Evolutionary Modeling Predicts a Decrease in Postcopulatory Sperm Viability as a Response to Increasing Levels of Sperm Competition

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abstract: Sperm competition has been found to have a strong influence on the evolution of many male and female reproductive traits. Theoretical models have shown that, with increasing levels of sperm competition, males are predicted to increase ejaculate investment, and there is ample empirical evidence supporting this prediction. However, most theoretical models concern sperm number, and although the predictions are likely to apply to other sperm traits that will affect the sperm competitive ability of males, substantiated predictions are difficult unless the evolution of specific traits is explicitly modeled. Here I present a novel theoretical model aiming at predicting evolutionarily stable sperm viability in relation to female mating frequency in a mating system with internal fertilization. At odds with verbal arguments, this model demonstrates that sperm viability is expected to decrease with increasing female remating rates and thus to decrease with increasing levels of sperm competition. The major reason for this is that, with increasing female remating rates, the prospects of future fertilization success will decrease, which acts to reduce the benefit of long-lived viable sperm. An additional interesting result is that, as the cost of sperm viability increases, the overall energy investment in ejaculates will decrease. These novel results should have a strong impact on future sperm competition studies and will also have implications for our understanding of the evolution of female polyandry.

Keywords: ESS, fertility, sexual selection, sperm quality, theoretical model, trade-offs.

Introduction

Since Parker's (1970) seminal paper, sperm competition (i.e., the competition between sperm from two or more males for the fertilization of a given set of ova) has been identified as a strong selective force shaping the evolution of many male reproductive traits (Smith 1984; Eberhard 1996; Birkhead and Møller 1998; Simmons 2001; Arnqvist and Rowe 2005). Sperm competition will therefore also

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have profound effects on the selection on sperm traits and thus the evolution of different sperm phenotypes (Pitnick et al. 2009; Pizzari and Parker 2009). In many cases, the outcome of sperm competition is mediated by the relative numbers of sperm from competing males (Dziuk 1996; Simmons 2001; Gage and Morrow 2003; Engqvist et al. 2007). It will be favorable to have many sperm, and sperm competition is therefore believed to be the main reason for the evolution of many tiny sperm cells (Parker 1982). Theoretical analyses have also shown that, with increasing levels of sperm competition, the ability to produce a large amount of spermatozoa will be increasingly important (Parker et al. 1996, 1997; Williams et al. 2005; Engqvist and Reinhold 2006; Fromhage et al. 2008), a prediction that is well supported empirically (see Engqvist and Reinhold 2005). However, sperm quality differences will also affect fertilization success (García-González and Simmons 2005). Sperm may differ in quality attributes, such as motility (Birkhead et al. 1999; Gage et al. 2004), size (Ward 1998; Morrow and Gage 2001), or longevity (Gage et al. 1995; Neff et al. 2003), and these factors may all influence the probability that males' sperm will fertilize the females' eggs (Snook 2005; Pizzari and Parker 2009).

Why do males not manufacture sperm of the highest possible quality? One reason might be that producing high-quality sperm will be costly (Pitnick et al. 2009). Therefore, males will have to balance these costs against the benefits gained by investing in something else. Thus decreased sperm viability may be advantageous, given that males gain some other benefit (Pitnick et al. 2009). There is some empirical evidence on the costs associated with investment in increased sperm survival. It is possible that there will be a trade-off with sperm number, given that larger sperm are more viable than smaller sperm (Parker 1998; Helfenstein et al. 2008; Pizzari and Parker 2009). Alternatively, at least in some insects, sperm viability has been shown to be accomplished by seminal fluids (Holman 2009a), which may be costly to produce. Trade-offs may also be

present between investment in sperm quality and other aspects of male reproductive success, such as investment in traits affecting attractiveness and mating success (Evans 2010; Simmons et al. 2010; Engqvist 2011; Preston et al. 2011). Yet not much is known concerning which factors may benefit sperm viability. Furthermore, recent studies on crickets, for example, have shown that males invest differentially in sperm quality under different environmental settings, suggesting that the advantage of viable sperm may be dependent on the situation or environment (Simmons et al. 2007; Thomas and Simmons 2007). Authors have therefore emphasized the importance of incorporating variation in sperm quality in theoretical and empirical studies of sperm competition (García-González and Simmons 2005; Pizzari and Parker 2009). The aim of this theoretical analysis will be to explore the influence of the level of sperm competition on the predicted investment in sperm viability in species in which females store sperm between matings and fertilizations.

In every animal mating system, there is a time lag between mating and fertilization. For external fertilizers (e.g., most fish species), this time lag can be extremely short, whereas for species with internal fertilization, this time period can be very long (weeks or even months). This is especially the case in species (e.g., many insects) in which females store sperm in specific sperm storage organs (spermathecae) until the eggs are fertilized shortly before egg laying. In any case, it seems crucial that sperm stay viable and survive for a certain time period. The longer a male's sperm can survive, the longer the male can participate in the competition for egg fertilization. Following the logic that male sperm traits will be more important at high levels of sperm competition, it has repeatedly been suggested that the benefit of high sperm survival should increase with an increase in the level of sperm competition (Hunter and Birkhead 2002; Pizzari et al. 2008; Fitzpatrick et al. 2009; Pitnick et al. 2009; Pizzari and Parker 2009; Ota et al. 2010). However for internal fertilizers, there is at present no theoretical analysis of this hypothesis. Moreover, this verbal prediction that sperm viability should be more important at high levels of sperm competition may well be a fallacy. One could also use arguments from life-history theories of ageing (Kirkwood and Austad 2000) to make the opposite prediction. The fertilization chances of sperm will decrease more strongly with time if females mate with many males than if females mate with fewer males. Under such situations, it should thus be less beneficial for males to invest in increased sperm survival. It may instead be more advantageous to increase the competitive ability of young sperm (e.g., to produce a greater amount of sperm whose viability diminish more rapidly). This is quite analogous to the evolutionary forces affecting senescence and ageing of organisms (Williams 1957; Rose 1991; Kirkwood and Austad 2000). To resolve this apparent contradiction, the aim here is to develop new theoretical models to understand the fundamental selective forces acting on sperm viability under different levels of sperm competition.

The Model

Here I will use an evolutionary game-theoretic approach to find the evolutionarily stable strategy (ESS; Maynard Smith and Price 1973) with respect to male investment in sperm viability. The main focus is to investigate how the sperm viability ESS is expected to change with increasing female mating frequency. The basic assumptions concern the female mating pattern determining the level of sperm competition, as well as the mortality and the raffle of sperm determining fertilization success. I will briefly describe these assumptions before continuing with the derivation of the model (see also table 1).

Female Mating and Reproduction

The model presented here focuses on female matings and reproduction during a single reproductive bout. This must not automatically exclude mating systems in which there is more than one such reproductive period in a female's lifetime (e.g., recurrent estrus cycles and breeding seasons). However, in such cases, it must be assumed that the interval between bouts is relatively long, compared with the duration of the bout itself, so that no sperm survive from previous reproductive periods. Within a reproductive bout, females can mate with several males; thus sperm competition is only occurring between males that mate with a female within the same reproductive period. The probability that the female reproductive bout will come to an end (i.e., that females will stop mating and producing offspring) is modeled as a constant rate (m), rendering an expected reproductive duration per bout that is exponentially distributed. Under the assumptions described above, it will suffice to consider only one reproductive bout per female, and therefore the explicit model here describes the common case in which there is one reproductive period in a female's lifetime, which will end with the death of the female. In this specific case, the parameter m will correspond to a constant female mortality rate.

An important additional assumption is that females are in control of mating rate, which is independent of male sperm investment. After females' first mating, the time between subsequent matings (τ) is assumed to be constant. As a consequence, female number of matings, and thus the level of sperm competition, will be determined by the time between female rematings in relation to the female mortality rate (m). All females will mate at least once, and

Table 1: Definition of variables and parameters used

Variable	Definition
r	Female mating rate
$\tau, \tilde{\tau}$	Time between female matings
m	Female mortality rate
$q = e^{-mr}$	Female remating probability
P_i	Probability that a female mates i times per lifetime
p_i	Probability that a male mates with a female that has mated i times previously
ϕ	Male fertilization success per mating
$s, \hat{s}$	Sperm number (mutant, resident)
$v, \hat{v}$	Sperm survival between matings (mutant, resident)
$\mu, \hat{\mu}$	Sperm mortality rate (mutant, resident)
σ_i	No. resident sperm in females immediately after a female's ith mating exclusively with resident males
$\tilde{\sigma}_{i+j}$	No. resident sperm in females immediately after a female's mating with i resident, one mutant, and j resident males (in that specific order)
D	Cost of each sperm cell
α	Cost of an ejaculate
a	Shape parameter for the cost of sperm viability function
β	Scaling parameter for the cost of sperm production
c	Nonejaculate costs of a mating
R	Male energy allocation on reproduction
n	Male number of matings
$\tilde{n}$	Male relative mating success
w	Male reproductive success

the probability of survival from one mating to next, and hence the probability of remating (q), will be given by the expression $q = e^{-mr}$. Under these assumptions, female total number of matings will follow a geometric distribution, and the probability that a female will mate i times (P_i) can be represented by the formula $P_i = (1 - q)\, q^{i-1} = (1 - e^{-mr}) \times (e^{-mr})^{i-1}$. In addition, the mean female number of matings can be expressed as $1/(1 - q) = 1/(1 - e^{-mr})$. An additional assumption is that the fertilization of ova is occurring continuously at a constant rate in the time between matings. Because matings occur in discrete time steps and fertilization is a continuous process, it will later be crucial to view time on both discrete and continuous scales.

Sperm Viability and Male Fertilization Success

In all analyses, fertilization probability is assumed to follow a "fair raffle" (see Parker 1990). In other words, the expected male fertilization success (ϕ) will be equal to $\phi(s_f) = s_f/(s_f + s_r)$. Here s_f is the number of the focal male's sperm, and s_r is the total number of rival sperm present at the time and place of fertilization. An additional fundamental assumption is that sperm mortality rate is constant. Sperm viability, which is defined as the survival probability per time unit of a given spermatozoa within the female, will thus also be constant. To find an expression for total male fertilization success as a function of sperm

viability, we now need to consider how the focal male's sperm number will change with time. However, we also need to consider how many rival sperm will be present at the time of mating and how this amount will change with time. Let us assume that most males in the population (the resident population) transfer $\hat{s}$ number of sperm in each mating and that these sperm have a viability denoted by $\hat{v}$. Here $\hat{v}$ represents the proportion of sperm that stay alive until the next female mating. With constant sperm mortality rate and a fixed time between female matings, the change in female sperm storage from one female mating to the next can be represented by the recurrence equation $\sigma_{n+1} = \sigma_n \hat{v} + \hat{s}$. Here σ_n gives the number of resident sperm immediately after the nth mating. Because the number of sperm present in nonmated females, σ_0, is equal to zero, the recurrence has the solution

$$\sigma_n = \frac{\hat{s}}{1 - \hat{v}}(1 - \hat{v}^n). \tag{1}$$

Consider now a mutant male mating with a female that has previously mated with N resident males. The total number of sperm immediately after this mating will equal $\sigma_{N+1} + s - \hat{s}$, where s denotes the ejaculate size of mutant males. The number of rival resident sperm after N matings with resident males and one with a male following a mutant strategy will thus not equal σ_{N+1}, but rather $\tilde{\sigma}_N = \sigma_{N+1} - \hat{s}$. In the matings that eventually will follow, the female will again mate with resident males. Hence, after

n additional matings, the number of rival sperm from resident males will equal $\tilde{\sigma}_{N+n} = \sigma_{(N+1+n)} - \hat{s}\hat{v}^n$, which can also be written as (see eq. [1])

$$\tilde{\sigma}_{N+n} = \frac{\hat{s}}{1-\hat{v}}(1 - \hat{v}^{N+n+1}) - \hat{s}\hat{v}^n. \qquad (2)$$

The number of sperm from the mutant male at the same time point will equal sv^n, where v represents the viability of the mutant male's sperm.

Because it is assumed that eggs are fertilized continuously in the time between matings, it is also essential to model how sperm amount changes, not only at each female mating, but also in continuous time between matings. It is therefore more natural to consider sperm viability in terms of mortality rate. With constant sperm mortality, the number of sperm will decrease exponentially. Let μ and $\hat{\mu}$ denote the mortality rates of mutant and resident sperm, respectively. How can we express v and $\hat{v}$, the proportion of sperm surviving from one female mating to the next, in terms of μ and $\hat{\mu}$? The time between matings is given by τ. In this time, the number of male mutant sperm decreases by the factor v, and the number of resident males' sperm decreases by a factor $\hat{v}$, hence $e^{-\mu\tau} = v$ and $e^{-\hat{\mu}\tau} = \hat{v}$. Figure 1 illustrates how, in this model, the amount of resident males' sperm stored by females changes at each female mating and in the time from one mating to the next. Consider now a female that mated with N males before mating with the mutant male and has mated with n additional males since then. The expected fertilization success for the mutant male in the time between this mating and the next possible female mating will equal

$$\int_0^\tau \frac{sv^n e^{-\mu t}}{sv^n e^{-\mu t} + \left[\frac{\hat{s}}{1-\hat{v}}(1 - \hat{v}^{N+n+1}) - \hat{s}\hat{v}^n\right]e^{-\hat{\mu}t}} e^{-mt}\, \mathrm{d}t.$$

$$(3)$$

Here the numerator and denominator equal the mutant male's sperm amount and total sperm amount, respectively. However, there is also a chance that the nth additional mating will be the females last mating and that she will die and stop reproducing before the next mating. This is accounted for in equation (3) by the term e^{-mt}, which represents the survival probability of the female until time t ($0 \le t \le \tau$) after the last mating, given that she survived until this mating. Before we can come up with a final expression for male total fertilization success, we must also consider the probability that females will survive from one mating to another ($e^{-m\tau}$) and the probability (p_N) that a male will mate with a female that has mated N times previously. This last probability requires some consideration (see also Engqvist and Reinhold 2006). In a popu-
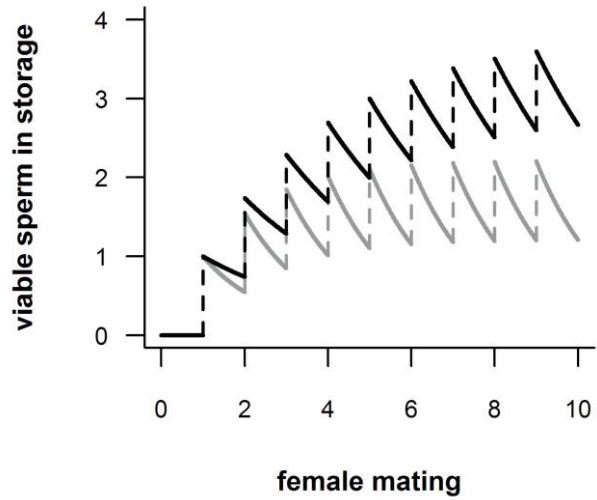
Figure 1: Illustration showing how the number of viable sperm in storage changes at each female mating and in the time between matings. The lines depict two populations in which males have identical ejaculate sizes, which equal one unit, but different sperm mortality rates. The gray line is from a population with a sperm mortality rate that is twice as high as that of the population represented by the black line.

lation consisting of x females, there will be an expected number of $x(1-q)^{-1}$ matings in total, because it is given that the average number of matings per female will equal $(1-q)^{-1}$. Furthermore, because all females mate exactly once as virgins, there will be x matings with virgin females, and thus a probability of $p_0 = x/x(1-q)^{-1} = (1-q)$ that a given mating will involve a previously unmated female. Similarly, the expected number of matings involving a once-mated female will equal xq. Hence, the probability that a given mating will be with a once-mated female will equal $p_1 = q(1-q)$. It can thus be shown that the probability that a given mating will be with a female that has mated exactly N times previously will equal $p_N = q^N(1-q) = e^{-m\tau N}(1 - e^{-m\tau})$. Using this and expressing sperm viability in terms of mortality rate ($v = e^{-\mu\tau}$, $\hat{v} = e^{-\hat{\mu}\tau}$) in equation, we get

$$\phi(\{\mu, \hat{\mu}\}, \{s, \hat{s}\}) = \sum_{N=0}^{\infty} e^{-m\tau N}(1 - e^{-m\tau}) \sum_{n=0}^{\infty} e^{-m\tau n} \times$$

$$\int_0^\tau \frac{se^{-\mu\tau n}e^{-\mu t}}{se^{-\mu\tau n}e^{-\mu t} + \left[\frac{\hat{s}}{1-e^{-\hat{\mu}\tau}}(1 - e^{-\hat{\mu}\tau(N+n+1)}) - \hat{s}e^{-\hat{\mu}\tau n}\right]e^{-\hat{\mu}t}} e^{-mt}\, \mathrm{d}t.$$

$$(4)$$

The term inside the first summation sign gives the probability that a male will mate with a female that has mated N times previously. The term inside the next summation

sign gives the probability that a female will survive n additional matings, and the expression within the integral sign gives mutant male fertilization success in the time between the nth additional female mating and the next possible one. Equation (4) describes the fertilization success of a mutant male potentially differing from the resident population both with respect to sperm number $s \neq \hat{s}$ and sperm mortality rate $\mu \neq \hat{\mu}$. Here I assume both sperm number and viability to be costly and will next consider two potential trade-offs: (i) a direct trade-off between sperm number and viability and (ii) a trade-off between investment in winning fertilizations and investment in obtaining matings.

Direct Trade-Off between Sperm Number and Viability

In the simplest case, there is a direct trade-off between sperm number and viability. There is some empirical evidence for such a trade-off (see Levitan 2000; Helfenstein et al. 2008). Let us assume that the cost (D) of each sperm cell is a function of the expected sperm lifetime. Furthermore, we assume that the resources available for sperm production are limited, and this limitation is given by the expression $sD = \alpha$, where α represents the reproductive resources allocated to sperm per mating. In this model, the expected sperm longevity equals $1/\mu$, thus $D = f(1/\mu)$. Here I will assume $D = \beta (1/\mu)^a$. The parameter a specifies whether the cost of sperm is a decelerating ($\alpha < 1$), linear ($\alpha = 1$), or accelerating ($\alpha > 1$) function of sperm longevity. We see that $s = \alpha/D$ and therefore $s = \mu^a \alpha / \beta$. Because this model does not assume female sperm limitation, sperm amount is only measured in relation to the sperm amount of other males. The parameters α and β therefore cancel out in all equations. Hence, for simplicity, it suffices to assume $s = \mu^a$, which can be inserted into equation (4) to get the fitness function $w(\mu, \hat{\mu}) = \phi(\mu, \hat{\mu})$.

Trade-Off between Investment in Winning Fertilizations and Investment in Obtaining Matings

Here we assume that there is no direct trade-off between sperm number and viability, but a trade-off between investment in winning fertilizations (i.e., ejaculate investment) and investment in obtaining matings (for empirical evidence see Warner et al. 1995; Danielsson 2001; Evans 2010; Simmons et al. 2010; Engqvist 2011). As in many previous sperm competition models (e.g., Ball and Parker 1996; Parker et al. 1996, 1997; Tazzyman et al. 2009), this trade-off is expressed as $R = n(c + \alpha)$. Here R is the males' fixed total energy budget, n is the number of matings, c is the cost of achieving matings, and α (as above) is the cost of the ejaculate. The relative mating success of

a mutant male in a population consisting of resident males can thus be expressed as

$$\tilde{n}(\alpha, \hat{\alpha}) = \frac{R/(c + \alpha)}{R/(c + \hat{\alpha})} = \frac{c + \hat{\alpha}}{c + \alpha}. \tag{5}$$

As above, the cost of the ejaculate (α) is the product of the number of sperm and the cost of each sperm. Thus $\alpha = sD = s\beta(1/\mu)^a$. By expressing the cost of achieving matings in units of β, hence $c/\beta = \tilde{c}$, we get

$$\tilde{n}(\{\mu, \hat{\mu}\}, \{s, \hat{s}\}) = \frac{\tilde{c} + \hat{s}\hat{\mu}^{-a}}{\tilde{c} + s\mu^{-a}}. \tag{6}$$

Male total reproductive success will be given by the number of matings times fertilization success per mating, thus by $w(\{\mu, \hat{\mu}\}, \{s, \hat{s}\}) = \tilde{n}(\{\mu, \hat{\mu}\}, \{s, \hat{s}\}) \times \phi(\{\mu, \hat{\mu}\}, \{s, \hat{s}\})$, where ϕ and $\tilde{n}$ are given in equations (4) and (6), respectively. The first necessary criteria for an evolutionarily stable strategy ($x^* = \{\mu^*, s^*\}$) are that

$$\left. \frac{\partial}{\partial \mu} w(\{\mu, \hat{\mu}\}, \{s, \hat{s}\}) \right|_{\substack{s = \hat{s} = s^* \\ \mu = \hat{\mu} = \mu^*}} = 0 \tag{7}$$

and

$$\left. \frac{\partial}{\partial s} w(\{\mu, \hat{\mu}\}, \{s, \hat{s}\}) \right|_{\substack{s = \hat{s} = s^* \\ \mu = \hat{\mu} = \mu^*}} = 0. \tag{8}$$

Numerical Solutions

It was not possible to find analytical solutions of the potential ESS for either of the two models that resulted from assuming different trade-offs. Instead, I applied an iterative method using a Newton-Raphson procedure to find the values of μ^* and s^* that satisfied equation (7) and thus represented evolutionarily singular strategies. In all cases, this method converged quickly. Subsequently, invasion and convergence stability criteria (see Eshel 1983; Dieckmann and Law 1996; Geritz et al. 1998; Leimar 2009) were evaluated numerically. In all cases, it was verified that equilibria were both evolutionarily and convergence stable.

Results

The major interest here is the ESS sperm viability in relation to female mating frequency. Female number of matings is ultimately determined by two parameters: the time between matings (τ) and female mortality rate (m). The latter will determine the average length of the female reproductive period. To reduce the number of parameters,

it is convenient to measure time in units of the expected length of the female reproductive period m^{-1}. By defining the new parameters $\tilde{\mu} = \mu m^{-1} = \mu/m$, $\tilde{\tau} = \tau/m^{-1} = \tau m$, and $\tilde{t} = tm$ (in contrast to τ and t, μ is a rate), m will, as a consequence, cancel out in all equations. We are thus left with two parameters influencing the resulting ESS, the remating time $\tilde{\tau}$ determining the average female number of matings $1/(1 - e^{-\tilde{\tau}})$, and a describing the cost of sperm production as a function of sperm viability.

### Direct Trade-Off between Sperm Number and Viability

The results of the first model with a direct trade-off between sperm viability and sperm number are shown in figure 2. Most importantly, the ESS sperm mortality rate will increase with an increase in female mating rate, thus leading to less viable sperm. This general pattern is independent of the parameter a, which describes the cost of producing highly viable sperm. However, it does affect the overall investment in sperm viability. The higher the cost of sperm viability (a), the higher the sperm mortality rate will be (fig. 2a). The adjustment of sperm mortality in response to female mating frequency has an interesting effect on the number of surviving sperm between matings (fig. 2b). As expected, if females remate more rapidly, a larger proportion of sperm will survive between matings. However, at higher female remating rates, the intermating sperm survival (v) soon seems to reach an asymptote, where the effect of time reduction between matings is

counterbalanced by the change in sperm mortality rate, and over evolutionary time an increased remating rate does not lead to a considerable increase in sperm survival rate between matings. Overall, sperm survival between matings is much lower than would be expected if sperm viability would not evolve in relation to female mating frequency (fig. 2b).

Trade-Off between Investment in Winning Fertilizations and Investment in Obtaining Matings

The second model, which assumed an allocation trade-off between ejaculate investment and investment in achieving matings, produced results that were identical to those of the direct trade-off model concerning the ESS with respect to sperm viability. At first, this outcome seems unreasonable. However, whenever the population is at an ESS with respect to sperm amount s, this term cancels out in equation (4). Thus the ESS sperm mortality rate is, first and foremost, dictated by female mating rate, and the trade-off details between investment in sperm viability and other aspects of male reproductive success are less important.

In the second model, we can also draw some conclusions on overall ejaculate investment. This can conveniently be expressed as the ESS relative ejaculate expenditure, measured as the proportion of total reproductive effort that is spent on the ejaculate (see Ball and Parker 1996; Parker et al. 1996, 1997), and thus $s^* \mu^{*-a}/(\tilde{c} + s^* \mu^{*-a})$. As expected, an increase in female mating frequency is predicted to lead
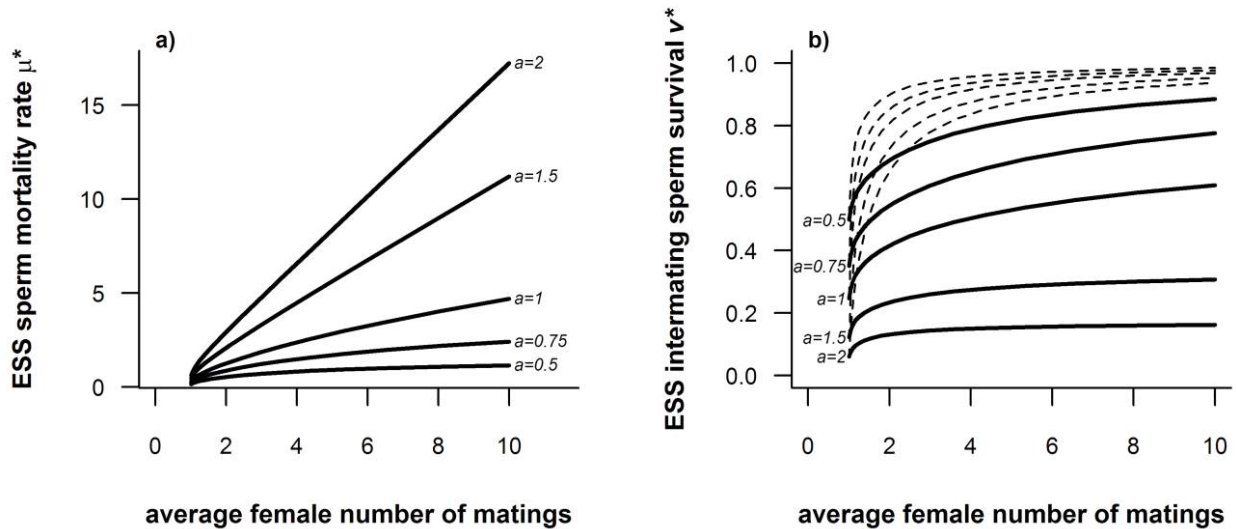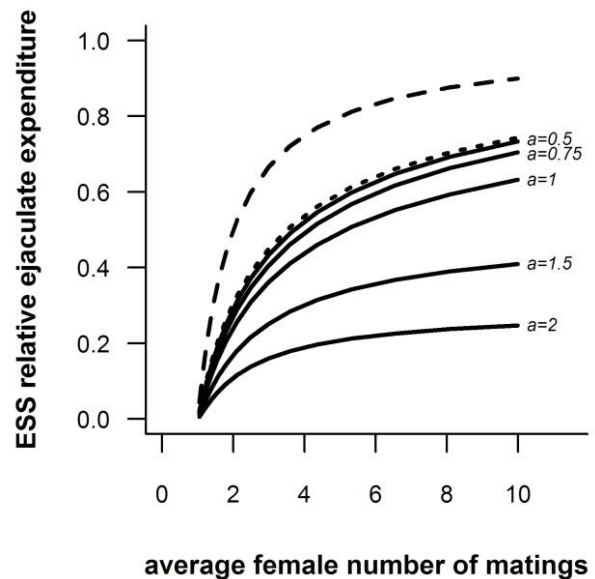

Figure 2: Results showing (*a*) the evolutionarily stable strategy (ESS) for sperm mortality rate μ^* in relation to average female number of matings $1/(1 - e^{-m\tau})$ ($= 1/(1 - e^{-\tilde{\tau}})$) for different values of the parameter *a*. In *b*, μ^* is expressed as the proportion of sperm surviving between female matings (i.e., $v^* = e^{-\mu^*\tau}$). The dashed lines depict expected values if sperm mortality remains at values predicted at minimal levels of sperm competition (average female number of matings equals 1.01).

to an increase in relative ejaculate investment (fig. 3; see also Parker 1998; Williams et al. 2005; Engqvist and Reinhold 2006; Fromhage et al. 2008). Interestingly, relative ejaculate expenditure decreases with increasing values of *a* and thus decreases with an increasing cost of sperm viability (fig. 3). Presumably, this is because ESS sperm mortality rate changes with *a*. Thus, the parameter *a* will change the effective level of sperm competition. This will be higher the lower the value of *a*, because then sperm mortality rate will be very low. Thus, as *a* approaches zero, relative ejaculate expenditure converges towards values that are equal to those predicted from a model without sperm mortality (see appendix; fig. 3).

Discussion

The analyses here demonstrate that, in species and/or populations in which females mate with many males, sexual selection is predicted to drive sperm viability to lower levels, compared with sperm viability in species and/or populations in which females mate less frequently. This particular result did not depend on whether sperm viability is traded off against sperm number or mating success against investment in ejaculates. This contrasts with the verbal predictions that have been presented so far (García-González and Simmons 2005; Pizzari et al. 2008; Pizzari and Parker 2009) and also with the limited empirical evidence at hand (Hunter and Birkhead 2002).

At first glance, it might seem a highly unreasonable result that an increase in female mating rate, and thus an increase in the level of sperm competition, would lead to a decrease in the benefit of viable sperm. How can we make more sense out of this prediction? If females mate frequently, males are very likely to face sperm competition. Investing in highly viable sperm is analogous to investing in future fertilization opportunities. However, when females have a high remating rate, these future fertilization chances will be continuously decreasing, because a male's sperm will compete against an increasing number of rival sperm. In analogy with a lottery, the value of buying extra tickets in a raffle for a fixed prize is greater if there are fewer competitors (Parker 1998). The number of competing sperm will be lower at the time of mating than it will be after future female matings. Hence, the more males that a female will mate with in the future, the steeper the increase in sperm competition intensity and the less beneficial it will be to invest in viable sperm that can fertilize eggs in the future. On the other hand, if females mate at a very low frequency, most male matings will be with virgin females. Sperm competitiveness will thus be less important for male reproductive success. In this case, the only reason to invest any excess energy in sperm is to enable them to

Figure 3: Results showing the evolutionarily stable strategy (ESS) for relative ejaculate expenditure $s^*\mu^{*-a}/(\tilde{c} + s^*\mu^{*-a})$ in relation to average female number of matings $1/(1 - e^{-m\tau})$ $(= 1/(1 - e^{-\tilde{\tau}}))$ for different values of the parameter *a*. The dotted line shows the result assuming an ESS sperm mortality rate of $\mu^* = 0.001$. This curve cannot be distinguished from the theoretical predictions assuming no sperm mortality (see appendix). In comparison, the dashed line shows the ESS relative ejaculate expenditure predicted from the intensity model (Parker et al. 1996).

survive until the female remates and sperm competition occurs.

In the models presented here, a fair raffle was assumed. However, the above line of reasoning will not change if different sperm competition mechanisms are assumed (i.e., sperm precedence). In this case, future fertilization opportunities will also decrease with an increase in female mating rate. However, they will do so more strongly when second- or last-male sperm precedence occurs and less strongly when first-male precedence occurs. Consequently, one would expect sperm ageing to become more prominent moving from first-male advantage, to a fair raffle, to last-male sperm precedence. Nevertheless, more precise predictions must await future research.

Evolutionary research regarding sperm viability is still a relatively recent development (Holman 2009*b*). Therefore, there are relatively few empirical data on the relationship between female polyandry and sperm viability. In a comparative study, Hunter and Birkhead (2002) demonstrated convincingly that sperm viability, measured as the proportion of living sperm in an ejaculate, was considerably higher in polyandrous species than in monandrous species. This outcome seems to be in sharp contrast

to the outcome of my analysis. However, here we must differentiate between different properties of sperm viability: the proportion of living sperm in an ejaculate versus the ability of sperm to maintain its function after ejaculation. The proportion of living sperm in an ejaculate at the time of mating will be affected by, for instance, quality control during spermatogenesis and sperm mortality within the male. Actually, this is a sperm amount property (closely related to s in the current model), because it determines how many functional sperm a male will transfer. With increasing female mating frequency, there will be an increasing selection on this precopulatory aspect of sperm quality (Parker et al. 1996, 1997), and this might be what the study by Hunter and Birkhead (2002) demonstrates. However, this must not necessarily be associated with the ability to survive within the female sperm storage organ (see also Pizzari et al. 2008), which is the attribute of sperm viability analyzed in the present model. The synthesis of this and previous sperm competition models (reviewed in Parker 1998; Parker and Pizzari 2010) is thus that, compared with relatively monandrous species, males in polyandrous species are predicted to produce a larger number of living sperm, but with a higher mortality rate after ejaculation within the female.

To test the present model, empirical studies must take this time perspective into account. However, in internally fertilizing species, it is difficult to measure the viability of sperm in storage without, at the same time, disrupting that storage (Stewart et al. 2007). Measures of sperm survival are thus either made in vitro (Helfenstein et al. 2008) or must rely on a very small number of time observations (Locke and Peng 1993; Bernasconi et al. 2002; Phiancharoen et al. 2004; Civetta et al. 2008; Holman 2009*b*) and, in most cases, on a single observation (Hunter and Birkhead 2002; Snook and Hosken 2004; García-González and Simmons 2005). However, to make a survival analysis of sperm, and thus to measure the mortality rate, we need repeated measurements of sperm survival from the same ejaculate. This will be a major challenge for future studies on sperm viability.

In the present model, a scenario with internal fertilization was explicitly assumed. Related theoretical models have been made for situations with external fertilization (Ball and Parker 1996). Here, the situation is fundamentally different, because inherently the prospect of future fertilization success will decrease strongly as soon as most eggs have already been fertilized. The predictions, however, are similar in both systems. For species with external fertilization, Ball and Parker (1996) predicted that the males' investment in sperm survival should decrease with an increasing average number of males participating in an spawning event. Everything else being equal, the more competitors, the faster all eggs will already be fertilized,

and the less beneficial it will be investing in long-lived sperm (but see Fitzpatrick et al. 2009). Thus, in both internal and external fertilizers, an increase in the number of males that females mate with leads to a decrease in the prospects of future fertilization chances and, hence, to a lower benefit of long-lived sperm. Nevertheless, the mechanisms responsible for the respective outcomes are fundamentally different.

A critical assumption of the model presented here is that fertilizations are not limited by the amount of sperm present in the females' sperm storage organs. In some natural systems, sperm limitation has been reported to occur (see Wedell et al. 2002); thus, this assumption can sometimes be violated. A crucial question concerns how the outcome of this analysis is influenced by this assumption. At high female mating rates, sperm limitation is an unlikely event, and consequently it should not have a large influence. However, at low female mating rates, female sperm limitation might be a strong selective force. But will this influence the conclusions drawn here? It is apparent that, in mating systems in which females usually mate with one male exclusively, there will be weak sexual selection on sperm viability because of the absence of sperm competition. Yet, here natural selection will act on sperm survival; enough sperm should survive for as long as most females survive and reproduce, because otherwise males would lose fertilization opportunities. This effect of sperm limitation on selection on sperm viability should be weaker the more frequently that females mate. Thus, a first naive prediction would be that the additional effect of female sperm limitation would reinforce the effect of female mating rate on sperm viability found here.

Nonetheless, even though sperm limitation might not change the qualitative predications from this model, it might well change the quantitative predictions. Furthermore, because sperm limitation will influence the benefit of female remating, it will most certainly change the underlying model dynamics. Here, I assumed a fixed female and male mating rate independent of the male strategies. This assumption is justified (but see Williams et al. 2005 and Fromhage et al. 2008 for alternative approaches) as long as male reproductive behavior does not influence female reproductive success. However, if females are sperm limited, changes in sperm viability will directly influence females as well. As seen here, an increase in female mating frequency would, in the long run, lead to lower levels of sperm viability. Thus, if there is selection on females to remate for viable sperm, this will lead to an even higher sperm mortality rate. The reduction in sperm viability will most likely not lead to even higher levels of sperm limitation, but it will drastically affect the strength of selection on female multiple mating (fig. 2). Furthermore, as soon as male sperm viability is adjusted to female mating rate,

a future decrease in female mating rate will become less likely. If this scenario turns out to be true, the evolution of female polyandry might be regarded as an evolutionary one-way street. To gain additional insights into these interesting processes, future models should therefore also aim to take female sperm limitation, the dynamic of male sperm investment, and changes in the level of female polyandry into account.

APPENDIX

Ejaculate Expenditure with Low Sperm Mortality

When there is no sperm mortality, the reproductive success of a mutant male in a resident population is characterized by the equation

$$w(s, \hat{s}) = \frac{c + D\hat{s}}{c + Ds} \sum_{N=1}^{\infty} p_N \frac{s}{s + (N-1)\hat{s}}, \tag{A1}$$

where p_N is the probability that a male mating will result in competition between the sperm of N different males. The ESS can be found using conventional techniques, such as

$$\frac{\partial}{\partial \mu} w(s, \hat{s}) \Big|_{s=\hat{s}=s^*} = 0 \Leftrightarrow -\frac{D}{c + Ds^*} \sum_{N=1}^{\infty} p_N \frac{1}{N} +$$

$$\sum_{N=1}^{\infty} p_N \frac{N-1}{N^2 s^*} = 0. \tag{A2}$$

The ESS expressed as relative ejaculate expenditure can be found as

$$\frac{Ds^*}{c + Ds^*} = \frac{\sum\limits_{N=1}^{\infty} p_N [(N-1)/N^2]}{\sum\limits_{N=1}^{\infty} p_N N^{-1}}. \tag{A3}$$

This is the general solution to the ESS $Ds^*/(c + Ds^*) = (N-1)/N$, found in Parker et al. 1996, which is the special case with no variation in expected sperm com-

petition intensity (i.e., when competition is always exactly between N ejaculates). It is also the general solution to the risk model ESS $Ds^*/(c + Ds^*) = q/2$ shown by Parker et al (1997), where q represents the proportion of eggs that are fertilized in competition with a rival male. In the present model, female number of matings follows a geometric distribution, and this determines p_N in the equation above. When sperm mortality is close to zero, the equation describing male reproductive success can be simplified to

$$w(s, \hat{s}) = \frac{c + D\hat{s}}{c + Ds} \sum_{N=0}^{\infty} (e^{-m\tau})^N (1 - e^{-m\tau}) \times \tag{A4}$$

$$\sum_{n=0}^{\infty} e^{-mn\tau} \int_0^{\tau} \frac{s}{s + (N+n)\hat{s}} e^{-mt} \, dt.$$

Hence, the ESS relative ejaculate expenditure can be expressed as

$$\frac{Ds^*}{c + Ds^*} = \frac{\sum\limits_{N=0}^{\infty} e^{-m\tau N} \sum\limits_{n=0}^{\infty} e^{-m\tau n} [(N+n)/(N+n+1)^2]}{\sum\limits_{N=1}^{\infty} e^{-m\tau N} \sum\limits_{n=0}^{\infty} e^{-m\tau n} (N+n+1)^{-1}}. \tag{A5}$$

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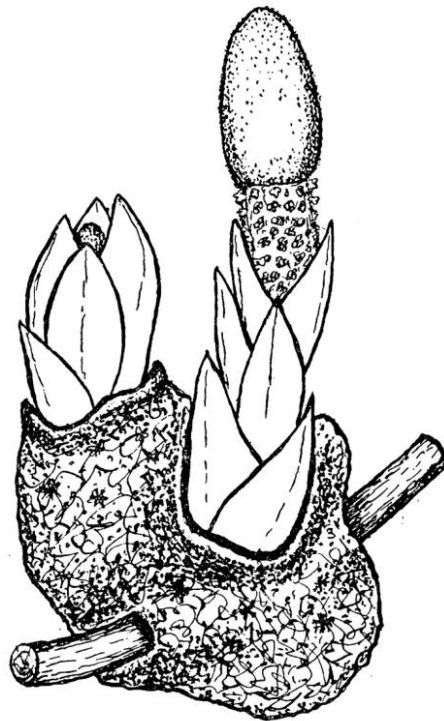
Balanophora hildendrandtii, a native of the Comoro Islands. "Some of them have large and showy flowers and some of them have so great a resemblance to fungi that the older botanists regarded them as such. All of them are parasitic upon roots of woody hosts." From "The Phenogamous Parasites" by Charles A. White (*American Naturalist*, 1908, 42:12–33).