

Sperm Competition and the Evolution of Ejaculate Composition

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ABSTRACT: We present a model of sperm competition that incorporates both sperm and nonsperm parts of the ejaculate. Our primary focus is on determining how ejaculate composition and size evolves as a function of the effects of seminal fluid on male reproductive success and as a function of asymmetry in sperm usage by females. The model predicts that different patterns of investment in sperm and seminal products are expected to evolve as a function of the bias in sperm usage by females. It also predicts the evolution of distinct patterns in ejaculate composition depending on the function of seminal fluid. In the discussion, we highlight a number of potential approaches for testing the theory that we develop.

Keywords: sperm precedence, male-male competition, seminal fluid, multiple mating.

Sperm competition is competition among the ejaculates of different males for fertilization of a given set of female ova; it is equivalent to interejaculate competition (Parker 1970, 1998). This phenomenon has formed the focus of a number of theoretical analyses aimed at understanding the evolution of male ejaculate expenditure strategies over the past decade. However, although models of sperm competition have explored ejaculate allocation patterns under a variety of conditions, they have typically been concerned only with the sperm-containing portion of the ejaculate

(e.g., Parker 1984, 1990*a*, 1990*b*, 2000; Parker et al. 1996; Ball and Parker 1998, 2000, 2003; Mesterton-Gibbons 1999*a*, 1999*b*; Greef and Parker 2000; Williams et al. 2005; but see Kura and Yoda 2001).

In many species, substances other than sperm make up a substantial portion of the ejaculate transferred to females during copulation (Eberhard and Cordero 1995). For example, sperm constitutes only 1%–5% of the total volume of human ejaculate (Mortimer 1994), and the spermatophylax (a sperm-free mass that attaches to the sperm-containing portion of the spermatophore) can represent up to 22% of the male bush cricket's body mass (Vahed and Gilbert 1996). The chemical content of this nonsperm portion of the ejaculate is typically complex, containing numerous substances with a wide variety of actions (Simmons 2001). The actions of some of these substances are concerned directly with sperm, including effects on sperm survival, probability of fertilization, and sperm transport (Eberhard and Cordero 1995). Seminal product substances are also known to influence important physiological and behavioral responses in females (Leopold 1976; Chen 1984; Eberhard 1996; Simmons 2001).

The effects of ejaculatory proteins on female reproduction are beginning to be documented in a number of animals (Eberhard 1996; Simmons 2001; Wolfner 2002; Arnqvist and Rowe 2005). In insects, evidence for the role of seminal products as fecundity-enhancing substances is particularly convincing (Gillot 2003). Ejaculatory products identified as having stimulatory effects on female egg laying include substances such as juvenile hormone transferred in lepidopteran seminal fluid (e.g., Webster and Carde 1984; Ramaswamy et al. 1997; Park et al. 1998) and the accessory gland proteins (Acp 26Aa and Acp 70A) found in *Drosophila* ejaculates (Chapman 2001). In other taxa, seminal products are known to have important effects on patterns of female sperm utilization (Simmons 2001). For example, Sakaluk (1986) has argued that male fertilization success in the cricket *Gryllodes supplicans* is proportional to the size of the spermatophylax transferred. Similarly, Sumption (1961) has suggested that the large volume of seminal fluid transferred during copulation in

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agricultural swine may function to swamp out the sperm of previous males, providing the last male with a reproductive advantage (sperm flushing; Simmons 2001). Thus, even though non-sperm-containing elements cannot have any direct role in fertilization, they clearly do influence the fertilization success of competing males and thereby the outcome of sperm competition.

Here we examine the evolution of male sperm investment strategies when the whole of the ejaculate is considered. We model sperm competition by partitioning the male ejaculate into two parts: a sperm-containing and a non-sperm-containing component (sperm and seminal fluid). We assume that sperm compete numerically for fertilization of eggs to be oviposited by a female and that seminal fluids affect the rate of female oviposition. Under these conditions, investment in sperm affects the proportion of a female's offspring sired by a male, and investment in seminal fluid affects the number of offspring produced by that female. We seek the evolutionarily stable strategy (ESS) level of energy investment in the ejaculate, including expenditure on both the sperm-containing and non-sperm-containing parts. We then extend this model to consider how other potential functions of seminal fluid are expected to affect investments.

The Model

The model developed below is meant to be an abstraction of the general kinds of trade-offs thought to be important in the evolution of male ejaculates rather than a model for a particular species. Most of the available empirical data on ejaculates comes from insects, however; therefore, many of our assumptions are motivated by this group of organisms. Nevertheless, our primary goal when choosing various assumptions was to develop a model that is as close as possible to previous theory on sperm competition to facilitate clear comparisons with this previous work.

We assume that mating in the population occurs during two independent reproductive bouts (i.e., there are two temporally discrete breeding events). Male ejaculates compete within a reproductive bout but do not compete across bouts. Such a scenario is easy to imagine when breeding is seasonal and females produce broods during two different seasons; we might also imagine a case where two breeding bouts are temporally separated within a season. We model the probability that a male survives from one bout to the next as a decreasing function of his ejaculate expenditure during the current bout. This reflects the fact that ejaculate production can be costly to males (Van Voorhies 1992; Olson et al. 1997; Simmons 2001; Wedell et al. 2002). Since reproduction is costly, a male must therefore weigh the value of current matings against the value of potential future matings. Although we include two repro-

ductive bouts in our model, we focus only on the evolution of male ejaculate characteristics in the first bout and treat ejaculate characteristics in the second bout as parameters. Indeed, the "second bout" could be considered to consist of multiple bouts. It is only essential to our model that decisions in the first bout affect subsequent fitness through the costs of reproduction. Consequently, only the investment strategy in the first reproductive bout evolves in our model.

For simplicity, we suppose that all females mate exactly two times during a reproductive bout. Males, on the other hand, mate on average $2N_f^{(1)}/N_m^{(1)}$ times, where $N_f^{(1)}$ and $N_m^{(1)}$ represent the numbers of females and males present during the first bout, respectively. We assume that the population structure is such that a male never mates with any given female more than one time. For all numerical examples presented, we use the sex ratio $N_f^{(1)}/N_m^{(1)} = 1/2$ because at this ratio all equilibria are convergent stable fitness maximums (Taylor 1989; Christiansen 1991; Otto and Day 2007; E. Cameron and T. Day, unpublished data). Similar results can be derived with other sex ratios, but some parameter values then also permit the evolution of a dimorphism in male reproductive strategies (E. Cameron and T. Day, unpublished data). The analysis of these cases is outside the scope of this article.

We assume that all male ejaculates enter into competition and that competition always occurs between the ejaculates of two males. Similar to previous models of sperm competition, males in our model occupy one of two mating roles (favored or disfavored), and they have full information about the roles they occupy. Ejaculate allocation strategies are thus role dependent. We assign male roles randomly by assuming that the number of matings a male achieves in a given role is a binomial random variable with parameters $1/2$ and $2N_f^{(1)}/N_m^{(1)}$. The amount of energy invested in the sperm-containing and non-sperm-containing portions of the ejaculate is denoted by s and b , respectively, with favored males having ejaculate strategy (s, b_1) and disfavored males having strategy (s_2, b_2) . We follow Parker (1990a, 1990b) by using the sperm discounting parameter r to ensure that sperm compete according to an unfair raffle. The parameter r may take any value between 0 and 1 so that the disfavored male's sperm are worth only r of the favored male's sperm. It is this discounting parameter that defines the difference between favored and disfavored males.

Different male roles may arise as a result of differences in the order or timing of mating. In *Drosophila*, for example, males mating first have a disadvantage in fertilization relative to later-mating males (there is high last-male sperm precedence). Thus, in this group, the first male to copulate occupies the disfavored role, and the last male to copulate occupies the favored role. We assume that the

probability that a male occupies either of these roles is independent of his role during any previous matings; all males are always equally likely to occupy either the favored or disfavored roles, regardless of their roles during previous matings. Thus, we assume that role is not dependent on some property of that male (e.g., condition or size). Additionally, males have plasticity in their ejaculate allocation strategies between these two roles so that a single male can play two strategies: one in the favored role and one in the disfavored role. There is evidence in some insects that males alter their ejaculate transfer depending on female mating history (Simmons 2001; Wedell et al. 2002)

Females in our model always mate once with a male in each of these two roles. We assume that the size of a female's clutch, F , is a function of the total amount of male seminal products she receives, $F(b_1 + b_2)$. This function increases monotonically with diminishing returns in a graded, dose-dependent manner (e.g., Riemann and Thorson 1969; Destephano and Brady 1977; Murtaugh and Denlinger 1985). Although there is little direct evidence that the gonadotropic effects of ejaculates are dose dependent, there is some indirect evidence in insects suggesting that this is so (Arnqvist and Rowe 2005).

Now let us first consider the fitness of a mutant male at the first reproductive bout in the context of a resident male population. After some simplification (see app. A), we find that the expected male fitness at the first bout can be described by

$$2 \frac{N_f^{(1)}}{N_m^{(1)}} \left[\frac{1}{2} F(b_1 + \hat{b}_2) \frac{s_1}{s_1 + r\hat{s}_2} + \frac{1}{2} F(\hat{b}_1 + b_2) \frac{rs_2}{\hat{s}_1 + rs_2} \right], \quad (1)$$

where (\hat{s}_1, \hat{b}_1) and (\hat{s}_2, \hat{b}_2) denote the population-wide investment strategies and (s_1, b_1) and (s_2, b_2) denote the mutant investment strategies for each of the two roles. The first factor in expression (1) represents the number of matings a male achieves, on average, and the expression in the parentheses represents the average value he receives for each of these matings; the first term inside the parentheses corresponds to male fitness in the favored role and the second term to the disfavored role. For example, to calculate the first term, consider a mutant male in the first (favored) role. The mutant male will transfer b_1 seminal fluid to the female while its competitor (who will be using the resident strategy) transfers \hat{b}_2 . This results in the female producing a total of $F(b_1 + \hat{b}_2)$ offspring. However, only a fraction $s_1/(s_1 + r\hat{s}_2)$ of these will be sired by the mutant male, since it transfers s_1 sperm while its competitor transfers \hat{s}_2 sperm (which are then discounted by the factor r since its competitor is in the disfavored role). This yields the first term in the brackets of equation (1). Analogous considerations can be used to arrive at the second term,

where the mutant male is then in the second (disfavored) role.

The probability that a male survives to compete in the second reproductive bout depends on both the number of matings he achieved at the first bout and his average ejaculate expenditure for each mating in that bout. We let the decreasing function $\beta(s + b)$ represent the probability that a male with ejaculate investment strategy (s, b) survives a single mating at the first bout; a male must survive all such matings in order to compete in the second bout. We assume that the survival cost a male incurs with mating increases as the male's ejaculate expenditure increases. In order to determine the probability that a male mates during the second reproductive bout, we must account for the probability distribution of investment strategies a male might use in the first bout, factoring in the randomness of roles and number of matings that the male might have had. Again, after some simplification (see app. A), we find that a male's probability of survival to the second bout is approximately

$$\left[\frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2} \right]^{\frac{2N_f^{(1)}}{N_m^{(1)}}}. \quad (2)$$

Given that a male survives to the second bout, the number of matings he can then expect to have is

$$\frac{2N_f^{(2)}}{N_m^{(2)}} = \frac{Q}{\left[\frac{\beta(\hat{s}_1 + \hat{b}_1) + \beta(\hat{s}_2 + \hat{b}_2)}{2} \right]^{(2N_f^{(1)}/N_m^{(1)})}} \frac{2N_f^{(1)}}{N_m^{(1)}}, \quad (3)$$

where Q is the probability that a female survives from the first to the second reproductive bout and $N_f^{(2)}$ and $N_m^{(2)}$ represent the numbers of females and males present at the second reproductive bout.

We denote the ejaculate expenditure of favored and disfavored males during the second bout of mating by $(s_{1,2}, b_{1,2})$ and $(s_{2,2}, b_{2,2})$, respectively; recall that investment strategies in each of the two roles in this second reproductive bout are treated as parameters. Each mating achieved by a male during the second reproductive bout brings a payoff of

$$\frac{1}{2} F(b_{1,2} + b_{2,2}) \frac{s_{1,2}}{s_{1,2} + s_{2,2}r} + \frac{1}{2} F(b_{1,2} + b_{2,2}) \frac{s_{2,2}r}{s_{1,2} + s_{2,2}r} = \frac{F(b_{1,2} + b_{2,2})}{2}. \quad (4)$$

For notational simplicity, we denote expression (4) by K , in which case the final expression for male fitness is

$$\begin{aligned}
 W_m = & 2 \frac{N_f^{(1)}}{N_m^{(1)}} \left[\frac{1}{2} F(b_1 + \hat{b}_2) \frac{s_1}{s_1 + \hat{s}_2 r} + \frac{1}{2} F(\hat{b}_1 + b_2) \frac{s_2 r}{\hat{s}_1 + s_2 r} \right] \\
 & + \left[\frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2} \right] \frac{2N_f^{(1)}}{N_m^{(1)}} \frac{2N_f^{(2)}}{N_m^{(2)}} K.
 \end{aligned} \tag{5}$$

In words, equation (5) is

$$\begin{aligned}
 W_m = & \text{no. matings} \times \frac{\text{value}}{\text{mating}} \\
 & + \text{probability of survival} \times \text{no. matings} \times \frac{\text{value}}{\text{mating}}.
 \end{aligned} \tag{6}$$

Results

The direction in which selection acts on sperm-containing and non-sperm-containing portions of the ejaculate is given by the sign of the selection gradients for each of these traits. This can be found by differentiating the male fitness (expression [5]) with respect to the trait of interest and evaluating these expressions at the population-wide investment strategies (Taylor 1989; Christiansen 1991; Otto and Day 2007). Doing so, we obtain

$$\left. \frac{\partial W_m}{\partial s_1} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r \hat{s}_2 F(\hat{b}_1 + \hat{b}_2)}{2(\hat{s}_1 + r \hat{s}_2)^2} + 2 \frac{N_f^{(1)}}{N_m^{(1)}} \frac{Q\beta'(\hat{b}_1 + \hat{s}_1)}{\bar{\beta}}, \tag{7a}$$

$$\left. \frac{\partial W_m}{\partial s_2} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r \hat{s}_1 F(\hat{b}_1 + \hat{b}_2)}{2(\hat{s}_1 + r \hat{s}_2)^2} + 2 \frac{N_f^{(1)}}{N_m^{(1)}} \frac{Q\beta'(\hat{b}_2 + \hat{s}_2)}{\bar{\beta}} \tag{7b}$$

for the change in male fitness that occurs with an increase in investment in sperm in each of the two male roles and

$$\left. \frac{\partial W_m}{\partial b_1} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{\hat{s}_1 F(\hat{b}_1 + \hat{b}_2)}{\hat{s}_1 + r \hat{s}_2} + 2 \frac{N_f^{(1)}}{N_m^{(1)}} \frac{QK\beta'(\hat{b}_1 + \hat{s}_1)}{\bar{\beta}}, \tag{8a}$$

$$\left. \frac{\partial W_m}{\partial b_2} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r \hat{s}_2 F(\hat{b}_1 + \hat{b}_2)}{\hat{s}_1 + r \hat{s}_2} + 2 \frac{N_f^{(1)}}{N_m^{(1)}} \frac{QK\beta'(\hat{b}_2 + \hat{s}_2)}{\bar{\beta}} \tag{8b}$$

for the change in male fitness that occurs with an increase in investment in seminal products. The quantity $\bar{\beta}$ represents the average probability of male survival to the second reproductive bout; that is, $\bar{\beta} = [\beta(\hat{b}_1 + \hat{s}_1) + \beta(\hat{b}_2 + \hat{s}_2)]/2$. Similarly, we can calculate the change in male fitness that occurs with an increase in total ejaculate investment (see app. B).

Seminal Products as Fecundity-Enhancing Substances

We begin by assuming that male seminal products function only to elevate female fecundity so that equation (5) describes the total fitness of a male. We are interested in determining how males occupying different roles are expected to invest energy in total ejaculates and their components (both sperm and seminal fluid). In order to understand exactly how selection acts on the ejaculate components of males in each of the two role types, it is useful to first consider the evolution of sperm and seminal products independently, even though they actually co-evolve simultaneously in our model, and to begin by imagining a population in which favored and disfavored males are using the same strategies.

First let us examine the selection gradients associated with seminal fluid production (expressions [8]). Since $F' > 0$ and $\beta' < 0$, the first term in each of these expressions is positive, whereas the second term is negative. The first term in both expressions (8a) and (8b) represents the benefit associated with increased investment in seminal fluid (increased female fecundity), and the second term represents the cost (decreased likelihood of surviving to the second reproductive bout). If we suppose that all males in the population initially have the same ejaculate investment strategy (i.e., $\hat{s}_1 = \hat{s}_2$, $\hat{b}_1 = \hat{b}_2$), then the magnitude of the benefit in expression (8b) will be smaller than that in expression (8a) owing to the discounting factor r in expression (8b). The benefit of investing in seminal products is less for the disfavored male than for the favored male because he is less likely to sire the additional offspring produced. We can also see that the cost term will be identical in both expressions (8a) and (8b) under these conditions, since both types of male face the same survival prospects. Thus, as long as the raffle is not fair, selection will drive the favored male to invest more in seminal products than the disfavored male (i.e., $\hat{b}_2 < \hat{b}_1$).

With this in mind, we can next examine the selection gradients associated with sperm production (expressions [7]). The first term in both expressions (7a) and (7b) represents the benefit associated with increased investment in sperm, and the second term represents the cost. The benefit terms are identical in this case for both male roles (Parker 1990a; Mesterton-Gibbons 1999b); therefore, the only way that selection will drive differences in sperm investment is if the cost terms differ between expression (7a) and expression (7b). Keeping in mind from the above results that we expect selection to drive the evolution of nonsperm investment to a point where favored males invest more than disfavored males (i.e., $\hat{b}_2 < \hat{b}_1$), we can see that these cost terms will then differ. In particular, the cost associated with investment in sperm is greater in the favored role than in the disfavored role. In other words, the magnitude of the second term is greater in expression (7b) than it is in expression (7a) when $\hat{b}_2 < \hat{b}_1$ since $|\beta'(\hat{s}_1 + \hat{b}_1)| > |\beta'(\hat{s}_2 + \hat{b}_2)|$ when $\beta'' < 0$ (meaning that there are accelerating costs of investment in terms of survival). This suggests that selection will then drive sperm allocation by the disfavored male to larger values than that of the favored male. Therefore, disfavored males will spend more on sperm but less on seminal fluid than favored males. This results from the fact that the favored male (their competitor) will benefit most from the increased fecundity of the female. When the raffle is fair ($r = 1$), the selection gradients associated with the ejaculate components of both favored and disfavored males are identical, and as a result, all males share the same ejaculate allocation strategy at the ESS.

A comparison of our model with previous models illustrates the importance of including seminal fluid substances in models of sperm competition. Although males in the disfavored role are predicted to always spend more on sperm than males in the favored role in our model, this has not been the prediction of previous models. Parker's (1990a) original analysis of sperm competition under an unfair raffle suggested that, even if males have full information about their roles, but if these roles are assigned randomly (as we assume here), favored and disfavored males should invest equally in sperm. Interestingly, when $\hat{s}_1 = \hat{s}_2$ and $\hat{b}_1 = \hat{b}_2$, the selection gradients associated with sperm investment by males in both roles are equivalent in our model (see expressions [7]). Therefore, if it were the case that all males invested the same amount of energy in seminal fluids (i.e., $\hat{b}_1 = \hat{b}_2$), then no difference between the sperm investment strategies of the males in the two role types is predicted to evolve. The selection gradients associated with seminal fluid investment, however, are never equivalent (see expression [8]), even when $\hat{s}_1 = \hat{s}_2$ and $\hat{b}_1 = \hat{b}_2$. Therefore, if we allow for the evolution of differential investment in seminal fluid by males

in the two roles, then this will always indirectly result in the evolution of differential investment in sperm as well. It is the difference in seminal product investment that drives the evolution of differences in sperm investment as a result of the effect it has on the relative cost of sperm investment in the two different male roles.

Recently, Mesterton-Gibbons (1999a, 1999b) has shown that including a risk of incomplete fertilization, differential mating costs, or varying levels of unfairness in models of sperm competition can cause males in the favored role to spend more on sperm than males in the disfavored role. Curiously, this is exactly opposite from the prediction found in our model. Although our finding of greater sperm expenditure in the disfavored role may initially seem paradoxical, when the expenditure on all components of the ejaculate is considered, the reason for this result becomes clear. By examining the selection gradients associated with total ejaculate investment (see app. B), we can see that disfavored males always spend more on sperm compared with favored males but less on the ejaculate as a whole. Reduced total expenditure on the ejaculate is favored because the marginal benefit to investing in ejaculates is reduced in the disfavored compared with the favored role (fig. 1).

An interesting question to consider next is how the ejaculate investment strategies of favored and disfavored males change as the raffle for fertilization becomes increasingly biased. In a fair raffle, all males invest the same amount of energy in each of the ejaculate components, but as the raffle becomes biased, investment patterns in the two roles begin to diverge. As we move from a fair raffle to a slightly biased one, disfavored males increase the amount of energy they invest in sperm but decrease the amount of energy they invest in seminal products. Favored males, on the other hand, decrease the amount of energy they invest in sperm but increase the amount of energy they invest in seminal products. Overall, disfavored males decrease their total investment in the ejaculate, and favored males increase their total investment (fig. 1; see also app. C). When the raffle is completely unfair (i.e., when the disfavored male's sperm never fertilize any of the female's ova), disfavored males will not spend any energy on ejaculate production because ejaculate investment cannot have any positive effect on fitness.

Mesterton-Gibbons's (1999a) model of sperm competition similarly explores how favored and disfavored male sperm investment changes when the raffle for fertilization moves from fair to biased. His model suggests that for slightly biased raffles, disfavored males will decrease—and favored males will increase—the amount of energy they invest in sperm as fertilization becomes more biased. Again, this is opposite of the prediction of our model. What is interesting, however, is that when we consider

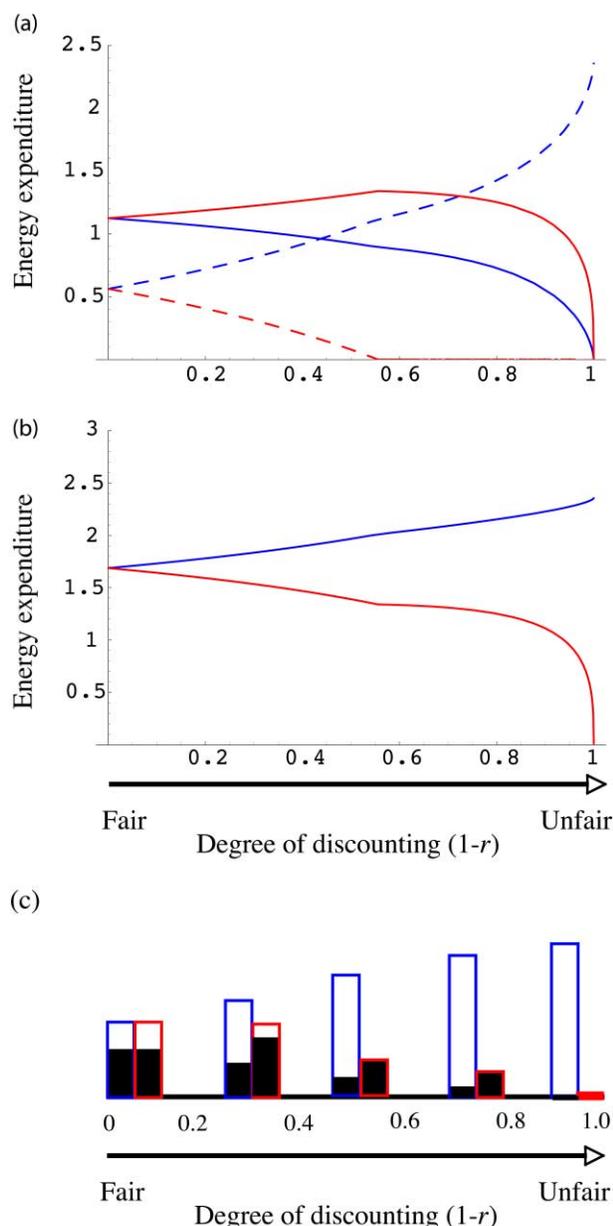


Figure 1: Evolutionarily stable strategy male ejaculate expenditure on sperm and seminal products (a) and the total ejaculate (b). Expenditure on sperm is always greater in the disfavored role (a, solid red line) than in the favored role (a, solid blue line), but expenditure on seminal products is always greater in the favored role (a, dashed blue line) than in the disfavored role (a, dashed red line). Favored males always spend more on total ejaculate production (b, blue line) than disfavored males (b, red line). Calculations use the functions $\beta(s+b) = 1 - (s+b)^2\phi$ and $F(b_1+b_2) = (b_1+b_2)^{1/2}$, along with parameter values $N_m^{(1)} = 1$, $N_m^{(2)} = 2$, $\phi = 0.1$, $K = 0.5$, and $Q = 1$. For nearly fair raffles, males in the disfavored role spend increasingly more on sperm and less on seminal fluids as fertilization becomes more biased. Once the equilibrium level of investment in seminal fluid by disfavored males reaches 0, the amount of energy these males spend on sperm begins to decrease. c is an alter-

total ejaculate investment, our model makes precisely the same prediction as Mesterton-Gibbons's (1999a). Although disfavored males increase their investment in sperm in our model, they decrease their investment in seminal fluid. Thus, overall, total ejaculate expenditure by disfavored males falls even though sperm investment rises (fig. 1). The differences in the predictions of these two models reflect differences in the assumptions made about the way in which the male ejaculate evolves: Mesterton-Gibbons (1999a) assumes only ejaculate size evolves, while we allow both ejaculate size and ejaculate composition to evolve (fig. 1c).

Effects of the Ejaculate on Fertilization Success

We now explore how other functions of seminal products might influence ejaculate investment patterns. Specifically, we consider the possibility that ejaculatory products affect both the fecundity of the female and the fairness of the raffle. Males might influence the fairness of the postcopulatory fertilization raffle in a number of ways. As Mesterton-Gibbons (1999b) has suggested, fertilization bias might vary with sperm investment. For example, in *Drosophila melanogaster*, Price et al. (1999) found that later-mating males could physically displace the sperm of earlier-mating males but that displacement occurred only when sperm were transferred. In this case, investment in sperm by the last male to mate should increase his fertilization success more than would be expected with simple numerical sperm mixing. An additional possibility is that the fairness of the raffle depends on non-sperm-containing substances in the ejaculate. For example, in a number of species, males transfer ejaculatory substances during mating that act to form mating plugs in females following copulation. Although these mating plugs usually function to inhibit remating, they may also have important effects on male fertilization success when remating does occur (Simmons 2001).

We model the effects of the favored male's ejaculate expenditure on his probability of fertilization success in two ways. We assume that fertilization bias becomes increasingly skewed in favor of the favored male as his investment in (1) sperm or (2) seminal fluids rises. We consider male ejaculate investment strategies under these two possible scenarios in order to determine how assumptions

native graphical portrayal of the qualitative patterns depicted in a and b. The shaded regions represent investment in sperm, and the open regions represent investment in seminal products; red bars denote ejaculate expenditure by disfavored males, and blue bars denote ejaculate expenditure by favored males.

regarding which component of the male ejaculate influences competitive processes affect patterns of investment.

We assume that male fitness is still determined by equation (5) but that r is now a decreasing function of s_1 in the case where sperm affects discounting (i.e., $r(s_1)$) and r is a decreasing function of b_1 in the case where seminal fluid affects discounting (i.e., $r(b_1)$). We can then calculate the selection gradients associated with the male ejaculate components (i.e., expressions [7], [8]) for this model when r is a function of s_1 and b_1 (see app. D).

If we examine the selection gradients associated with sperm and seminal fluid investment for the case where r decreases with sperm investment, $r(s_1)$, we find that which of the two male types invests most in sperm at equilibrium depends on the combined balance of the costs and benefits associated with sperm investment in the favored role (see app. D). Favored males spend more on seminal products than disfavored males, but they may spend more or less on sperm depending on the shapes of the functions $r(s_1)$ and $\beta(s + b)$. Interestingly, when there is no evolution of seminal fluids (i.e., when $\hat{b}_1 = \hat{b}_2$), favored males will always evolve to spend more on sperm than disfavored males. In a previous model of sperm competition, Mesterton-Gibbons (1999b) likewise explored the possibility that the degree of sperm discounting is dependent on a male's investment in sperm. He found that when the fertilization bias increased with increasing sperm investment by the favored male, favored males always spent more on sperm than disfavored males. Thus, although the details of our model differ from those of Mesterton-Gibbons's (1999b), it nevertheless makes the same predictions whenever seminal product investment does not evolve.

If we examine the selection gradients associated with sperm and seminal fluid investment for the case where r decreases with seminal fluid investment, $r(b_1)$, we find that the benefits associated with investment in seminal fluid are always greatest for the favored male, while the benefits associated with investment in sperm are always greatest for the disfavored male. Therefore, when r is a function of seminal product investment, selection ensures that favored males spend less on sperm and more on seminal fluid than disfavored males (see app. D).

To demonstrate more clearly how ejaculate investment patterns can differ when the fairness of the raffle is influenced by different ejaculate components, we present two numerical examples. By explicitly defining the functions of the model and using numerical simulations, we determine the equilibrium ejaculate investment strategies of favored and disfavored males when r is a function of s_1 or when r is a function of b_1 . In each of the two cases, we use the same parameter set so that differences between the equilibrium ejaculate allocation strategies reflect differences in the part of the ejaculate affecting fertilization

success only. In this example, when the fairness of the raffle is influenced by sperm ($r(s_1)$), favored males spend more on sperm than disfavored males (see fig. 2a). Favored males also invest more energy in both seminal fluids and total ejaculate production (see fig. 2b). When fairness is influenced by seminal products ($r(b_1)$), disfavored males spend more on sperm than favored males (see fig. 2a). However, favored males invest more energy on both seminal products (fig. 2b) and total ejaculate production (not shown).

These results highlight the importance of assumptions about the way in which the ejaculate affects reproductive responses. In particular, we arrive at the opposite prediction about the outcome of sperm competition, depending on which ejaculate component affects the fairness of sperm competition. In nature, this interaction is likely even more

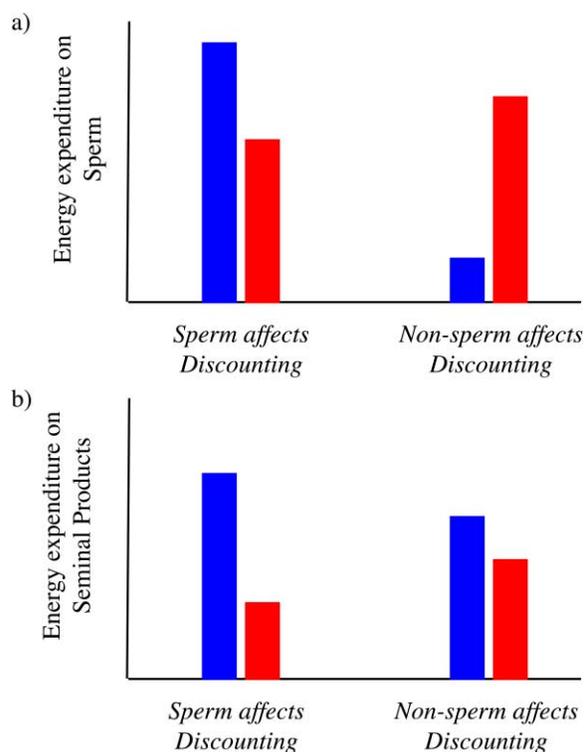


Figure 2: a, Energy expenditure on sperm in both the favored (blue) and disfavored (red) roles when the degree of discounting is affected by either the sperm-containing ($r(s_1)$) or non-sperm-containing ($r(b_1)$) portion of the ejaculate. Although total ejaculate expenditure is always greater for males in the favored role (not shown), the rank order of expenditure on sperm by males in the two roles depends on which component of the ejaculate affects discounting. b, Same as in a but now depicting the energy expenditure on nonsperm components of the ejaculate. Results use the same functions as in figure 1, along with $r(s_1) = 1 - s_1^2\gamma$ or $r(b_1) = 1 - b_1^2\gamma$. Parameter values: $N_f^{(1)} = 1$, $N_m^{(1)} = 2$, $\phi = 0.5$, $K = 0.75$, $Q = 1$, $\gamma = 1.2$.

complex, and we suggest that an understanding of the evolution of sperm competition strategies will therefore require knowledge of which part of the ejaculate affects competition between males and the manner in which this occurs.

Discussion and Qualitative Predictions

The composition of male ejaculates can affect not only the paternity of competing males but also the fertility of females as well (Gillot 2003). From a strategic standpoint, a male must ensure that his ejaculate contains not only enough sperm to effect high levels of paternity but also high levels of nonsperm components that enhance female fertility. To the extent that there are energetic trade-offs between these two components of the ejaculate (e.g., see Moore et al. 2004), males are expected to evolve different ejaculate compositions under different conditions. Males that invest heavily in fecundity-enhancing components have the advantage of stimulating high female fertility, but this must be traded off against the possibility that these offspring might be preferentially sired by competing males that invest more heavily in sperm.

We have attempted to make some progress in developing predictions about how we expect this evolutionary dynamic to play out using relatively simple game theoretic models. Our starting point was the development of a model of male-male competition in which both total ejaculate investment and ejaculate composition were allowed to evolve. At the most fundamental level, this model predicts that competing males should evolve different ejaculate compositions whenever there is differential sperm utilization by females. For example, if, when females mate twice, they preferentially use the sperm of one of the males (e.g., the second male in *Drosophila*), and if males can respond plastically in their ejaculate composition to the role that they are in (e.g., first vs. second male), then differential investment should evolve (fig. 1).

The first model also makes some specific qualitative predictions about the relative investment in sperm versus fertility-enhancing seminal products for each of the two males at evolutionary equilibrium. Favored males are predicted to have a greater total ejaculate investment than disfavored males, but the disfavored male should nevertheless invest a greater amount in sperm production than the favored male (fig. 1c). In extreme cases of very biased sperm utilization by females, these patterns are expected to be extreme as well, with disfavored males investing almost completely in sperm, with little or no investment in fertility-enhancing seminal products, and favored males displaying the opposite pattern. As described in "Results," these predictions differ from previous theory that does not incorporate the effects of seminal fluid.

The above qualitative predictions are relatively easy to intuit, and doing so helps to shed some light on their generality. A male in the disfavored role is predicted to spend more on sperm than a male in the favored role because increased investment in sperm improves his probability of fertilization success, whereas an investment in fertility-enhancing seminal products would benefit the competing (favored) male more than himself. The production of fertility-enhancing components by any given male enhances the reproductive success of all competing males (i.e., it is a "communal" or shared resource since it translates into unfertilized eggs), whereas the production of sperm enhances the reproductive success of the particular male that produced the sperm alone. Since favored males benefit more from the communal resource, they invest more in this resource. Disfavored males instead specialize on enhancing their reproductive success through investment in sperm. The disfavored male nevertheless invests less on the ejaculate as a whole because the potential for future fitness gains outweighs the advantages of current reproductive costs for such males as compared with a male that is currently in the favored role.

The predictions from this first model should be amenable to empirical investigation. From a conceptual standpoint, the best way to begin addressing the issues raised by this model and to begin testing its predictions is to quantify both total ejaculate investment and ejaculate composition in studies of sperm competition. The extent to which this is possible will, of course, depend on the logistical details of the study organism in question. Figure 1c then presents a qualitative guide for how investment strategies in the two male roles are predicted to vary with the degree of preferential sperm utilization by females. The simplest studies might test whether patterns of relative investment by males of a given population conform to the predictions in this figure. For example, for a species with high first-male precedence, do first males invest more overall in ejaculates, whereas second males nevertheless still invest a greater absolute amount in sperm? More substantive tests could then explore whether the patterns of relative investment in the two roles change as predicted by this figure when the degree of bias in sperm usage changes.

The second model represents an attempt to account for other ways in which male ejaculate composition might affect fertilization success. In particular, investment in sperm or seminal fluid products might also alter the extent to which there is bias in sperm usage by females. For example, investment in seminal fluid products might affect fertilization bias, r , and thereby be another strategy by which males compete for paternity. Similarly, investment in sperm might alter paternity not only as a result of increased sperm representation but also by affecting fer-

tilization bias r (i.e., it might affect the likelihood that a single spermatozoon achieves fertilization). Our second model predicts that favored males will tend to spend more on sperm than disfavored males when sperm increases the fertilization bias but less on sperm when seminal fluid (or some other ejaculate component) increases this bias. The reason for this result is not difficult to interpret conceptually: when investment in sperm increases fertilization success, it will generally pay to invest in sperm, and when investment in seminal fluid increases fertilization success, it will generally pay to invest in seminal fluid.

Since this second model yields varied predictions about the outcome of sperm competition, it will be more challenging to test directly. A key finding of the model is that theoretical predictions regarding the outcome of sperm competition depend critically on the way in which ejaculatory products (sperm and otherwise) influence how females utilize the sperm of rival males. The model makes very different predictions about the evolutionary outcome of sperm competition when different parts of the male ejaculate influence female sperm use, in some cases resulting in exactly opposite predictions. These results provide a strong argument for attempting to quantify the way in which ejaculates affect the likelihood of fertilization in sperm competition. Obtaining a detailed understanding of how ejaculatory components mediate sperm utilization in the female reproductive tract would be a first step toward an empirical test of our results. Once we understand how ejaculates shape sperm precedence patterns for a particular organism, the relative sperm investment of competing males (in both the favored and disfavored roles) could be measured to determine whether the investment patterns conform to theoretical expectations.

The results presented here are a first step toward de-

veloping a more general theory of male ejaculate evolution. It would be very valuable, however, to extend the results presented here to allow for female coevolution (see also Greef and Parker 2000; Ball and Parker 2003; Gavrillets and Hayashi 2006). For example, in *Drosophila melanogaster*, the receipt of seminal fluid has been associated with decreased female longevity (Chapman et al. 1995; Wolfner 1997; Chapman 2001), and if this results in decreased female fitness, then there will be strong selection on females to evolve counterresponses. In fact, there is evidence of genetic variation among female *Drosophila* in their responses to ejaculates (Clark and Begun 1998; Clark et al. 1999), and comparative data suggest that the female reproductive tract evolves in response to aspects of the male ejaculate (Miller and Pitnick 2002; Pitnick et al. 2003). Preliminary theoretical results (E. Cameron, T. Day, and L. Rowe, unpublished data) reveal that such female coevolution can result in changes in fertilization bias, r , and can thereby significantly affect the evolution of male ejaculate composition. This interface between male-male competition and sexually antagonistic coevolution is an interesting one for future study.

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APPENDIX A

Derivation of Equation (5)

Let $N_{\text{matings}}^{(1)}$ be the number of matings a male obtains in the first reproductive bout, with n representing the number of matings in the first role and $N_{\text{matings}}^{(1)} - n$ representing the number of matings in the second role. The probability that a male has n matings in the first role (and $N_{\text{matings}}^{(1)} - n$ matings in the second role) is then

$$\text{Binomial}\left(N_{\text{matings}}^{(1)}, \frac{1}{2}\right) \equiv \binom{N_{\text{matings}}^{(1)}}{n} \left(\frac{1}{2}\right)^n \left(\frac{1}{2}\right)^{N_{\text{matings}}^{(1)} - n}. \quad (\text{A1})$$

To calculate male fitness at the first bout, we must weigh the number of matings a male receives in each role by the value of each mating. This gives

$$\sum_{n=0}^{N_{\text{matings}}^{(1)}} \binom{N_{\text{matings}}^{(1)}}{n} \left(\frac{1}{2}\right)^n \left(\frac{1}{2}\right)^{N_{\text{matings}}^{(1)} - n} \left[nF(b_1 + \hat{b}_2) \frac{s_1}{s_1 + r\hat{s}_2} + (N_{\text{matings}}^{(1)} - n)F(\hat{b}_1 + b_2) \frac{rs_2}{s_1 + rs_2} \right], \quad (\text{A2})$$

which simplifies to

$$N_{\text{matings}}^{(1)} \left[\frac{1}{2} F(b_1 + \hat{b}_2) \frac{s_1}{s_1 + \hat{s}_2} + \frac{1}{2} F(\hat{b}_1 + b_2) \frac{rs_2}{\hat{s}_1 + s_2} \right]. \tag{A3}$$

Now the number of matings that a male has in the first reproductive bout (i.e., $N_{\text{matings}}^{(1)}$) is itself a random variable with the following probability distribution:

$$\text{Binomial} \left(2N_f^{(1)}, \frac{1}{N_m^{(1)}} \right) \equiv \binom{2N_f^{(1)}}{N_{\text{matings}}^{(1)}} \left(\frac{1}{N_m^{(1)}} \right)^{N_{\text{matings}}^{(1)}} \left(1 - \frac{1}{N_m^{(1)}} \right)^{2N_f^{(1)} - N_{\text{matings}}^{(1)}}. \tag{A4}$$

Therefore, averaging equation (A3) over the total number of matings obtained by a male gives

$$\sum_{N_{\text{matings}}^{(1)}=0}^{2N_f^{(1)}} \binom{2N_f^{(1)}}{N_{\text{matings}}^{(1)}} \left(\frac{1}{N_m^{(1)}} \right)^{N_{\text{matings}}^{(1)}} \left(1 - \frac{1}{N_m^{(1)}} \right)^{2N_f^{(1)} - N_{\text{matings}}^{(1)}} N_{\text{matings}}^{(1)} \left[\frac{1}{2} F(b_1 + \hat{b}_2) \frac{s_1}{s_1 + \hat{s}_2} + \frac{1}{2} F(\hat{b}_1 + b_2) \frac{rs_2}{\hat{s}_1 + s_2} \right], \tag{A5}$$

which simplifies to

$$2 \frac{N_f^{(1)}}{N_m^{(1)}} \left[\frac{1}{2} F(b_1 + \hat{b}_2) \frac{s_1}{s_1 + \hat{s}_2} + \frac{1}{2} F(\hat{b}_1 + b_2) \frac{rs_2}{\hat{s}_1 + s_2} \right]. \tag{A6}$$

Since the above distribution is valid only when $2N_f^{(1)}/N_m^{(1)}$ is an integer, we assume that the number of males and females in the population are such that this is always true.

To determine how the number of matings at the first bout translates into the probability of survival, we assume that each time a male mates, his probability of surviving to the next bout is multiplied by β . A male's expected probability of survival to the second bout is then

$$\sum_{n=0}^{N_{\text{matings}}^{(1)}} \binom{N_{\text{matings}}^{(1)}}{n} \left(\frac{1}{2} \right)^n \left(\frac{1}{2} \right)^{N_{\text{matings}}^{(1)} - n} [\beta(s_1 + b_1)^n \beta(s_2 + b_2)^{N_{\text{matings}}^{(1)} - n}], \tag{A7}$$

which simplifies to

$$\left[\frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2} \right]^{N_{\text{matings}}^{(1)}}. \tag{A8}$$

Averaging equation (A8) over the total number of matings obtained by a male gives

$$\sum_{N_{\text{matings}}^{(1)}}^{2N_f^{(1)}} \text{Binomial} \left(N_{\text{matings}}^{(1)}, \frac{1}{N_m^{(1)}} \right) \left[\frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2} \right]^{N_{\text{matings}}^{(1)}}, \tag{A9}$$

which simplifies to

$$\left[\frac{N_m^{(1)} + \frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2} - 1}{N_m^{(1)}} \right]^{2N_f^{(1)}}. \tag{A10}$$

Equation (A10) is exact, but we can obtain a more useful approximation by first letting $N_m^{(1)} = N_{\text{total}}^{(1)} p$ and $N_f^{(1)} = N_{\text{total}}^{(1)} (1 - p)$, where $N_{\text{total}}^{(1)}$ represents the total population size at the first reproductive event and p represents the

proportion of that population that is male. Taking the limit of equation (A10) as the total population size becomes large then gives

$$\lim_{N_{\text{total}}^{(1)} \rightarrow \infty} \left[\frac{N_{\text{total}}^{(1)} p + \frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2} - 1}{N_{\text{total}}^{(1)} p} \right]^{2N_{\text{total}}^{(1)}(1-p)} = \left\{ e^{-\left[1 - \frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2}\right]} \right\}^{\frac{2N_{\text{total}}^{(1)}}{N_{\text{m}}^{(1)}}}. \quad (\text{A11})$$

Expanding the term in the braces on the right-hand side in equation (A11) in a Taylor series, we arrive at the approximation

$$\left[\frac{\beta(s_1 + b_1) + \beta(s_2 + b_2)}{2} \right]^{\frac{N_{\text{total}}^{(1)}}{N_{\text{m}}^{(1)}}}$$

for the probability that a male will survive to the second reproductive bout.

APPENDIX B

Calculating the Selection Gradient Associated with Total Ejaculate Investment

We can calculate the change in male fitness that occurs with an increase in total ejaculate investment in each role, $E_i = s_i + b_p$, as

$$\left. \frac{\partial W_{\text{m}}}{\partial E_1} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r\hat{s}_2 F(\hat{b}_1 + \hat{b}_2) + \hat{s}_1(\hat{s}_1 + r\hat{s}_2)F'(\hat{b}_1 + \hat{b}_2)}{(\hat{s}_1 + r\hat{s}_2)^2} + 2 \frac{N_{\text{f}}^{(1)}}{N_{\text{m}}^{(1)}} \left[\frac{QK\beta'(\hat{s}_1 + \hat{b}_1)}{\bar{\beta}} \right], \quad (\text{B1})$$

$$\left. \frac{\partial W_{\text{m}}}{\partial E_2} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r\hat{s}_1 F(\hat{b}_1 + \hat{b}_2) + r\hat{s}_2(\hat{s}_1 + r\hat{s}_2)F'(\hat{b}_1 + \hat{b}_2)}{(\hat{s}_1 + r\hat{s}_2)^2} + 2 \frac{N_{\text{f}}^{(1)}}{N_{\text{m}}^{(1)}} \left[\frac{QK\beta'(\hat{s}_2 + \hat{b}_2)}{\bar{\beta}} \right], \quad (\text{B2})$$

for favored and disfavored males, respectively.

APPENDIX C

Determination of the Effect of r on Predictions

Here we determine how the equilibrium values of the ejaculate components of both favored and disfavored males change as the bias in the fertilization raffle increases (i.e., as the parameter r decreases). We begin by considering the case where the raffle is almost fair (when r is close to 1). We define the functions $H_1(s_1, s_2, b_1, b_2, r)$, $H_2(s_1, s_2, b_1, b_2, r)$, $H_3(s_1, s_2, b_1, b_2, r)$, and $H_4(s_1, s_2, b_1, b_2, r)$ as being given by expressions (7a), (7b), (8a), and (8b). Each of these functions is equal to 0 at the joint ESS. Implicitly differentiating each of these with respect to r , treating the ESS trait values as functions of r , then gives

$$\begin{bmatrix} \frac{\partial H_1}{\partial s_1} & \frac{\partial H_1}{\partial s_2} & \frac{\partial H_1}{\partial b_1} & \frac{\partial H_1}{\partial b_2} \\ \frac{\partial H_2}{\partial s_1} & \frac{\partial H_2}{\partial s_2} & \frac{\partial H_2}{\partial b_1} & \frac{\partial H_2}{\partial b_2} \\ \frac{\partial H_3}{\partial s_1} & \frac{\partial H_3}{\partial s_2} & \frac{\partial H_3}{\partial b_1} & \frac{\partial H_3}{\partial b_2} \\ \frac{\partial H_4}{\partial s_1} & \frac{\partial H_4}{\partial s_2} & \frac{\partial H_4}{\partial b_1} & \frac{\partial H_4}{\partial b_2} \end{bmatrix} \times \begin{bmatrix} \frac{ds_1}{dr} \\ \frac{ds_2}{dr} \\ \frac{db_1}{dr} \\ \frac{db_2}{dr} \end{bmatrix} + \begin{bmatrix} \frac{\partial H_1}{\partial r} \\ \frac{\partial H_2}{\partial r} \\ \frac{\partial H_3}{\partial r} \\ \frac{\partial H_4}{\partial r} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}. \quad (C1)$$

Substituting $r = 1$, $s_1 = s_2 = s$, and $b_1 = b_2 = b$ (since investment in the ejaculate components is the same in both roles when the raffle is fair) into equation (B1), rearranging, and solving, we obtain

$$-\frac{ds_1}{dr} \propto -\frac{s^2 F'(2b)}{F(2b) + 2sF'(2b)}, \quad (C2a)$$

$$-\frac{ds_2}{dr} \propto \frac{s^2 F'(2b)}{F(2b) + 2sF'(2b)} \quad (C2b)$$

for the direction of change in ESS sperm investment with increasing fertilization bias and

$$-\frac{db_1}{dr} \propto -\frac{F'(2b)[N_m^{(1)}F(2b)\beta(s+b) - 4N_f^{(1)}Qs^2\beta''(s+b)]}{8N_f^{(1)}Q[F(2b) + 2sF'(b)]\beta''(s+b)}, \quad (C3a)$$

$$-\frac{db_2}{dr} \propto \frac{F'(2b)[N_m^{(1)}F(2b)\beta(s+b) - 4N_f^{(1)}Qs^2\beta''(s+b)]}{8N_f^{(1)}Q[F(2b) + 2sF'(b)]\beta''(s+b)} \quad (C3b)$$

for the direction of change in the ESS seminal product investment. The direction of change in total ejaculate investment is

$$-\frac{d(s_1 + b_1)}{dr} \propto -\frac{N_m^{(1)}F(2b)\beta(s+b)F'(2b)}{8N_f^{(1)}Q[F(2b) + 2sF'(2b)]\beta''(s+b)}, \quad (C4a)$$

$$-\frac{d(s_2 + b_2)}{dr} \propto \frac{N_m^{(1)}F(2b)\beta(s+b)F'(2b)}{8N_f^{(1)}Q[F(2b) + 2sF'(2b)]\beta''(s+b)}. \quad (C4b)$$

Since we assume that $F' > 0$ and $\beta'' < 0$, we can see that equations (C2a), (C3b), and (C4b) are negative, while equations (C2b), (C3a), and (C4a) are positive. We can also see that these functions are symmetric since the expressions are the same (only their signs differ).

APPENDIX D

Details of the Model When $r(s_1)$ or $r(b_1)$

When r is a function of s_1 , the selection gradients associated with the male ejaculate components (i.e., expressions [7], [8]) become

$$\left. \frac{\partial W_m}{\partial s_1} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{[r(\hat{s}_1) - r'(\hat{s}_1)\hat{s}_1]\hat{s}_2 F(\hat{b}_1 + \hat{b}_2)}{[\hat{s}_1 + r(\hat{s}_1)\hat{s}_2]^2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_1 + \hat{s}_1)}{N_m^{(1)} \bar{\beta}}, \quad (\text{D1a})$$

$$\left. \frac{\partial W_m}{\partial s_2} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r(\hat{s}_1)\hat{s}_1 F(\hat{b}_1 + \hat{b}_2)}{[\hat{s}_1 + r(\hat{s}_1)\hat{s}_2]^2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_2 + \hat{s}_2)}{N_m^{(1)} \bar{\beta}} \quad (\text{D1b})$$

for investment in sperm and

$$\left. \frac{\partial W_m}{\partial b_1} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{\hat{s}_1 F'(\hat{b}_1 + \hat{b}_2)}{\hat{s}_1 + r(\hat{s}_1)\hat{s}_2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_1 + \hat{s}_1)}{N_m^{(1)} \bar{\beta}}, \quad (\text{D2a})$$

$$\left. \frac{\partial W_m}{\partial b_2} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r(\hat{s}_1)\hat{s}_2 F'(\hat{b}_1 + \hat{b}_2)}{\hat{s}_1 + r(\hat{s}_1)\hat{s}_2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_2 + \hat{s}_2)}{N_m^{(1)} \bar{\beta}} \quad (\text{D2b})$$

for investment in seminal products by males in each of the favored and disfavored roles, respectively. When r is a function of b , these gradients become

$$\left. \frac{\partial W_m}{\partial s_1} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r(\hat{b}_1)\hat{s}_2 F(\hat{b}_1 + \hat{b}_2)}{[\hat{s}_1 + r(\hat{b}_1)\hat{s}_2]^2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_1 + \hat{s}_1)}{N_m^{(1)} \bar{\beta}}, \quad (\text{D3a})$$

$$\left. \frac{\partial W_m}{\partial s_2} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r(\hat{b}_1)\hat{s}_1 F(\hat{b}_1 + \hat{b}_2)}{[\hat{s}_1 + r(\hat{b}_1)\hat{s}_2]^2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_2 + \hat{s}_2)}{N_m^{(1)} \bar{\beta}} \quad (\text{D3b})$$

for investment in sperm and

$$\left. \frac{\partial W_m}{\partial b_1} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{\hat{s}_1 F'(\hat{b}_1 + \hat{b}_2)}{\hat{s}_1 + r(\hat{s}_1)\hat{s}_2} - \frac{\hat{s}_2 r'(\hat{b}_1) F(\hat{b}_1 + \hat{b}_2)}{[\hat{s}_1 + r(\hat{s}_1)\hat{s}_2]^2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_1 + \hat{s}_1)}{N_m^{(1)} \bar{\beta}}, \quad (\text{D4a})$$

$$\left. \frac{\partial W_m}{\partial b_2} \right|_{\substack{s_1 = \hat{s}_1; b_1 = \hat{b}_1 \\ s_2 = \hat{s}_2; b_2 = \hat{b}_2}} \propto \frac{r(\hat{b}_1)\hat{s}_2 F'(\hat{b}_1 + \hat{b}_2)}{\hat{s}_1 + r(\hat{s}_1)\hat{s}_2} + 2 \frac{N_f^{(1)} QK\beta'(\hat{b}_2 + \hat{s}_2)}{N_m^{(1)} \bar{\beta}} \quad (\text{D4b})$$

for investment in seminal fluid.

We begin by considering the evolution of sperm and seminal products independently. Suppose that initially there is no difference between the investment strategies of the two types of males in the population so that $\hat{s}_1 = \hat{s}_2$ and $\hat{b}_1 = \hat{b}_2$. The first term in each of the fitness gradients represents the benefit associated with investment in that ejaculate component, and the second term represents the cost; in equation (D4a), the first two terms represent the benefit, and

the last term represents the cost. Similar to the parameter r , the function $r(x)$ must lie between 0 and 1, where x is either s_1 or b_1 .

We first consider the case where r decreases with sperm investment, $r(s_1)$. If we examine the selection gradients associated with seminal product production (eq. [D2]), we can see that whenever $r(\hat{s}_1) \neq 1$, the benefit of investing in seminal products is greater in equation (D2a) than in equation (D2b); the first term of the selection gradient in equation (D2b) is weighted by $r(\hat{s}_1)$. Thus, since the costs in each of these two expressions are equal, selection will drive \hat{b}_1 to be lower than \hat{b}_2 . If we examine the selection gradients associated with sperm production (eq. [D1]), we can see that the benefit in equation (D1a) is greater than it is in equation (D1b) (since $r' < 0$). However, since $\hat{b}_1 > \hat{b}_2$, the magnitude of the cost in equation (D1a) is also greater than it is in equation (D1b); $|\beta'(\hat{s}_1 + \hat{b}_1)| > |\beta'(\hat{s}_2 + \hat{b}_2)|$ since $\beta'' < 0$. Which of the two male types invests most in sperm at equilibrium therefore depends on the combined balance of the cost and benefit terms in equation (D1a). Favored males spend more on seminal products than disfavored males, but they may spend more or less on sperm depending on the shapes of the functions $r(s_1)$ and $\beta(s + b)$. The costs in equations (D1a) and (D1b) will be the same when $b_1 = b_2$, but the benefit will still be greater in equation (D1a), so favored males always spend more on sperm than disfavored males.

Next, we consider the case where r decreases with seminal fluid production, $r(b_1)$. If we examine the selection gradients associated with seminal fluid production (eq. [D4]), we can see that the benefit of investing in seminal fluid is always greater in equation (D4a) than in equation (D4b); the first term in equation (D4a) is greater than the first term in equation (D4b) (since it is weighted by $r(\hat{b}_1)$ in eq. [D4b]) and the second term in equation (D4a) is positive (since $r' < 0$). As a result, selection always drives greater investment in seminal fluid by favored males at equilibrium. If we examine the selection gradients associated with sperm production (eq. [D3]), we can see that whenever $\hat{b}_1 \neq \hat{b}_2$, the costs associated with sperm production in the two roles differ. These costs are greater in equation (D3a) than in equation (D3b) when $\hat{b}_1 > \hat{b}_2$ since $|\beta'(\hat{s}_1 + \hat{b}_1)| > |\beta'(\hat{s}_2 + \hat{b}_2)|$ when $\beta'' < 0$. As a result, selection drives greater investment in sperm by disfavored males at equilibrium.

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