Theoretical influence of female mating status and remating propensity on male sperm allocation patterns

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Abstract

Theoretical models predict that males should allocate more sperm in matings where the immediate risk of sperm competition is high. It has therefore often been argued that males should invest less sperm in matings with virgin females compared with matings with already mated females. However, with relatively polyandrous females, high sperm competition risk will covary with high sperm competition intensity leading to more unpredictable conditions, as high competition intensity should favour smaller ejaculates. With the use of a genetic algorithm, we found that males should allocate more sperm in matings with virgin females when female mating frequency is relatively high, whereas low remating rates will select for higher effort in matings with nonvirgin females. At higher remating rates, first male sperm precedence favours larger ejaculates in matings with virgin females and second male precedence favours the reverse. These results shed some light on several findings that have been difficult to explain adaptively by the hitherto developed theory on sperm allocation.

Introduction

The realization that females of many animal species often mate with several males within the same reproductive period, with sperm competition as a result (e.g. Parker, 1970), has had enormous influences on evolutionary theory in the last decades. Sexual conflict over matings and fertilizations, cryptic female choice, variance in male post-copulatory reproductive success are all fields of research which have attracted considerable interest from students of sexual selection (for reviews see Eberhard, 1996; Simmons, 2001; Arnqvist & Rowe, 2005).

A fruitful sub-discipline of research on sperm competition, often termed sperm competition games, concerns male total allocation of reproductive investment and strategic male allocation of sperm in response to sperm competition. If sperm compete numerically, males transferring large ejaculates with many sperm will have an increased fertilization gain in specific matings compared with other males. However, as sperm production

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costs are nontrivial (e.g. Dewsbury, 1982; Nakatsuru & Kramer, 1982; Olsson *et al.*, 1997), allocation of sperm in one mating is likely to affect sperm resources available for subsequent matings. Furthermore, as the gain of investing sperm is likely to differ between matings, sperm competition will often select for male strategic allocation of sperm (see Parker, 1998; Wedell *et al.*, 2002). Several factors have been shown to have an influence. First, if females differ for instance in fecundity, males are expected to increase investment with increasing female quality (Galvani & Johnstone, 1998; Parker *et al.*, 1999; Engqvist & Sauer, 2001; Reinhold *et al.*, 2002). Second, matings may differ with respect to the number of other sperm the focal male's sperm have to compete against in the raffle for fertilizations, and this has been shown to have profound effects on male mating decisions. Theoretical analyses of variation in the level of sperm competition have focused on two discrete situations, variation in sperm competition risk (i.e. probability that sperm competition between different ejaculates will occur) and variation in sperm competition intensity (the number of competing ejaculates). These analyses have shown that males should allocate more sperm in matings with an increased risk of sperm competition if males are able to differentiate between situations of, for instance, high vs.

low or certain vs. mean risk (Parker, 1990b, Parker et al., 1997; Ball & Parker, 1998). Furthermore, if males are able to adequately assess the number of competing ejaculates, males should allocate less sperm with an increasing intensity of sperm competition above the level of one competing ejaculate. In the case of exactly one competing ejaculate males should invest maximally (Parker et al., 1996).

In mating systems with internal fertilization and frequent female multiple mating (i.e. more than twice), a formal analysis of how males should respond to variance in female mating status (e.g. virgin vs. mated vs. multiply mated) has not yet been attempted. It may appear that it should be straightforward to draw conclusions on this issue from the analyses previously mentioned (Parker, 1990b; Parker et al. 1996, 1997). But this is only partially true. If females maximally mate with two males or at least very rarely mate more than twice, it is obvious that males should invest more sperm in matings with already mated females compared with matings with virgin females. The reason is that mated females represent a certain risk of sperm competition whereas virgin females represent a lower risk of sperm competition which equals the average remating probability in the female population. This situation has already been thoroughly and adequately analysed (Parker et al., 1997; Ball & Parker, 1998). However, there is an entirely different situation if there is a possibility that females may mate more than twice before egg laying. In this case virgin females still represent a lower risk of sperm competition compared with females that have already mated. However, if females remate again with a reasonably high probability virgin females on average represent situations of lower sperm competition intensity than already mated females (see also Engqvist & Reinhold, 2005). The justification of the previous statement is motivated by the rules of conditional probability: the probability that a female will mate at least x times given that the female has already mated once is equal to or higher than the probability that a female will mate x times given that the female has not already mated, or mathematically:

$$P(x|x \geq 1) \geq P(x)\{x \geq 1\}.$$

Therefore, in situations of frequent female multiple remating, predictions from sperm competition intensity models may seem appropriate, and we would expect males to invest more sperm in matings with virgin females. The sperm competition intensity model by Parker et al. (1996) was originally developed to mimic the mating system of group spawners with external fertilizations. It is of course generally possible to extend the predictions from this model and make predictions on situations with sequential copulations and internal fertilization. The analysis of male response to female mating status in these systems is however likely to violate several critical assumptions of the original sperm

competition intensity model. Most importantly, an information asymmetry between males regarding probable number of competing ejaculates is likely to exist in the situation discussed here. Males mating with a virgin female will have less of information on the intensity of sperm competition at egg laying than a male, which 'knows' that the female has already mated once or twice. This is different from the original model of external fertilizers in which all males are assumed to have equal information, a realistic assumption under these circumstances.

This discrepancy between predictions from the two models on the one hand and between model assumptions and realistic conditions on the other hand motivated us to perform an analysis on optimal sperm allocation in relation to female mating status in species with sequential copulations, subsequent sperm storage and internal fertilization. We assume one parameter, average female remating propensity, which determines both the risk and intensity of sperm competition. Our model thus provides a general framework on how both variation in risk and intensity of sperm competition can be combined in one analysis. We assume that males can assess female mating status and analyse how this should influence male sperm allocation for different levels of female remating propensity. A fair raffle (e.g. Parker, 1990a; Parker et al., 1997) for fertilization was assumed in the intensity model (Parker et al., 1996) of group spawner behaviour. In situations of sequential copulations, however, male mating order often has considerable effects on paternity success (Boorman & Parker, 1976; Simmons & Siva-Jothy, 1998). The magnitude of this mating order effect on paternity is often expressed in terms of the P_2-value, which is defined as the proportion of offspring sired by the second male to mate in controlled double-mating trials (Boorman & Parker, 1976). This pattern of sperm precedence may also affect optimal sperm allocation depending on which 'role' (e.g. mating first or mating last) a male will have. For instance in the analysis of sperm competition risk by Parker et al. (1997), the relative difference between sperm allocation in matings with virgin and already mated females was predicted to be higher the more paternity is biased towards the first male to mate. We therefore also included variation in sperm precedence in our analysis.

Materials and methods

Basic model assumptions

In our model all females are assumed to mate at least once. Subsequent to this mating females are assumed to remate with a different male with a probability of q. Thus $(1 - q)$ represents the probability that a female following this mating will produce offspring and never mate again. Further, twice-mated females are again assumed to

remate with another male with the same probability q and so on. Hence, female mating frequency will follow a geometric distribution, and the probability that a female will mate i times $[P(i)]$ is represented by the formula:

$$P(i) = (1 - q)q^{i-1}. \qquad (1)$$

Thus in our model, the propensity of a female to remate will shape both the average risk and intensity of sperm competition for males.

Males are assumed to follow a heritable strategy $I = \{s_0, s_1, \ldots, s_i\}$, where s_i denotes sperm expenditure in each mating with females of different mating status. Males were assumed to have a fixed and identical amount of resources for reproduction. Further, analogous to previous models (Parker, 1998), we assumed a trade-off between sperm expenditure and mating success. There is some empirical evidence supporting this assumption (e.g. Warner *et al.*, 1995; Danielsson, 2001). The relative mating success n_r of a mutant male J, whose strategy deviates from an evolutionary stable strategy (ESS, e.g. Maynard Smith, 1982) $I^* = \{s_0^*, s_1^*, \ldots, s_m^*, \ldots\}$ only by $s_j \neq s_j^*$ in copulations with females of mating status j were assumed to equal

$$n_r(s_j, I^*) = \frac{1 - (p_j s_j + \sum p_i s_i^* - p_j s_j^*)}{1 - \sum p_i s_i^*}, \qquad (2)$$

where the values of p_i denote the probabilities that a male mates in contexts of i expending the sperm amount s_i. This trade-off between effort spent on sperm and effort spent on obtaining matings is similar to some previous models (Parker, 1982, 1990a, b). We also assumed an equal sex ratio. Most importantly this means that assumed mean female mating frequency will equal mean male mating frequency $\bar{n}$. Therefore, female mating frequency will not only determine risk and intensity of sperm competition but also determine average male mating success. It can easily be shown that

$$\bar{n} = \frac{1}{1 - q} \qquad (3)$$

and, thus the equation

$$n(s_j, I^*) = \frac{1 - (p_j s_j + \sum p_i s_i^* - p_j s_j^*)}{1 - \sum p_i s_i^*} \times \frac{1}{1 - q} \qquad (4)$$

will express total male mating success.

Individual male fertilization success (v) following each mating will depend on how many sperm a male expends on a specific mating in relation to the total amount of sperm present in the female sperm storage organ at fertilization. Hence, under all our assumptions sperm compete numerically. Nevertheless, male mating order with a female may affect fertilization. In our simplest model, we assume a 'fair raffle' between sperm, but in further models we allow a certain degree of unfairness [(loaded raffle) (see e.g. Parker, 1990a; Parker *et al.*, 1997)] in the raffle depending on the order in which males mate with a female.

Male sperm allocation and fertilization success in different situations will further depend on how accurate they (and other males) may assess female mating status. We model three different situations: (i) males are unable to assess female mating status, (ii) males can differentiate between virgin and already mated females only and (iii) males are able to differentiate between virgin, once-mated and multiply mated females. In the first situation there is only one strategy for each male. The mating status of females will affect the fertilization success of males. We must therefore first estimate the probability that males will compete against a certain number of other ejaculates in order to compute the fertilization of males following different strategies. In a population consisting of x females, eqn 1 gives us that there will be

$$x \times \sum_i i \times (1 - q)q^{i-1} = \frac{x}{1 - q} \qquad (5)$$

matings. As all females mate exactly once as virgins, there will be x matings with virgin females, and thus a probability of

$$\frac{x}{x/(1 - q)} = 1 - q \qquad (6a)$$

that a given female will be virgin at the time of mating. Similarly, the number of matings involving a once mated female will equal xq. Thus, the probability that a given female will have mated exactly once will equal $q(1 - q)$. It can be shown that the probability to mate with a female that have mated exactly i times will equal

$$P(i) = (1 - q)q^i. \qquad (6b)$$

However, females will remate with a probability of q. Thus, in a fair raffle the average fertilization success of a male $[v(s, s^*)]$ mating with a female having E offspring will equal:

$$v(s, s^*) = E\left[(1 - q)^2 + 2q(1 - q)^2 \frac{s}{s + s^*} + \cdots \right.$$
$$\left. + kq^{(k-1)}(1 - q)^2 \frac{s}{s + N_{k-1}s^*} + \cdots \right]. \qquad (7)$$

The first term $[(1 - q)^2]$ is the probability that the female will be virgin $(1 - q)$ and will not mate again $(1 - q)$, hence the male will sire all the offspring. The second term $[2q(1 - q)^2]$ is the sum of two probabilities. Either the female is virgin $(1 - q)$ and will mate exactly once more $[q(1 - q)]$ or the female had already mated with one male $[q(1 - q)]$ and will not mate again $(1 - q)$. Thus in this case the male's sperm will compete against the sperm from exactly one other male and will sire $[s/(s + s^*)]$ of the female's offspring, and so on.

In the situation where males are able to tell the difference between virgin and mated females only, the male sperm allocation strategy will constitute two values $[I = \{s_0, s_1\}]$. Let s_0 denote the number of sperm allocated

to matings with virgin females and s_1 the number of sperm in matings with already mated females. A male mating with a virgin female will then have the fertilization success

$$v_0(s_0, s_1^*) = E\left[(1-q) + q(1-q)\frac{s_0}{s_0 + s_1^*}\right.$$
$$\left. + \cdots + q^k(1-q)\frac{s_0}{s_0 + N_k s_1^*} + \cdots\right], \quad (8a)$$

whereas the fertilization success of a male mating with an already mated female can be expressed by

$$v_1(s_1; s_0^*, s_1^*) = E\left[(1-q)^2\frac{s_1}{s_0^* + s_1} + 2q(1-q)^2\frac{s_1}{s_0^* + s_1 + s_1^*}\right.$$
$$\left. + \cdots + kq^{(k-1)}(1-q)^2\frac{s_1}{s_0^* + s_1 + N_{k-1}s_1^*} + \cdots\right].$$
$$(8b)$$

This formula is derived in analogy to eqn 7 from the fact that, given that males know that a females has mated at least once, the probability to mate with a female that has mated exactly i times previously will equal

$$P(i|i \geq 1) = (1-q)q^{i-1}, \quad (9a)$$

which may be generalized to

$$P(i|i \geq j) = (1-q)q^{i-j}. \quad (9b)$$

For instance, the probability to compete against exactly one ejaculate, in which case the male will fertilize $[s_1/(s_1 + s_0^*)]$ of the female's offspring, will equal $(1-q)^2$. This is the combined probability that a mated female will have mated exactly once previously $(1-q)$ and not remate again $(1-q)$.

Assuming that males are able to differentiate another level of female mating status will add yet another value to a males sperm allocation strategy $[I = \{s_0, s_1, s_2\}]$. In the case that males are able to differentiate virgin (s_0), once-mated (s_1) and multiply (s_2) mated females, the fertilization success of a male mating with a virgin female will average

$$v_0(s_0; s_1^*, s_2^*)$$
$$= E\left[\begin{array}{l}(1-q) + q(1-q)\dfrac{s_0}{s_0 + s_1^*} + q^2(1-q)\dfrac{s_0}{s_0 + s_1^* + s_2^*} \\ + \cdots + q^k(1-q)\dfrac{s_0}{s_0 + s_1^* + N_{k-1}s_2^*} + \cdots\end{array}\right],$$
$$(10a)$$

a male mating with a once mated female will have the fertilization success

$$v_1(s_1; s_0^*, s_2^*) = E\left[(1-q)\frac{s_1}{s_0^* + s_1} + q(1-q)\frac{s_1}{s_0^* + s_1 + s_2^*}\right.$$
$$\left. + \cdots + q^k(1-q)\frac{s_1}{s_0^* + s_1 + N_k s_2^*} + \cdots\right], \quad (10b)$$

and finally a male mating with a multiply mated female will sire

$$v_2(s_2; s_0^*, s_1^*, s_2^*)$$
$$= E\left[\begin{array}{l}(1-q)^2\dfrac{s_2}{s_0^* + s_1^* + s_2} + 2q(1-q)^2\dfrac{s_2}{s_0^* + s_1^* + s_2 + s_2^*} \\ + \cdots + kq^{(k-1)}(1-q)^2\dfrac{s_2}{s_0^* + s_1^* + s_2 + N_{k-1}s_2^*} + \cdots\end{array}\right]$$
$$(10c)$$

of the offspring, in analogy with eqns 7 and 8b.

A loaded raffle, i.e. a certain degree of unfairness in the raffle with respect to male mating order, can also be assumed in our model. Suppose each of a male's sperm count r ($r > 0$) for each sperm of the preceding male in the female mating sequence. Then for instance a male mating with a virgin female will sire

$$v_0(s_0; s_1^*, s_2^*)$$
$$= E\left[\begin{array}{l}(1-q) + q(1-q)\dfrac{s_0}{s_0 + rs_1^*} + q^2(1-q)\dfrac{s_0}{s_0 + rs_1^* + r^2 s_2^*} \\ + \cdots + q^{k+1}(1-q)\dfrac{s_0}{s_0 + rs_1^* + r^2 s_2^* + \cdots + r^{k+1}s_2^*} + \cdots\end{array}\right]$$
$$(11)$$

of the female's eggs assuming males may differentiate between virgin, mated and multiply mated females. Hence, a 'loading factor' r will represent a P_2-value of $[r/(r+1)]$ and a value $r = 1$ will thus represent a fair raffle between sperm, independent of male mating order. This assumption closely resembles the outcome of sperm competition including three males in *Drosophila melanogaster* (Morrow *et al.*, 2005).

The fitness (W) of an individual male will be a product of the number of matings secured times the fertilization gain of each mating. Thus, the fitness for a mutant male with strategy J will equal

$$W(J, I^*) = n(J, I^*) \times \sum p_i v_i(J, I^*). \quad (12)$$

Strategy I^* will be an ESS against alternative strategies J if the fitness of I^* in a population of I^* will be greater than J in the same population (Maynard Smith, 1982), thus if $W(I^*, I^*) > W(J, I^*)$.

The genetic algorithm

We used a genetic algorithm to estimate the evolutionary stable sperm allocation strategy of males. Genetic algorithms are tools used to find optima in complex systems (Holland, 1975). They are based on genetic systems and natural evolution (Sumida *et al.*, 1990; Mitchell & Taylor, 1999), which also means that genetic algorithms can be a very effective search technique to find solutions for game theory problems, such as sperm competition games (see e.g. Reinhold *et al.*, 2002), in which the optimal behaviour of a male will depend on what other males are doing.

We assumed discrete generations that consisted of populations of 100 males. In all simulations, 100 random allocation strategies (I) were generated at the beginning

of the first generation. Such a strategy comprises a certain number of values determining the sperm number a male should allocate in different situations depending on the female mating status. The number of sperm allocation values in a strategy will depend on how accurately a male can assess female mating status (see above).

The reproductive success of a male with strategy I was calculated using the formulas described above, with two slight modifications: first, the mean values of all s_i-values was used as a substitute for the different s_i^*. Second, to avoid the endless succession of a consistently smaller fraction females' remating (cf. formulae 7–11), the maximum female remating frequency was set at the smallest value m not satisfying the inequality $q^m (1 − q) > 10^{−6}$. The strategies of the 50 most successful strategies were used to generate the allocation strategies of the next generation. Preliminary strategies were first generated by randomly choosing one of the 50 selected strategies from the previous generation. With a recombination rate of 0.2 one of its strategy values (s_i) altered by selecting the corresponding value at random from one of the 50 most successful strategies. This process was repeated 100 times to result in 100 strategies.

Following selection and recombination, we randomly selected 10% (mutation rate) of all preliminary strategy values and changed them by randomly adding or subtracting a random number from a uniform distribution (±0.1). If this process, which was included to simulate mutation, rendered negative values or values larger than 100%, the respective allocation value was altered to zero or 100% respectively. The strategy values now obtained were used to calculate male fitness in the next generation and so on.

Generally, the simulations obtained an equilibrium corresponding to the ESS relatively fast (within the first 40 generations). To be on the safe side we ran all our simulations for 100 generations. A sub sample of simulations was additionally run for 500 generations. These did not differ qualitatively from the 100-generation simulations. All simulations were repeated 10 times to calculate mean values and confidence intervals for the different ESS.

We performed simulations for three different levels of male ability to discriminate female mating status: (i) males are unable to discriminate between females, (ii) males can discriminate between virgin and mated females and (iii) males are able to discriminate between virgin, once mated and multiply mated females. For each of these conditions we performed simulations assuming five different values for the 'loading factor' r (0.1, 0.5, 1, 2, 10) representing average sperm precedence. These values correspond to P_2-values in double-matings of 0.091, 0.333, 0.5, 0.667 and 0.909, respectively (1/11, 1/3, 1/2, 2/3, 10/11). We chose these values because they represent cases of a fair raffle, of medium first and last sperm precedence and extreme first and last sperm

precedence. For each of these $3 \times 5 = 15$ situations we performed simulations for the 19 values of female remating rate q ranging between 0.05 and 0.95 with an interval of 0.05. In summary we performed 285 simulations that were repeated ten times.

Results

Our simulations generated stable and highly repeatable results. Relatively small uncertainties were only found for the ESS male sperm allocation strategies in matings with mated or multiply mated females in situations of extremely low average female remating rates, or accordingly, for male sperm allocation strategies in matings with virgin females in situations of extremely high average female remating rate (cf. Fig. 2). This is because these are relatively rare situations, so selection will not act as strongly on these strategy values generating higher variance (see also Reinhold *et al.*, 2002).

Males are unable to assess female mating status

In this case a male's strategy can be expressed by one value determining how much of its resources a male should spend on sperm production and how much on traits influencing mating success. As expected, for all five different values of sperm precedence we found that males should spend an increasing amount of resources on spermatogenesis with an increasing average female remating rate (Fig. 1). Our simulations also show that with a decreasing fairness of the sperm raffle, males should spend fewer resources on sperm allocation. In addition, males should spend more resources on sperm in situations of last male sperm precedence compared with situations of equally strong first male sperm precedence.
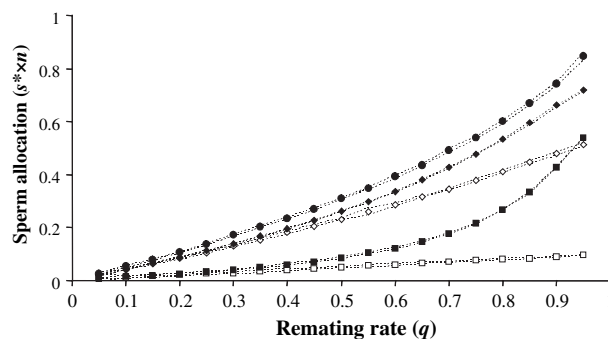

Fig. 1 Predicted influence of average female remating frequency on allocation of resources on sperm production. Each value represents the mean value from 10 simulations and the lines show the corresponding standard deviation. The five different symbols stand for five different values of loading factor r: 0.1 (open squares), 0.5 (open diamonds), 1 (closed circles), 2 (closed diamonds) and 10 (closed squares).

Males can differentiate between virgin and mated females

Our model predicts that at low female remating rates, males should always spend more sperm in matings with mated females compared with matings with virgin females (Fig. 2). The magnitude of this effect is larger the more the paternity is skewed towards the first male to mate. However, at higher but still realistic remating rates, males are under certain conditions predicted to allocate more sperm in matings with virgin females. This effect is most pronounced when there is first male sperm precedence (Figs 2a, b). In cases of last male precedence on the other hand males are always, even under extremely high remating rates, predicted to spend more on matings with mated females (Figs 2d, e). When there is a fair raffle of sperm from different males, the remating rate at which males should spend an equal amount of sperm in all matings irrespective of female mating status, is situated at a female remating rate of slightly <0.75 (cf. Fig. 2c). This corresponds to a median female mating frequency close to three and a mean female mating frequency close to four matings. At intermediate to high first male sperm precedence this point moves closer to an average female mating frequency of two ($q = 0.5$).

Males can estimate three different levels of female mating status: virgin, once-mated and multiply mated females

In situations of last male sperm precedence our model predicts males to always allocate sperm in the sequence virgin < once mated < multiply mated, irrespective of female remating rate (Fig. 3). When there is strong first
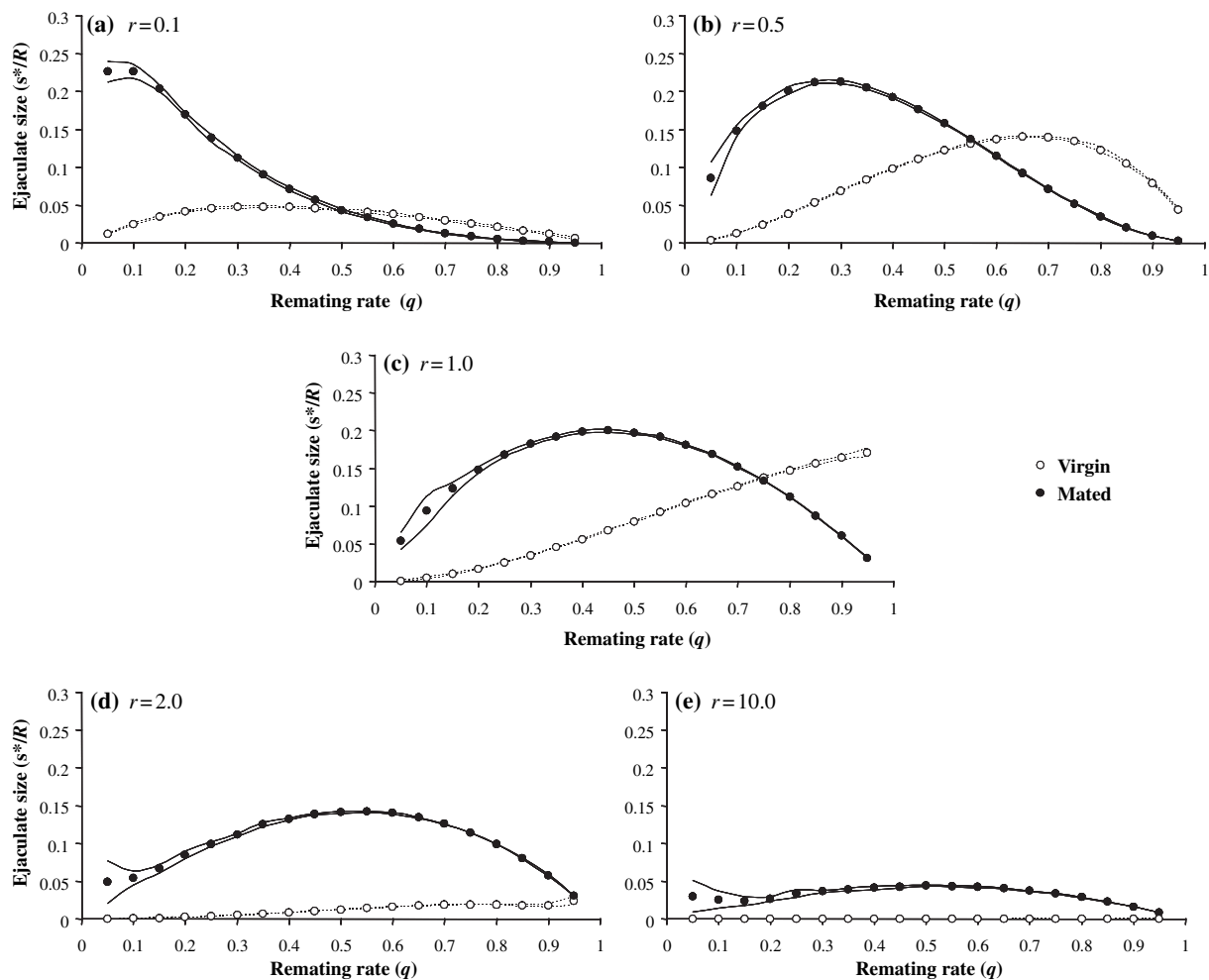

Fig. 2 Results of simulations showing optimal sperm expenditure on matings with virgin (open circles) and already mated females (closed circles) in relation to the average female remating propensity of the population. Each value represents the mean value from 10 simulations and the lines show the corresponding standard deviation. The five subfigures stand for five different levels of sperm precedence: (a) strong first male precedence, (b) medium first male precedence, (c) a fair raffle, (d) medium second male precedence and (e) strong second male precedence.
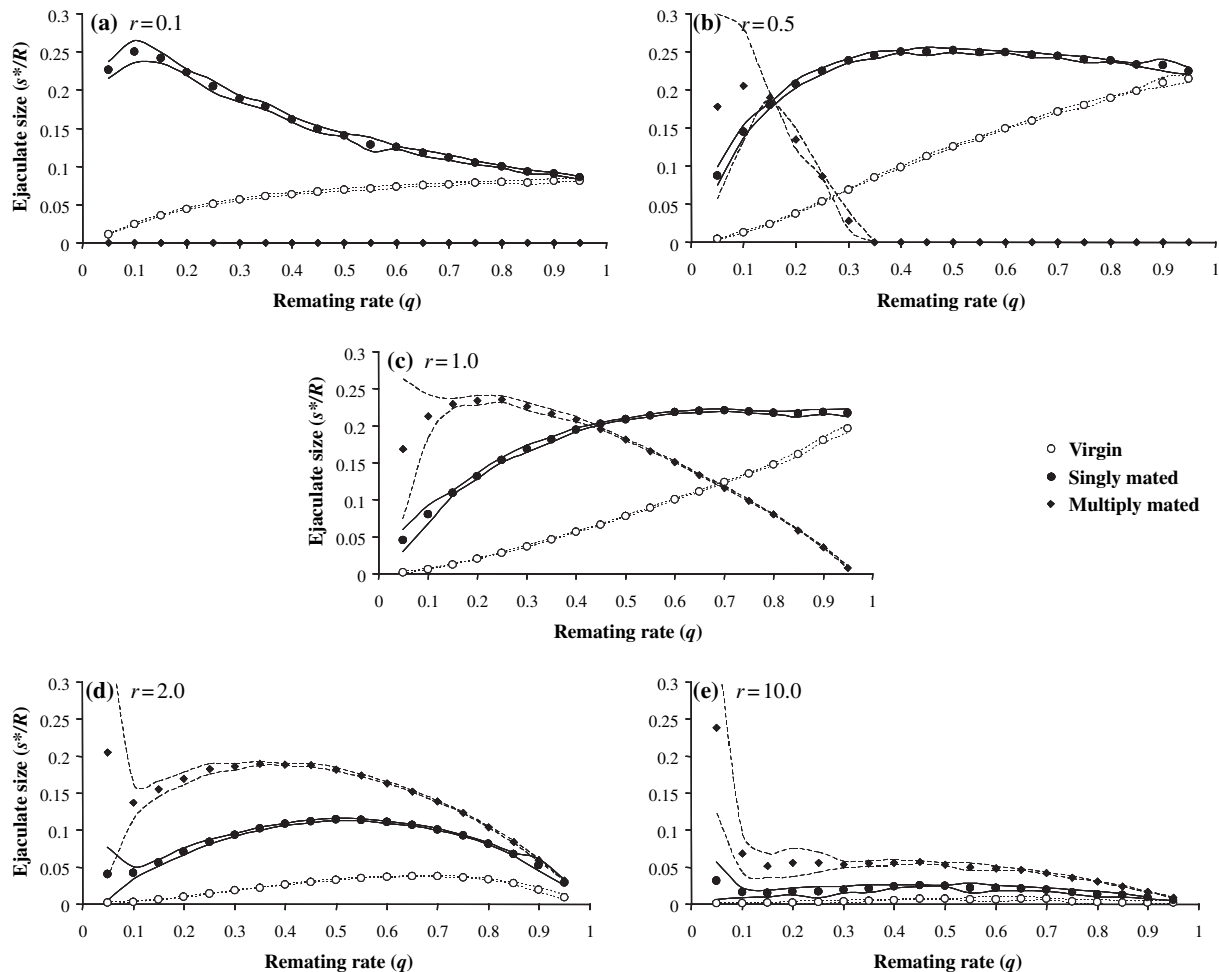
Fig. 3 Results of simulations showing optimal sperm expenditure on matings with virgin (open circles), singly mated (closed circles) and multiply mated females (closed diamonds) in relation to the average female remating propensity of the population. Each value represents the mean value from 10 simulations and the lines show the corresponding standard deviation. The five subfigures stand for five different levels of sperm precedence: (a) strong first male precedence, (b) medium first male precedence, (c) a fair raffle, (d) medium second male precedence and (e) strong second male precedence.

male precedence, on the other hand, males are predicted to reject multiply mated females. The system thus reduces to conditions similar to those assumed in sperm competition risk models (Parker *et al.*, 1997; Ball & Parker, 1998): virgin females may mate again, once mated females will not mate again. Therefore, in contrast to the previous model, males are predicted to spend a higher amount of sperm in matings with mated compared with virgin females over the whole range of female remating rates (Fig. 3a, b). In situations assuming a fair raffle or weak first male sperm precedence, the predictions become somewhat more complex. At low remating rates, males are assumed to allocate sperm in the following sequence: virgin < once mated < multiply mated, similar to the situation for last male sperm precedence. With increasing remating rates, male invest-

ment in matings with multiply mated females is predicted to continuously decrease until only a small or no amount of sperm is spent on these pairings. Therefore, under these conditions, the system equals a risk model and males are accordingly predicted to invest more in matings with once mated females compared with matings with virgin females. Nevertheless, at intermediate to high mating rates, males should allocate more to matings with virgin females than to matings with multiply mated females (Fig. 3c).

Discussion

With the use of a genetic algorithm we assessed the evolutionary stable sperm allocation strategy in response to female mating status. Our main aim was to address the

question how males should allocate their sperm reserves to matings if they are able to differentiate between already mated and virgin females only. Most importantly we found that the answer to this question depends on the average female remating propensity and the pattern of sperm precedence. As expected from previous models (Parker *et al.*, 1997; Ball & Parker, 1998), we found that the predominant pattern is to invest more in matings with mated females. Nevertheless, in contrast to what has been generally deduced from previous sperm allocation models, we found that males will be expected to invest more in matings with virgin females under a wide range of realistic conditions. Specifically, high female remating rates and first male sperm precedence will select for a higher allocation of sperm resources in matings with virgin females.

Empirical evidence

In many species, there is ample evidence that males may detect female mating status, predominantly via chemosensory cues (e.g. Mair & Blackwell, 1998; Simmons *et al.*, 2003; Siva-Jothy & Stutt, 2003; Carazo *et al.*, 2004). However thus far, empirical studies on sperm expenditure in relation to female mating status have produced very disparate results (see Wedell *et al.*, 2002). A number of studies do indeed support the predominant view that males should allocate most sperm to matings with nonvirgin females (e.g. Cook & Gage, 1995; Wedell, 1998; Wedell & Cook, 1999; Martin & Hosken, 2002), whereas in some studies no difference was found (e.g. Baur *et al.*, 1998; Bateman *et al.*, 2001). However, the opposite case, higher ejaculate expenditure in matings with virgin females, has been found in studies of the mite *Macrocheles muscaedomesticae* (Yasui, 1996), the orbweaving spider *Micrathena gracilis* (Bukowski & Christenson, 1997), the bushcricket *Decticus verrucivorus* (Wedell, 1992), the bedbug *Cimex lectularius* (Siva-Jothy & Stutt, 2003), the ceratopogonid midge *Culicoides melleus* (Linley & Hinds, 1975) and the stalk-eyed fly *Cyrtodiopsis whitei* (Lorch *et al.*, 1993). Hitherto, these data have been difficult to explain, but the predictions from our model allow interpreting these data from the perspective of optimal male sperm allocation. Furthermore, in eight of nine examined *Drosophila* species, males copulate longer with virgin than with mated females, in seven species significantly so (Snook, 1998; Singh & Singh, 2004). However, differences in copulation duration must not reflect differences in sperm transfer (for *D. melanogaster*, see Gilchrist & Partridge, 2000). For instance, longer copulations may reflect post-copulatory mate guarding (Sillén-Tullberg, 1981), or reflect an increase in the transfer of nonsperm substances in the ejaculate that will change female remating behaviour (e.g. Miyatake *et al.*, 1999). The significance of these male adaptations may differ entirely between virgin and nonvirgin matings.

Factors affecting sperm allocation

Sperm competition risk and intensity

The observed increase of sperm expenditure on virgin matings relative to nonvirgin matings with an increasing female mating frequency (q) observed from our model has at least two explanations. First, to withhold sperm in matings with virgin females should be promoted by the uncertainty of sperm competition following these matings (cf. Parker *et al.*, 1997). As sperm investment only makes sense when there is actual competition between different sperm, the proportion of virgin matings in which most of the invested sperm will be 'wasted' ($1 - q$) will be reduced as female mating frequency and thus sperm competition risk increases. Second, in our model virgin females have an expected average lifetime mating frequency of exactly one mating less than females that have already mated once, and of exactly two less than females that have already mated twice and so on, resulting in a reduced intensity of sperm competition following matings with virgin females. Crucial, however, is that males cannot distinguish between mated females. With an increasing female remating rate the discrepancy in expected sperm competition intensity between virgin and already mated females thus will strongly increase to a much higher degree than, for instance, the difference between virgin and once-mated females.

The effect of this last issue is underlined if we look at the results from the simulations in which males were assumed to be able to differentiate between once mated and multiply mated females. In this case the effect from the uncertainty of sperm competition risk following matings with virgin females was under no circumstances balanced by the effect from the marginally higher sperm competition intensity that follows copulations with once mated females. On the other hand, the effect from the much higher sperm competition intensity that follows matings with already multiply mated females will often out compete effects from sperm competition risk, at least when females remate readily or there is first male sperm precedence.

Sperm precedence

At high female remating rates, first male sperm precedence will favour an increased investment in matings with virgin females, whereas such an increase was not predicted under last male sperm precedence. But, first male precedence does not generally favour investment in virgin matings or disfavour investment in copulations with already mated females. When comparing predicted male sperm investment in matings with mated vs. virgin females at low mating rate the opposite pattern is envisaged. Here, the relative sperm expense on copulations with mated females compared with copulations with virgin females is predicted to be much larger when there is first rather than last male sperm precedence (cf. Fig. 2). This result is similar to the ones obtained in

previous models (Parker *et al.*, 1997). If first males are favoured and second males are unfavoured, a small investment by males mating with virgin females will be enough to secure paternity for a large proportion of the offspring. However, males mating with already mated females should attempt to equalize their handicap caused by mating order by increasing ejaculate size. This will cause the higher investment in matings with already mated females under first male sperm precedence compared with situations with second male sperm precedence. However, with increasing female mating frequencies, first male precedence will strongly penalize male sperm investment in matings with mated females, as on average the male's sperm will compete against several ejaculates from males that mated previously, and the disadvantage in the loaded raffle will increase in magnitude with $1/r$ for each previous male. Hence, first male sperm precedence does not *per se* select for an increasing allocation of sperm to virgin females (on the contrary). Only in combination with relatively high female mating rate first male sperm precedence leads to an increased sperm allocation to virgin females.

Models of loaded raffles have hitherto only regarded the outcome in the competition between two players (e.g. Parker, 1990a; Parker *et al.*, 1997; Ball & Parker, 1998; Mesterton-Gibbons, 1999). Nevertheless, we had to make some assumptions on the outcome of a loaded raffle involving *n* players. Reliable data on the outcome of sperm raffles when more than two males are involved is recently beginning to accumulate (Simmons, 2001), but is in many cases restricted only to the paternity success of the last male to mate. We used the simplest possible assumption that the male mating first will have the same advantage/disadvantage compared with the second male as the second male has compared with the male mating third and so on, a pattern that is realized, for instance, in *D. melanogaster* (Morrow *et al.*, 2005). This assumption would most closely mimic a situation of incomplete sperm displacement of previous sperm followed by sperm mixing between the sperm remaining from the previous males and the sperm added by the present mate (e.g. Parker *et al.*, 1990; Simmons *et al.*, 1999) for P_2-values greater than 0.5 or, for P_2-values smaller than 0.5, situations where female genital tracts are effectively but incompletely plugged by the ejaculates of previous males. However, the pattern of sperm precedence in loaded raffles with more than two males may be different. Therefore a notice of caution has to be made regarding the interpretation of some of the simulation outcomes. For instance Zeh & Zeh (1994) have reported that in the harlequin beetle-riding pseudoscorpion *Cordylochernes scorpioides*, the pattern of second male sperm precedence apparently breaks down to a state of fair raffle when the sperm from more than two males participate in the raffle (see also Drnevich, 2003). Likewise, it is possible that some sperm competition mechanisms will skew the favour in the raffle only

towards the last or the first male in the mating sequence. In these cases, the predictions from our model are not straightforwardly applicable. However, it seems reasonable to assume that the predictions in these cases will equal the ones for a loaded raffle when female remating propensity is low and those of a fair raffle when female mating frequency is high. It should also be noted, that estimates of P_2-values will be affected, and often exaggerated, if these estimates do not take male sperm allocation patterns into account. Thus, although there is for instance a fair raffle of sperm, the estimated P_2-value may differ from 0.5 depending on whether males are expected to spend more sperm in matings with virgin or already mated females.

Increasing male sensitivity

The results from the simulations in which males distinguished between mated females with different mating histories are in many aspects different from those obtained in the simulations where males were not able to distinguish once and multiply mated females (cf. Figs 2 and 3). Male capability to assess female mating status, thus, had an unforeseen strong effect on the resulting dynamic of the ESS-game. This is important to notice in interpretations of results from sperm allocation experiments. If sperm allocation patterns to once-mated and virgin females are compared, the expected effect of female mating status may be completely different depending on how good males can assess female mating history.

From the simulations assuming a 'fair raffle' of sperm we were initially surprised by the prediction that in some cases males mating with multiply mated females should invest more sperm than in matings with once-mated females only, as was the case when average female remating rate was low. This seemingly contradicts one of the central predictions from the intensity model (Parker *et al.*, 1996). When males are certain of sperm competition this model predicts that males should invest a decreasing amount of sperm with an increasing number of competing males. However, this model assumes that different males have the same amount of information on the number of competing males, as can be assumed in-group spawners with external fertilization. However, in our system it is clearly an asymmetry in the level of information males have on the final intensity of sperm competition. Males mating with once mated females are certain of sperm competition but have imperfect and uncertain information on the final number of competing males. Males mating with multiply mated females will have more exact information on the number of the females' mates. This asymmetry in information is analogous and has similar consequences as the asymmetry of information between males in the two-player sperm competition risk game (Parker *et al.*, 1997; Ball & Parker, 1998). When female remating is rare, the discrepancy between the estimations that preceding males make and the information that succeeding males have, will be the

greatest. Under these conditions succeeding males are thus in an advantage and therefore an increasing sperm allocation with an increase in female mating partners may be expected.

Female polyandry and male resource allocation

We found that males should increase resource allocation towards sperm production when female mating frequency is high (Fig. 1). This is analogous to the predictions from previous models (Parker *et al.*, 1996, 1997), which are further supported by a large set of empirical data (Harcourt *et al.*, 1981; Svärd & Wiklund, 1989; Gage, 1995; Kappeler, 1997; Stockley *et al.*, 1997; Hosken, 1998; Byrne *et al.*, 2002; Brown & Brown, 2003). When males are unable to differentiate female mating status, the model further predicts total sperm allocation to be higher the fairer the sperm raffle for fertilizations is (Fig. 1), also very similar to previous model predictions (Parker *et al.*, 1997).

Concluding remarks

Here we have reported results from a first attempt to theoretically predict expected sperm allocation patterns in relation to female mating status under varying conditions. Most importantly the effect of female mating status will be different depending on the average mating frequency of females and this fits well with the inability to find a prevalent pattern of sperm allocation in relation to female mating status in previous empirical studies (for a review see Wedell *et al.*, 2002). Nevertheless, further empirical studies are needed in order to evaluate the applicability of our model. However, as allocation patterns may drastically change depending on how accurately males may estimate female mating status, this should be accounted for when planning and interpreting future studies.

Acknowledgments

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