that surround the egg. Once sperm have passed through these many potential controls, they arrive at the outer egg surface, called the zona pellucida in mammals. Here, tight binding of sperm and possible passage to the egg's final protective membrane is roughly analogous to sperm interactions with the vitelline layer of sea urchin eggs.

Although the details of fertilization vary widely among taxa, the same general steps are followed in each case. First, the sperm traverse the outer protective layers or potential regulatory controls. This passage is often mediated by highly specific interactions between the sperm surface and the egg's jelly coat or female reproductive tract. Next, the sperm arrive at the protective layer just above the egg's inner membrane, for example the vitelline layer or the zona pellucida. Tight binding of sperm to specific egg receptors is required for passage through to the inner membrane and subsequent fertilization.

There are many close interactions between the sperm surface and the egg or female reproductive tract. These interactions may allow the female to influence the numbers of sperm arriving at the egg's membrane and the timing of arrival. In particular, females are favoured to avoid simultaneous fertilization and polyspermy. Sperm face competing selective pressures. On the one hand, winning the race to fertilize is required for the success of a particular sperm; on the other, simultaneous fertilization kills both the sperm and the egg.

I model the competing selective pressures on males and females as follows. I assume that two sperm arrive within T time units at the egg's outer protective layer. In sea urchins, the protective layer is the egg's jelly; in mammals, the protective controls include the various steps of sperm binding and passage towards the egg. For convenience, I use the sea urchin as a model and refer to sperm passage as boring through the egg's outer protective layer.

Interaction between a sperm character and an egg character determines the average passage of time of the sperm to the egg's inner membrane. The egg is favoured to reduce the probability of polyspermy by maximizing the expected difference in arrival times of the two sperm at the inner membrane. The sperm are favoured to win the race to fertilize, subject to losses that may be incurred by polyspermy.

SPERM–SPERM–EGG CONFLICT

In this section, I derive expressions for the fitnesses of two sperm and one egg that interact during fertilization. The egg has two barriers, an outer protective layer through which the sperm must bore and an inner membrane. The first sperm to arrive at the inner membrane wins the race to fertilize. Define $t = T + t_2 - t_1$ as the time difference between the arrival of the two sperm at the inner membrane. The term t_1 is the time to bore to the inner membrane for the first sperm to arrive at the outer layer. The term t_2 is the time to bore to the inner membrane for the second sperm to arrive at the outer layer. The term T is the arrival time of the second sperm at the outer layer relative to the first arrival.

If the two sperm attach to the egg's inner membrane within τ time units of each other, $|t| < \tau$, then the egg dies as a result of polyspermy and all fitnesses are zero. Otherwise, the egg and the winning sperm receive one fitness unit and the losing sperm receives zero.

For the first sperm to arrive at the outer protective layer, the time t_1 to bore through the protective layer and arrive at the inner membrane depends on the sperm's boring character, x, and the egg's physical structure in its protective layer, z. For the second sperm to arrive at the outer protective layer, boring time t_2 depends on the sperm's character, y, and the egg's character, z.

Define the probability distribution of t, the difference in arrival times at the inner membrane, as $f(t; x, y, z)$. The time difference depends on the boring characters of the first and second sperm, x and y, and the physical structure of the egg's protective layer, z.

The fitness of an egg with character z depends on the characters of the two sperm that attach to the outer protective layer of the egg. Let x be the character of the first arriving sperm. With probability r the second sperm also has character x, and with probability $1 - r$ the second sperm has character y uncorrelated with character x of the first sperm. Thus female fitness is

$$\phi(x, y, z) = 1 - \int_{-\tau}^{\tau} [rf(t; x, x, z) + (1 - r)f(t; x, y, z)]\, \mathrm{d}t \tag{1}$$

which is one minus the probability of death caused by polyspermy. Death occurs when the two sperm arrive within τ time units of each other, that is $|t| < \tau$, as expressed in the integral.

The term r is the kin selection coefficient of relatedness and comes into play as follows. With probability r the first and second sperm have the same allele determining their characters and hence the same character value, and with probability $1 - r$ the characters are determined by uncorrelated genotypes. In this case, r can be interpreted as the probability that alleles are identical by descent from a recent ancestor. For example, if both sperm come from a single diploid male who was produced by random mating, then $r = 1/2$ of the sperm have the same allele by descent from a recent common ancestor and $1 - r = 1/2$ of the sperm have uncorrelated alleles. If the sperm are chosen randomly from a pool produced by two males, then $r = 1/4$. I assume here that the sperm and egg characters are determined by their allele at their own haploid locus rather than by the diploid somatic genotype. This is realistic for some organisms (e.g. brown algae) but unrealistic for others (e.g. mammals). I summarize data on genetic control of gametic characters in a later section.

The fitness of a sperm with character v depends on the character, z, of the egg to which the sperm attaches and the character of the other sperm with which it competes. The competitor sperm is identical, having character v, with probability r, and is uncorrelated, having character w, with probability $1 - r$. Thus male fitness is

$$\mu(v, w, z) = \int_{\tau}^{\infty} [rf(t; v, v, z) + (1 - r)f(t; v, w, z)]\, \mathrm{d}t + \int_{-\infty}^{-\tau} [rf(t; v, v, z) + (1 - r)f(t; w, v, z)]\, \mathrm{d}t \tag{2}$$

Any particular sperm has an equal chance of being the first or second to arrive at the outer protective layer of the egg. The first integral assumes that the focal sperm, with character v, is first to arrive at the outer protective layer of the egg. In this case, the focal sperm wins the

race to fertilize if $t > \tau$; that is, the focal sperm arrives first at the inner membrane and the difference in arrival time between sperm is greater than τ. This first integral is divided into the case in which the characters of the first and second sperm are identical by descent from a recent common ancestor and the case in which the sperm are genetically uncorrelated.

The second integral assumes that the focal sperm, with character v, is second to arrive at the outer protective layer. The second sperm wins the race if $t < -\tau$; that is, the second sperm wins the race to the inner membrane by at least τ time units. The second integral is also divided into correlated and uncorrelated cases. Note that the factor $f(t; w, v, z)$ arises because, in that case, the second sperm has character v and the first sperm has character w.

ASSUMPTIONS ABOUT PROCESS

Let the time, T, between the arrival at the egg's outer layer of the second sperm relative to the first sperm have a normal distribution with mean m_T and variance σ_T. Note that normal variables may have negative values. The interpretation of a negative time, T, is that the sperm labelled as the first actually arrives second. For this particular problem, all times are relative measures and thus negative values pose no analytical problems. A similar interpretation arises for other time variables that take on negative values. In spite of this notational complication, I chose normal variables because they provide a reasonable description for many processes, analytical power, and the ability to control independently the mean and variance of the process. A common alternative approach for time-dependent processes uses exponential distributions and queuing theory (Feller, 1971).

The time, t_1, for the first-arriving sperm to bore through the outer layer to the inner membrane depends on the sperm's boring character, x, and the egg's physical structure in its outer protective layer, z. This time, t_1, follows a normal distribution with mean m_1 and variance σ_1, in which these parameters are functions of the match between x and z. Similarly, the time, t_2, for the second-arriving sperm to bore to the egg's inner membrane follows a normal distribution with mean m_2 and variance σ_2, in which these parameters are functions of the match between y and z.

Fitnesses depend on the difference in the arrival times at the egg's inner membrane between the first and second sperm, given above as $t = T + t_2 - t_1$. Based on the assumption that the three time variables are independent random processes, standard manipulation of normal variables leads to the distribution $f(t; x, y, z)$ as normal with mean $m = m_T + m_2 - m_1$ and variance $\sigma = \sigma_T + \sigma_1 + \sigma_2$.

I assume that the mean boring times from outer to inner membranes have a variable component that depends on the phenotypic match between sperm and egg plus an independent, fixed component. In particular, $m_1 = K + \alpha_1$ and $m_2 = K + \alpha_2$, where K is the fixed component and α is the variable component, such that

$$\alpha_1 = s|z - x|^a \tag{3}$$

$$\alpha_2 = s|z - y|^a \tag{4}$$

The values for the variable component of boring time depend on a scale parameter, s; the distance between the egg character, z, and the sperm character x or y; and the exponent a. The character values are all normalized to the interval [0, 1]. I assume that the variance

of boring time is equal to the part of the mean that depends on phenotypic interaction, thus $\sigma_1 = \alpha_1$ and $\sigma_2 = \alpha_2$. The value of a determines whether boring time increases at a decelerating ($a < 1$) or accelerating ($a > 1$) rate with the distance between sperm and egg characters.

Based on these assumptions, the mean time difference in the arrival of the two sperm at the egg's inner membrane is $m = m_T + \alpha_2 - \alpha_1$, and the variance is $\sigma = m_T + \alpha_1 + \alpha_2$. Note that the part of the mean boring times that is independent of phenotype, K, does not enter into the distribution of t. Thus we can, without loss of generality, set $K = 0$, and set $\alpha_1 = m_1$ and $\alpha_2 = m_2$. I do this for notational convenience because it allows me to refer to the mean boring times, m, rather than the portion of the mean boring times that depends on phenotype, α.

Figure 1 shows the shape of $f(t; x, y, z)$ for two parameter combinations, given that the sperm have the same character, $x = y$. The shaded area is the region of polyspermy, in which $|t| < \tau$. In this figure and all cases that follow, I assume that the minimum time separation required to avoid polyspermy is $\tau = 0.125$. I also assume that the average time between arrivals of the first and second sperm at the egg's outer protective layer is $m_T = 1$. These assumptions focus attention on the remaining parameters that control sperm–egg interaction and the difference in boring time between polymorphic sperm – in particular, the scale parameter, s, the exponent a, and the relatedness between sperm, r.

Figure 2 shows that the frequency of polyspermy declines as the difference in average boring times between the sperm increases.

EVOLUTION OF DIVERSITY

On the male side, selection favours sperm to minimize boring time and win the race to the egg's inner membrane. Sperm minimize boring time by matching their boring characters to the protective structural characters of the egg's outer layer. On the female side, selection favours eggs to increase the average difference in arrival times of the first and second sperm.

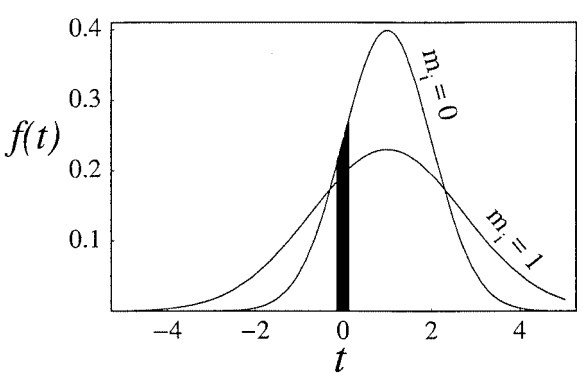

Fig. 1. Probability distribution of t, the time difference between the arrivals of the two sperm at the inner egg membrane. Here the two sperm have the same character, $x = y$. In the tall curve, both sperm match the egg perfectly with $m_1 = m_2 = 0$. In the wide curve, the average boring times caused by sperm–egg mismatch are $m_1 = m_2 = 1$. As noted in the text, I assume $\tau = 0.125$ for these plots and all other analyses. The probability of polyspermy, in which $|t| < \tau$, is 0.060 for $m_i = 0$ and 0.049 for $m_i = 1$. This comparison shows that, when the sperm are the same, sperm–egg mismatch by itself causes only a modest decline in the expected frequency of polyspermy.

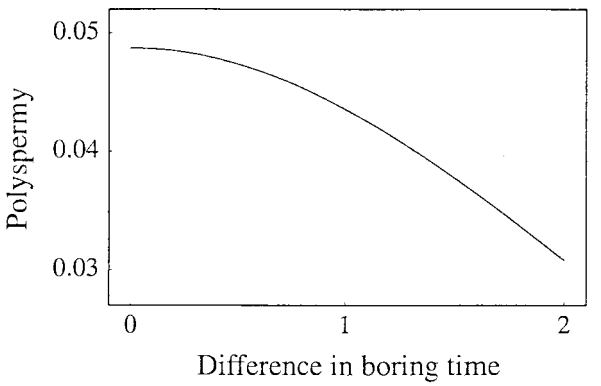

Fig. 2. Probability of polyspermy as a function of the difference in average boring times for the two sperm, $m_2 - m_1$. In this case, the sum of the average boring times is held constant at $m_1 + m_2 = 2$.

If the difference in arrival times is too short, then the egg is at risk of death by polyspermy. Eggs may increase the difference in arrival times by increasing the difference in boring speed between the sperm.

The sperm gain by matching the egg; the egg gains by matching some sperm better than others. Thus selection diversifies egg characters. Diversified eggs favour either matching diversification of sperm or a tendency of the sperm towards the centre of the egg's distribution of characters. Whether selection diversifies or stabilizes the sperm distribution depends on the exponent parameter a in a manner very similar to generic host–parasite models (Frank, 1993). I demonstrate this in the following analysis.

This verbal introduction sets the stage for the analysis and results, but should be taken only as a rough, intuitive guide. For example, sperm and egg interests are not completely opposed. Polyspermy not only destroys the egg, but the sperm as well. Thus selection favours sperm to win the race to fertilize but to avoid arriving too closely to opponents. In most cases, the race to fertilize dominates.

I analyse the evolution of diversity by studying the dynamics of sperm and egg characters. I assume that character values of both sperm and egg vary over the interval [0, 1], where x and y are the characters of the first and second sperm to arrive at the egg's outer protective layer, and z is the character of the egg's protective layer. To follow dynamics, I split the [0, 1] interval into $N + 1$ equally spaced values. Thus

$$x = i/N$$

$$y = j/N$$

$$z = k/N$$

$$i, j, k = 0, 1, \ldots, N$$

Let the frequency of sperm characters be p_i for $x = i/N$ or p_j for $y = j/N$. Similarly, let the frequency of egg characters be q_k for $z = k/N$. Next, use the fitness definition for eggs in equation (1) to abbreviate $\phi(x, y, z) = \phi(i/N, j/N, k/N)$ as ϕ_{ijk}. Similarly, use the definition for the fitness of sperm in equation (2) to abbreviate $\mu(v, w, z) = \mu(i/N, j/N, k/N)$ as μ_{ijk}. I assume $N = 50$ in the following analyses.

The complete set of recurrence relations for the dynamics of character frequencies can now be written as

$$\bar{\mu} p'_i = p_i \sum_j \sum_k p_j q_k \mu_{ijk}$$

$$\bar{\phi} q'_k = q_k \sum_i \sum_j p_i p_j \phi_{ijk}$$

where all sums run from 0 to N. The mean fitnesses $\bar{\mu}$ and $\bar{\phi}$ are defined so that frequencies always sum to one; that is, $\Sigma p'_i = 1$ and $\Sigma q'_k = 1$.

The fitnesses depend on various time processes, which in turn depend on the specific parameters defined above. In particular, I have set for all runs the window of polyspermy as $\tau = 0.125$ and the average time between the sperms' arrivals at the egg's outer protective layer as $m_T = 1$. I examine the role of relatedness, r, and the two key parameters that determine how sperm–egg match influences boring time, the scale, s, and the exponent, a.

To determine how these parameters influence diversity of sperm and egg characters, I iterated the system of recurrence equations until an equilibrium was achieved. For some parameters, there were many locally stable but globally unstable equilibria. For the first 100,000 iterates, I continued to perturb the system occasionally until the system could not be invaded by any mutant and the maximum change of new versus old frequency divided by old frequency, for any frequency greater than 10^{-4}, was less than 5×10^{-5}. For most parameters, the system converged by these criteria before the first 100,000 iterates.

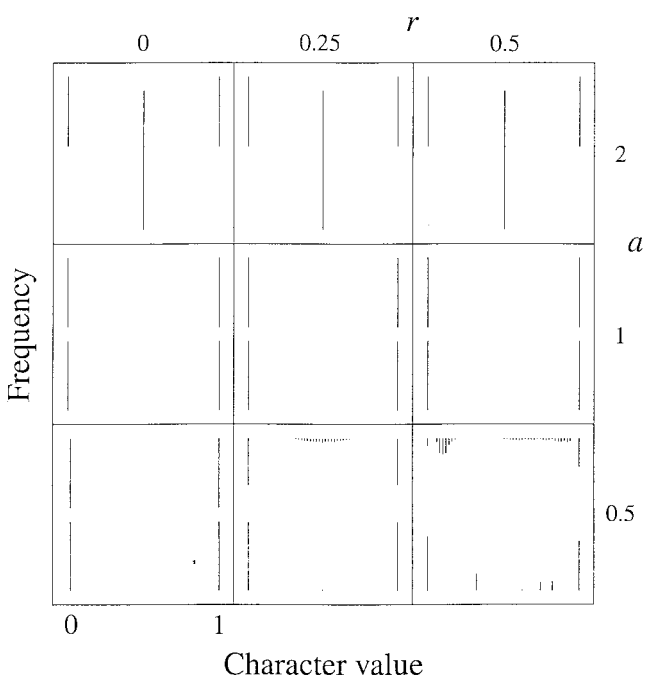

Fig. 3. Matching frequency distributions of sperm and egg character values. For all cases shown here, $\tau = 0.125$, $m_T = 1$ and $s = 1$.

If not, then I ran the system for another 100,000 iterates without perturbation and used the final iterate as the approximate equilibrium state.

Figure 3 shows the results for different values of relatedness, r, and the exponent, a, that control the effect of the sperm–egg match on boring time of the sperm. In each panel, the character value is shown on the horizontal axis ranging over the interval [0, 1]. The height shows the frequency of each particular character value. The lines starting at the bottom are the frequencies of each sperm character – in the lower-left panel, the lines moving up from the bottom reach a height that corresponds to a frequency of 0.5. The lines starting at the top are the frequencies of each egg character – in the lower-left panel, the lines moving down from the top reach a depth that corresponds to a frequency of 0.5.

Three conclusions can be drawn from Fig. 3. First, all cases show strong diversification of egg characters; typically, there is an even split between the extreme character values. Second, the sperm also tend to split between extreme character values when the exponent, a, is 0.5 or 1, but sperm converge to a middle value of 0.5 when the exponent is 2. Third, when the exponent is 0.5, increasing relatedness causes some spreading of character values and weak convergence to equilibrium. On the whole, relatedness values of $r \leq 0.5$ have relatively little effect.

Figure 4 explores the parameter space for different values of the exponent, a, and the scale, s. Most cases show a split in egg characters. Sperm characters split for exponents of 0.5 and 1, but converge for an exponent of 2. When the scale, s, is small and the exponent is 0.5 (left column, lower panels), convergence towards equilibrium is weak because sperm–egg interactions have relatively little effect on boring time.

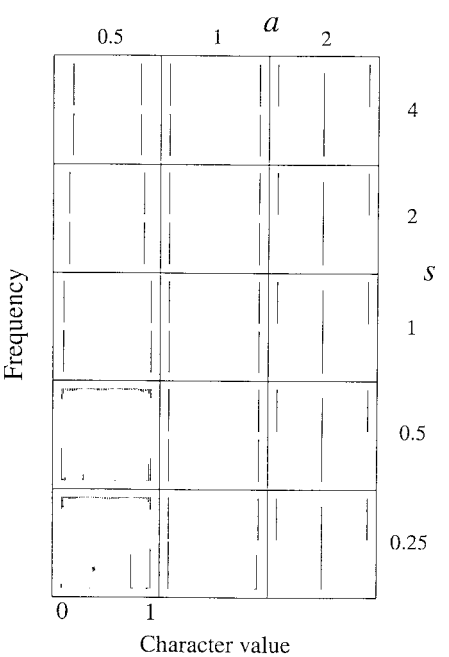

Fig. 4. Matching frequency distributions of sperm and egg character values. For all cases shown here, $\tau = 0.125$, $m_T = 1$ and $r = 0$.

GENETIC CONTROL OF GAMETIC CHARACTERS

I assumed that the gamete's haploid genotype controls its characters. This assumption is realistic for brown algae, in which the haploid gametophytic stage of the life cycle produces the gametes. Brown algae provide the only well-studied case of polyspermy in natural populations (Brawley, 1992), as mentioned in the review I provided earlier in this paper. One study on the colonial ascidian *Botryllus primigenus* suggested that the parental diploid genotype determines egg characters, whereas the sperm's own haploid genotype determines its recognition properties. However, studies on other colonial ascidians has provided contradictory results (see Grosberg, 1988, for a review of data on ascidians).

I did not find any mention of polyspermy in angiosperms. It is unclear whether polyspermy does not have an adverse effect on fertilization success or simply has not been studied because of technical difficulties. Pollen-style interactions in angiosperms may be influenced by sperm–sperm–egg conflicts (Mulcahy, 1979). A few studies have found that style genotype promotes or inhibits pollen tube growth rate from various donors because of genetic interactions between pollen and style (reviewed by Delph and Havens, 1998). The models presented above may be modified to study conflicts in seed plants. In this case, the 'egg surface' would have components from the diploid (sporophytic) style and the haploid (gametophytic) tissue surrounding the egg. The 'sperm surface' would be represented by the pollen characters. Some pollen characters are controlled by the diploid (sporophytic) tissue, but, in contrast to many animals, the haploid pollen also determines many of its own characters (reviewed by Delph and Havens, 1998).

Sperm and egg characters in insects and mammals are probably determined by the parental diploid genotype (Haig and Bergstrom, 1995). The control of gene expression in mammalian sperm is particularly interesting (Nayernia *et al.*, 1996). Several key sperm genes are expressed after meiosis by the haploid sperm. But during this stage of haploid gene expression, each sperm remains linked by cytoplasmic bridges to the other sperm cells in the meiotic lineage. The RNA transcripts appear to diffuse among sperm cells, leading to diploid expression of characters (reviewed by Hecht, 1993; Miller, 1997). Haig and Bergstrom (1995) argued that diploid expression in sperm is imposed by males to reduce potential conflicts among sperm.

Multicellular aggregations of developing sperm cells apparently occur in all mammals, but the details vary in interesting ways. For example, proacrosin is expressed during meiosis in mice, whereas proacrosin is expressed post-meiotically in rats, boars and bulls (reviewed by Miller, 1997). Proacrosin plays an important role in surface reactions during attachment and penetration of the egg. At present, there is no evidence to suggest that the variable timing of expression is important for sperm characters. But as more comparative evidence accumulates, the details of gene expression and control of sperm characters may prove to be an interesting problem in light of the potentially strong evolutionary conflicts in sperm–sperm–egg interactions.

I did not find any clear statements about mode of genetic control in other taxa. The patterns may vary in interesting ways. Evolutionary dynamics of sperm–sperm–egg conflict depend on the details of genetic control. The simple haploid models here only hint at the range of possible interactions.

DISCUSSION

The model shows that sperm competition and the risk of polyspermy can create strong diversifying selection on egg characters. In response to diversified eggs, the sperm may either diversify in a matching way or stabilize to intermediate characters. Sperm diversify when there is a linear ($a = 1$) or decelerating ($a = 0.5$) relation between sperm–egg match distance and the boring speed of sperm. Stabilizing selection occurs when the time for sperm-boring increases at an accelerating rate with match distance between sperm and egg ($a = 2$). These patterns of polymorphism are consistent with a generic co-evolutionary model of attack and defence in which phenotypic match distance determines the success of attack (Frank, 1993).

Clark and Begun's (1998) study and Rice's (1996) experiments, summarized in the Introduction, suggest that some fertilization characters may be polymorphic within populations. Metz and Palumbi (1996) found extensive genetic polymorphism within species of sea urchins for a sperm protein, bindin, used in binding to eggs. The variation within species did not appear to affect the ability of sperm to fertilize conspecific eggs, although no data were collected on sperm success under competitive circumstances. In contrast, the strong genetic divergence of bindin among closely related species does appear to act as a reproductive barrier – bindin attached to a heterospecific egg fails to elicit the required response for fertilization.

Species recognition probably plays an important role in the divergence of fertilization characters among species of free-spawning marine organisms (Vacquier, 1998). The reasons for the observed polymorphism of bindin within species of sea urchins is less clear. Such polymorphisms may be neutral if they do not interfere with fertilization, or they may be influenced by various forms of sexual selection (Metz and Palumbi, 1996). Recently, Swanson and Vacquier (1998) have shown that the egg receptor for sperm lysin varies among species of abalone, but appears to be homogenized within species by a mechanism such as gene conversion or unequal crossing over. Clearly, not all fertilization characters will be polymorphic within species.

Other studies have documented rapid divergence of fertilization characters among species, without providing data on variation within species. Eberhard (1996) summarized several reports on divergence of seminal products among various arthropods; Foltz (1995) reported preliminary evidence on divergence of proteins that mediate binding of mammalian sperm to the zona pellucida of conspecific eggs. The importance of species recognition is unclear in these groups with internal fertilization, in which heterospecific matings may occur but are likely to be rare.

The apparently widespread tendency of fertilization characters to diverge rapidly suggests that, in addition to species recognition, various forms of male–male and male–female conflict may be important for some traits (Eberhard, 1996; Rice and Holland, 1997). If mating conflicts promote variation in fertilization characters within species, then variation in reproductive compatibility within populations could initiate reproductive isolation (Rice and Holland, 1997).

Many authors have suggested that sexual selection may be important in causing rapid divergence among species and subsequent reproductive isolation (e.g. West-Eberhard, 1983; Wu and Davis, 1993). The biochemistry of fertilization characters appears to be an excellent system for pursuing these ideas.

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