

The sexually selected sperm hypothesis, maternal effects, and sperm competitive success in the bulb mite *Rhizoglyphus robini*

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ABSTRACT

Background: The sexually selected sperm hypothesis predicts that selection will favour female multiple mating by increasing the sperm competitive abilities of their sons.

Hypothesis: Sons of multiply mated females should have higher sperm competitive abilities than sons of singly mated females.

Organism: The bulb mite, *Rhizoglyphus robini* (Acari: Acaridae).

Methods: *Rhizoglyphus* is a highly promiscuous species in which females gain no direct benefits from multiple matings. Virgin females were mated to one or six males. Sons of singly and multiply mated females were allowed to compete with each other for access to females' ova. Sperm competitiveness was estimated by the sterile male method.

Results: Contrary to the predictions of the hypothesis, we found no difference in sperm competitiveness between sons of singly and multiply mated females. We suggest that non-genetic effects could explain this result.

Keywords: bulb mite, maternal effects, polyandry, sexually selected sperm hypothesis, sperm competition.

INTRODUCTION

For a long time, research on mating systems focused on sexual selection as a result of competition for access to a mating partner. Thirty years ago, the attention of evolutionary biologists turned to sexual selection that takes place after copulation. Post-copulatory sexual selection occurs when a female mates with more than one male during one reproductive cycle.

Male reproductive success increases with the number of mating partners, whereas female success is more or less the same (Bateman, 1948), because females usually produce bigger and less numerous eggs than the number of sperm available after a single copulation. So their

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reproductive success should depend on their fecundity, rather than on the number of her mating partners.

Female multiple mating is widespread among various animal taxa, although it is costly. At first sight, females do not benefit from multiple mating. Frequent copulations are costly in terms of time and energy (Keller and Reeve, 1995). Polyandry increases the risk of predation and transmission of diseases or parasites (Hamilton, 1990; Keller and Reeve, 1995), seminal fluids are deleterious for females (Chapman *et al.*, 1995), and multiple mating has been shown to decrease females' lifespan or the number of their offspring in several species (Radwan and Rysińska, 1999; Johnstone and Keller, 2000; Kolodziejczyk and Radwan, 2003).

Many hypotheses have been proposed to explain polyandry involving direct benefits, including replenishment of depleted sperm stores (Parker, 1970), receiving nutrients during copulation (Ridley, 1988; Fedorka and Mousseau, 2002) or in the ejaculate (Savalli and Fox, 1999), avoiding costs connected with resistance to male mating attempts (Clutton-Brock and Parker, 1995), parental care of offspring (Veiga *et al.*, 2002), and indirect, genetic benefits, such as avoidance of inbreeding (Stockley *et al.*, 1993), avoidance of genetic incompatibility (Zeh, 1997), and improvement of genetic quality of offspring (Moller, 1992, 1997).

One hypothesis relating to the improvement of the genetic quality of offspring is the sexually selected sperm hypothesis (Keller and Reeve, 1995). This hypothesis states that multiply mated females are more likely to have their eggs fertilized by the most competitive sperm; their sons will thus inherit high sperm competitiveness and their daughters a propensity to multiple matings. The required condition is heritability for sperm competition abilities. The heritable components that may impact sperm competitiveness include the quantity of sperm a male produces, success in reaching and fertilizing the eggs, the ability to displace the sperm from previous matings, and preventing other males from having subsequent matings (Keller and Reeve, 1995). Once polyandry evolves, a variety of specialized adaptations can arise in males that increase the chance of their sperm fertilizing their partner's eggs under the risk of competition from sperm of other males (Parker, 1970; Simmons and Siva-Jothy, 1998).

A direct prediction of the sexually selected sperm hypothesis is that sons of polyandrous females should have, on average, higher fitness than sons of females mated to a single male. This prediction has been confirmed in red flour beetles (Bernasconi and Keller, 2001; Pai and Yan, 2002) and yellow dung flies (Hosken *et al.*, 2003), but not in Australian field crickets (Simmons, 2001).

Orteiza *et al.* (2005) have shown that *Drosophila melanogaster* females gain a 6.13% increment in grand-offspring number through their sons when multiply mated, although sexy sons from re-mating do not recoup the cost of polyandry, which can be an approximate 10% decrease in lifetime fecundity. This highlights the need to take into account not only the benefits, but also the costs associated with polyandry. Such costs may include not only direct detrimental effects on female reproductive success, but also negative maternal effects of polyandry (Kozielska *et al.*, 2004).

In this study, using the bulb mite *Rhizoglyphus robini*, we investigate how the competitive abilities of males are affected by the number of mating partners of their mother. In this species, females mate several times a day with several males and sperm competitiveness is heritable (Radwan, 1998). Previous research has shown that mating number has several consequences. First, polyandrous females produce more fecund daughters than singly mated females (Konior *et al.*, 2001), probably as a result of the genetic correlation between sperm competitiveness and female fecundity documented in this species (Kozielska *et al.*, 2004). Second, Kozielska *et al.* (2004) showed that there are non-genetic costs associated with multi-male mating that decrease the reproductive success of males. Such costs may offset the benefits of

multi-male mating. To obtain a better picture of the consequences of multi-male mating, we compared the competitive abilities of females mated to either one or six males.

METHODS

Mite culture was derived from a population of about 200 individuals found on onions collected near Krakow, Poland in September 1998 (about 25 generations before starting this experiment). *Rhizoglyphus robini* occurs in nature on the bulbs of onions, garlic, and flowering plants. In the laboratory, the mites were kept in desiccators at room temperature (22–26°C) and at a relative humidity of 90%, maintained by KOH solution (153 g·l⁻¹), and fed on a 3:1 mixture of powdered yeast and wheat germ. Mites in base colonies were kept in three 2.5-cm diameter plastic jars, with more than 1000 individuals in each. A few hundred individuals were mixed between containers and moved to a fresh jar once a month to avoid genetic drift. The sex ratio in the laboratory populations was close to 1:1, which is similar to that in natural conditions (M. Konior, personal observations). Mating, egg laying, and rearing of individual mites took place in 0.5-cm diameter glass tubes with plaster and charcoal bases. During manipulations outside the desiccators, the tubes were placed on wet filter paper to maintain high humidity.

Virgin females used in the experiment, obtained by isolation of individual protonymphs, were randomly divided into two groups. Polyandrous females and monoandrous females were kept with males for the same period of 72 h. In the polyandrous treatment the male was replaced every 12 h, whereas in the monoandrous treatment the male was not replaced. Previous research had indicated that males kept with a female for 24 h were as likely to mate with her again as the newly introduced males that were kept with another female during the preceding 24 h (Konior *et al.*, 2001). As the females in the polyandrous and monoandrous treatments had access to males for the same amount of time, the probability of mating was the same in both groups.

After mating, females were left in the tubes to oviposit. When the eggs hatched, ten protonymphs from each female were isolated to new tubes and reared individually to adulthood. Then, one son of each female was chosen at random.

Sperm competitiveness was determined by means of the sterile male method (Parker, 1970). Male offspring from each treatment were randomly divided into two equal groups. One group was irradiated with 200 Gy of gamma radiation from Co⁶⁰, the smallest dose found to result in complete sterility (range tested: 50–550 Gy, $n = 10$ males per dose). The other group of males was not irradiated. Irradiated males are able to fertilize eggs, but the eggs do not develop. Bulb mite females lay only fertilized eggs, and the hatchability of eggs fertilized by non-irradiated males is about 100% (0.99 ± 0.02) (Radwan, 1998). Thus, paternity of a fertile male competing with a sterile male for fertilization of eggs can be calculated as the proportion of eggs hatched.

All males were then kept with females prior to the experiments, and separated 2 h before the start of mating. This was done to standardize the stored sperm reserves (Radwan, 1997), and is similar to the mating interval that usually occurs in nature (J. Radwan, personal observations). One group of virgin females, obtained by protonymph isolation, were mated to a sterile male (s) from the polyandrous (P) group for 2 h, after which the male was immediately replaced with a fertile male (f) from the monoandrous (M) group for another 2 h (Ps, Mf condition). A second group of virgin females was mated with a sterile male from the monoandrous group first, and then with a fertile male from the polyandrous group (Ms, Pf condition).

On the following day, a third group of virgin females were mated to a fertile male from the polyandrous group, and then with a sterile male from the monoandrous group (Pf, Ms condition). Finally, a fourth group of virgin females were mated to a fertile male from the monoandrous group, and then with a sterile male from the polyandrous group (Mf, Ps condition). Females that did not lay eggs, or died before we counted eggs and larvae, were excluded from the analysis. This is why the numbers of females in groups were not equal. Because egg and larvae counting is very laborious, the experiment was performed in two blocks, each lasting about 2 weeks. Data were analysed with a nested analysis of variance (ANOVA), with mother mating regime, irradiation, order of mating (i.e. as a first or as a second male), block, and pair of males (as a random factor) as independent variables (pair was nested within block), and number of eggs fertilized by the first and second male as the dependent variable. For estimating effect size, we computed partial eta-squared ($SS_{\text{factor}} / (SS_{\text{factor}} + SS_{\text{error}})$) (Cohen, 1973). The numbers of males tested for sperm competitiveness in each group, in the first and second block, were as follows: Ps, Mf = 31 and 42; Ms, Pf = 33 and 33; Pf, Ms = 24 and 35; Mf, Ps = 24 and 32. Altogether, we analysed 112 males in the first block and 142 males in the second block. All analyses were conducted with Statistica version 8.0 (Statsoft, Inc., 2007).

RESULTS

The mean proportion of sired offspring (P2) tended to be higher for sons of monoandrous females (Fig. 1). However, we did not observe a significant effect of the number of mother's

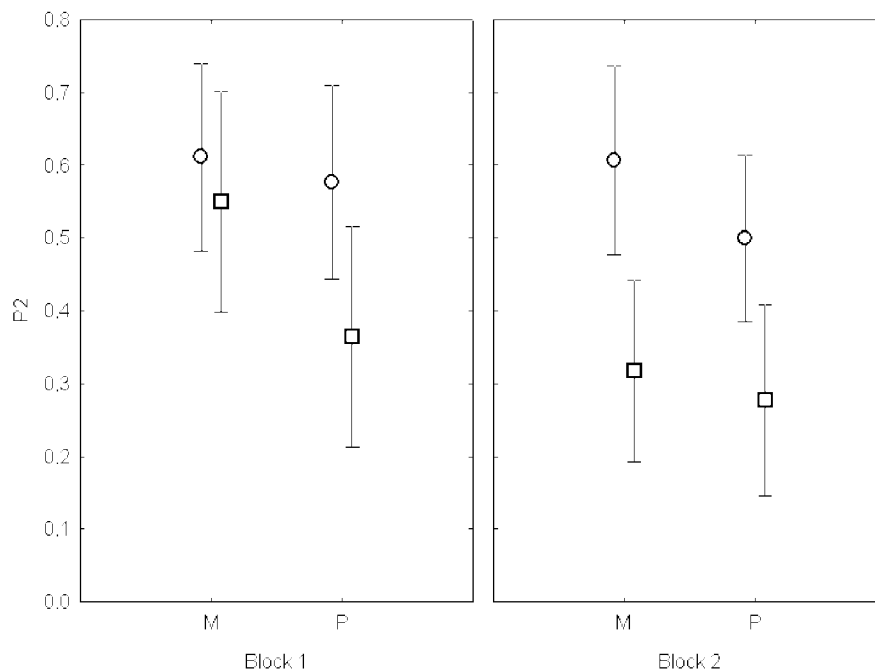


Fig. 1. Mean proportion ($\pm 95\%$ confidence interval) of offspring sired in double matings by the second mate (P2). M = sons of monoandrous females; P = sons of polyandrous females; □, sterile males; O, fertile males.

Table 1. Results of nested ANOVA for number of eggs fertilized by a male depending on the number of his mother's partners (mating system), on whether he mated as a first or a second female partner (mating order) or whether he was sterile or fertile (irradiation)

Effect	d.f.	MS	<i>F</i>	<i>P</i>
Block	1	48165	57.126	0.000
Mating system	1	1284.700	1.195	0.275
Irradiation	1	50,314.900	46.780	0.000
Mating order	1	162.300	0.151	0.698
Pair of males	252	843.100	0.784	0.973
Error	251	1075.500		

Note: Pairs of competing males were nested within blocks.

mating partners on the number of offspring sired by their sons (Table 1). The effect of irradiation on male sperm was significant and decreased sperm competitive abilities. Mating order had no significant effect, implying that the first and the second male to mate with a female fertilize similar proportions of eggs. Eta-squared for mother's mating regime was 0.004, implying that only about 0.4% of the variability in sperm competitiveness can be explained by the independent variable.

DISCUSSION

We found no significant effect of the number of mothers' mating partners on sperm competitiveness of their sons. Similar results were obtained in an experiment that examined the influence of mothers' polyandry on a rough measure of male sperm production rate, estimated from fertilization success with five females inseminated in a short period of time (Konior *et al.*, 2001). These results may appear surprising at first, given that sperm competition success has repeatedly been shown to be heritable in this species (Radwan, 1998; Konior *et al.*, 2005). However, a possible explanation might be that the benefits conferred by multiple matings are offset by non-genetic costs associated with multiple mating, transmitted to progeny via maternal effects, and/or females changing the investment in their offspring depending on the number of partners they mate with. Indeed, Kozielska *et al.* (2004) showed that the same male had lower reproductive success (measured as the proportion of eggs fertilized in competition with another male) when their mother also mated with three other, sterile males.

Thus, the lack of a significant effect of polyandry on sons' reproductive success in the present study, as well as in the study of Konior *et al.* (2001), might result from non-genetic costs and/or maternal effects balancing the positive genetic effects conferred by multi-male mating.

Negative effects of polyandry were reported in a study of the bean beetle *Callosobruchus maculatus*, where offspring resulting from polyandrous mating had lower egg-to-adult survival than offspring of females mated the same number of times to one male (Eady *et al.*, 2000). The authors suggested that this could have resulted from intra-specific variation in male seminal products having synergistic negative effects on brood development. Such intra-specific variation in accessory gland products has been reported in *Drosophila*

(Whalen and Wilson, 1986). Such negative effects of seminal fluids, if they lower the condition of males and decrease their competitive abilities, may thus explain the results of our study.

Quantitative genetic data in the field cricket *Teleogryllus oceanicus* are also consistent with maternal effects associated with polyandry. In this species, there was no significant correlation between paternity and polyandry, but there was a strong dam effect on paternity of sons, polyandry of daughters, and testes size in sons (Simmons, 2003). This result, however, may also be explained by X-linkage of sperm competitiveness. Indeed, there is some evidence to suggest that traits influencing sexual selection are situated at X-linked loci (Reinhold, 1998; Hurst and Randerson, 1999; Johns and Wilkinson, 2007). In such cases, when sperm competition inheritance is female biased, genetic benefits through sexually selected sperm are not likely to appear (Pizzari and Birkhead, 2002).

There are also increasing reports of females altering the investment in their eggs depending on environmental factors or characteristics of their mate. For instance, female zebra finches regulate the level of hormones transmitted to the eggs according to the attractiveness of their mate (Gil *et al.*, 1999). Similarly, Tregenza *et al.* (2003) showed that the interaction of the mother's environment with the number of her mating partners can influence fitness of offspring in the dung fly *Scatophaga stercoraria*. Experiments in the seed beetle *Callosobruchus maculatus* (Fox and Savalli, 1998) and the ant *Pogonomyrmex rugosus* also showed that maternal effects can influence the size of the offspring produced (Schwander *et al.*, 2008).

Bernasconi and Keller (2001) found that in the red flour beetle *Tribolium castaneum*, sons of polyandrous females increased their sperm competition success when males were mated as a second mate, but the proportion of sired offspring tended to decrease when mated as a first mate. Simmons and Kotiaho (2007) found a genetic correlation between sperm length and male condition in the dung beetle *Ontophagus taurus*. Females with large spermathecae select for males with short sperm. There was also additive genetic variance and heritability for spermathecae size and genetic covariance between sperm length and spermathecae size, as predicted by the sexually selected sperm hypothesis. Miller and Pitnick (2002) also showed that males with long sperm gained a fertilization advantage over males with short sperm, when they were both mated to females selected for long seminal receptacles. These results provide support for a sexually selected sperm process. In red flour beetles, Pai and Yan (2002) found that multiply mated females produce sons that sire more offspring than sons of females mated to one male, and their offspring have higher survival from egg to adult. However, these effects need not be genetic.

Finally, in contrast to a previous study (Radwan, 1997), we did not find a significant effect of mating order. This might be explained by the fact that the fertilization advantage of the second male was small [on average, 55% of eggs fertilized in double mating (Radwan, 1997)] compared with that in other acarid species (Radwan, 1991; Radwan and Witaliński, 1991), making it less likely that it will be detected.

In summary, we did not observe a significant effect of polyandry on the competitive ability of the males produced. In the light of previous studies, it is possible that the genetic benefits of multiple mating are offset by non-genetic effects associated with female polyandry, which cause a decrease in sperm competitiveness of their sons (Kozielska *et al.*, 2004). Unfortunately, there are very limited data about the cost of multiple mating and/or maternal effects and their influence on the competitive abilities of the males produced. It will be important to consider such effects in future studies testing the sexually selected sperm hypothesis.

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