

Polyandry produces sexy sons at the cost of daughters in red flour beetles

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Female mating with multiple males within a single fertile period is a common phenomenon in the animal kingdom. Female insects are particularly promiscuous. It is not clear why females mate with multiple partners despite several potential costs, such as expenditure of time and energy, reduced lifespan, risk of predation and contracting sexually transmitted diseases. Female red flour beetles (*Tribolium castaneum*) obtain sufficient sperm from a single insemination to retain fertility for several months. Nonetheless they copulate repeatedly within minutes with different males despite no direct fitness benefits from this behaviour. One hypothesis is that females mate with multiple partners to provide indirect benefits via enhanced offspring fitness. To test this hypothesis, we compared the relative fitness of F_1 offspring from females mated with single males and multiple males (2, 4, 8, or 16 partners), under the condition of relatively high intraspecific competition. We found that a female mating with 16 males enhanced the relative fitness of F_1 males (in two out of three trials) but reduced F_1 females' fitness (in two independent trials) in comparison with singly mated females. We also determined whether several important fitness correlates were affected by polyandry. We found that F_1 males from mothers with 16 partners inseminated more females than F_1 males from mothers with a single partner. The viability of the eggs sired or produced by F_1 males and females from highly polyandrous mothers was also increased under conditions of low intraspecific competition. Thus, the effects of polyandry on F_1 offspring fitness depend on environmental conditions. Our results demonstrated a fitness trade-off between male and female offspring from polyandrous mothers in a competitive environment. The mechanisms and biological significance of this unique phenomenon are discussed.

Keywords: polyandry; *Tribolium castaneum*; good genes; fitness; sexy sons

1. INTRODUCTION

Female multiple mating, defined as mating with more than one partner in the same fertile period, is a widespread phenomenon despite several potential costs, such as expenditure of time and energy, temporary infertility, reduced lifespan and increased risk of contracting sexually transmitted diseases (Drummond 1984; Parker 1984; Chapman *et al.* 1995; Watson *et al.* 1998; Yasui 1998). Female multiple mating may occur within a short period (short intermating interval, e.g. a few minutes) or a long period (long intermating interval, e.g. weeks or months). Because the benefits of multiple mating to females are not obvious, it has encouraged much research into the evolution of female multiple mating behaviour (Ridley 1988; Jennions & Petrie 2000).

The red flour beetle, *Tribolium castaneum*, is a species in which both sexes mate promiscuously (Sokoloff 1974). The fertile period of the female *T. castaneum* could be more than five months, and females readily remate within minutes as well as within months of the initial copulation (A. Pai, unpublished data). Because the intermating interval determines whether sperm from different males co-occur in the female tract and affects the age of the competing sperm, it could have a significant impact on the consequences of multiple mating (Simmons & Siva-Jothy 1998). Therefore, based on the length of intermating interval, multiple mating can be classified as rapid mul-

iple mating (the intermating interval is as short as a few minutes) and periodic multiple mating (the intermating interval is weeks or months). Female red flour beetles exhibit rapid multiple mating and can mate with up to 10 males within 1 h (A. Pai, unpublished data), but they do not gain direct fitness benefits from this rapid multiple mating behaviour (A. Pai, unpublished data). If polyandry is adaptive there should be some fitness advantage for females mating with multiple partners. One hypothesis to explain female multiple mating behaviour is that multiple copulations with different partners in a short period allows post-copulatory paternity-biasing processes, which in turn enhance offspring quality (Yasui 1997, 1998; Jennions & Petrie 2000). Thus, post-copulatory processes, such as sperm selection by females or sperm competition among ejaculates, may enhance the genetic quality of offspring if 'compatible sperm', 'good sperm' or 'sexy sperm' fertilize a majority of the female's ova (Keller & Reeve 1995; Yasui 1997, 1998; Jennions & Petrie 2000).

This study examined the hypothesis that rapid female multiple mating provides indirect benefits to offspring using *T. castaneum* as a model system. We tested whether the reproductive success of offspring of polyandrous (multiply mated) females is higher than that of monandrous (singly mated) females. Furthermore, we determined which fitness correlates of F_1 offspring were affected by polyandry. Three fitness correlates were studied, including mating behaviour, egg production and F_2 egg-to-adult viability. If females gain indirect fitness benefits from multiple mating and post-copulatory paternity-biasing mechanisms, the offspring from polyandrous

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matings would be expected to have higher fitness than offspring from monandrous matings.

2. METHODS

(a) *Beetle rearing*

The cSM strain of *T. castaneum* was used for all experiments (see Wade (1977) for strain origin). Beetles were raised in 28 mm diameter \times 95 mm shell vials containing 5 g of standard medium (95% by weight of fine-sifted wholewheat flour and 5% of dried powdered brewer's yeast). Vials were maintained in a dark incubator regulated at 29 °C and 70% relative humidity. Individuals used in the experiments were between two and eight weeks post-emergence.

(b) *F₁ offspring production and sex ratio from singly and multiply mated females*

In preliminary studies, we found that the female beetles mated with up to 16 males (10 males on average) in two 1 h observation periods that were 48 h apart (A. Pai, personal observation). Thus, we designed five treatments in which individual virgin females were given the opportunity to mate with 1, 2, 4, 8 or 16 males in a vial with 5 g of flour medium for 10 days. This experimental design allowed male–male interaction and female choice, which may be important in determining the indirect consequences of multiple mating, such as egg-to-adult viability of offspring (A. Pai, unpublished data). The overall copulation frequency of a female should be similar across the five mating treatments (A. Pai, unpublished data). Thus, the degree of polyandry for females with more males should be generally higher than those with fewer males. The actual number of males that a female copulated with was not recorded because the recording process would disrupt precopulatory female choice or male–male interactions.

After 10 days, all adults were removed from the vials and the eggs were allowed to grow. The sex ratio of offspring from each female was examined. We conducted three independent trials of this experiment and set up five replicates for each treatment in each trial. Approximately 10% of females did not produce any offspring. Because all of the females that did not produce offspring were exposed to multiple virgin males, and females were given ample opportunity to mate (10 days), the lack of offspring was probably due to female sterility; therefore, these females were excluded from the analysis.

(c) *F₁ offspring fitness assay*

Fitness was measured as the proportion of offspring contributed by a focal individual in a test population, using a genetic body colour marker (Yan & Stevens 1995). Briefly, the fitness assay involved placing the focal individual in a population with four other adults of the same sex and five of the opposite sex. The focal individual was the wild-type red body colour (genotype +/+), and the other individuals had a black body colour (genotype b/b). The black body colour is determined by a codominant allele. Thus, all the progeny of the focal individual would be heterozygous (+/b), brown and phenotypically distinguishable. The adults were allowed to mate and lay eggs for 12 days, after this they were removed. Eight weeks after the initial introduction of adults into the vials, the progeny had become adults, but no new offspring had been produced. The contents of each vial were sifted and the number of individuals of each genotype was recorded. The focal beetle is expected to produce one-fifth (0.20) of the offspring if all beetles in a

population have equal reproductive success. Thus, relative fitness of the focal beetle is defined as the proportion of the red beetle's offspring in a test population divided by the expectation (0.20). Relative fitness of more than one suggests that the focal beetle may have higher fitness than the black beetles. Our experimental design compared the fitness of sons and daughters from singly and multiply mated females. By keeping the environment (i.e. competing genotype) constant, we were in fact estimating the effect of multiple mating on the relative fitness of F₁ offspring.

Using the above design, we examined the relative fitness of F₁ males and females from monandrous and polyandrous mothers. We conducted three independent trials for F₁ males and two trials for F₁ females to test the generality of our results, and set up 20–25 replicates for each treatment in each trial. Trial 3 of the F₁ male fitness assay and trial 2 of the F₁ female assay used seven-week-old F₁ beetles, whereas other trials used three-week-old beetles. We excluded from the analysis any vials that contained less than 50 total offspring in the fitness assay, because beetle relative fitness estimation may be biased when the total number of beetles in the fitness assay is small.

(d) *F₁ offspring fitness correlates assay*

The above fitness assay measured the total number of genes that a beetle passed to the next generation in a population, but did not provide information on which factors caused fitness variation among treatments. Therefore, to understand the mechanism of fitness changes in F₁ offspring among different treatments, it is necessary to examine fitness correlates. The fitness correlates that we examined included mating behaviour, egg and adult progeny production, and egg-to-adult survival of F₂ offspring. The experiments described below compared fitness correlates for the same five mating treatments as those used in the fitness assay, but they were conducted under low intraspecific conditions. In the fitness assay, eggs from five females were present in 5 g flour medium, thus intraspecific competition was higher than the following experiments in which eggs from only one female were present in the same amount of flour medium.

(e) *Mating behaviour of sons and daughters*

Copulation behaviour of offspring from singly and multiply mated females was examined. Virgin beetles may be more motivated to copulate, therefore to avoid overestimating mating frequency of focal individuals we used non-virgin beetles in this experiment. Virgin beetles were kept in vials with an even sex ratio for one week prior to being used in the experiment, and thus all beetles had a comparable mating history. A single focal male or female offspring was allowed to habituate for 30 min in a 35 mm diameter plastic Petri dish, in dim light. A virgin beetle of the opposite sex was introduced into the Petri dish. All focal individuals were red and the mating partners were black so that the two sexes could be easily distinguished. During the 10 min observation period, we recorded: (i) time to first copulation; (ii) duration of each copulation; and (iii) number of copulation attempts (attempts to mount by the male). Thus, the frequency of remating and total time spent on copulation activities (the sum of copulation time and time on copulation attempts) were calculated. Time to first copulation may indicate an ability to locate a mate, an eagerness to mate and/or attractiveness. Likewise, total time spent on mating-related activity (copulation attempts and copulation) may indicate mating vigour (ability to copulate) (Otronen 1994). Fifteen replicates were conducted for each treatment for each sex. Mating behaviour was observed for

Table 1. Summary of ANOVA results comparing offspring production and relative fitness of F_1 offspring (the proportion of the focal beetle's offspring in a test population) from multiply and singly mated females.

experiment	source of variation	d.f.	<i>F</i>	<i>p</i>
F_1 offspring production	trial	1	43.80	< 0.001
	mating treatment	4	0.64	0.63
	trial \times mating treatment	4	3.17	0.02
	error	38	—	—
F_1 male and female fitness assay	trial	2	40.72	< 0.001
	sex	1	56.25	< 0.001
	mating treatment	4	2.99	0.02
	trial \times sex \times mating treatment	8	2.35	0.02
	error	453	—	—

10 min, an adequate period of time for beetles to copulate more than once (A. Pai, personal observation), and yet short enough to allow one observer to finish 150 observations within a few days to minimize day-to-day variation. In the observation period, 6 out of 75 (8%) daughters and 3 out of 75 (4%) sons did not copulate at all, and were therefore excluded from analyses.

(f) Insemination capacity of sons

Bloch Qazi *et al.* (1996) showed that *T. castaneum* males ejaculated less sperm in successive copulations and some males could not copulate more than twice. Thus, males may be limited in their ability to inseminate all available partners due to sperm depletion. An estimate of sperm quality or quantity is important because males differ in their fertilization capacity and a measure of sperm production may not be evident from mating behaviour such as duration of copulation (Bloch Qazi *et al.* 1996). Studies with zebra finches also suggest that sperm numbers and sperm quality decline in successive ejaculates and may limit the male's ability to mate (Birkhead *et al.* 1995).

To examine sperm depletion and production in male offspring, an individual non-virgin, male offspring was placed into a tube with five virgin black females in 1 g flour medium. Twenty-eight non-virgin sons were randomly selected from each treatment for this experiment. Males were removed from the tube after 30 min and females were transferred into individual tubes with 5 g flour. A successful insemination was defined as one that resulted in the female producing viable offspring. All females exposed to F_1 male offspring were virgins and therefore strongly motivated to mate, and thus female choice is unlikely to be a factor in determining male insemination success. After four weeks, we examined the presence or absence of larvae in each tube to determine how many of the five available females had been successfully inseminated by males. Overall, 39% of males from all treatments did not successfully inseminate any female and were thus excluded from the analysis. Our other preliminary studies have also shown that a high proportion of recently mated non-virgin males were not able to successfully inseminate females when they were paired with a female for 30 min (A. Pai, unpublished data). There was no significant difference among treatments in the proportion of females that were unsuccessfully inseminated.

(g) Egg production, F_2 adult production and F_2 egg-to-adult viability

This experiment aimed to determine whether the progeny from sons and daughters of singly and multiply mated mothers differ in egg-to-adult viability. The egg-to-adult viability is defined as the proportion of eggs that successfully emerged into

adults. One F_1 male or female was paired with a virgin beetle of the opposite sex for 24 h in a tube with 2 g of flour medium. As described in the above experiments, all focal individuals were one-week-old red beetles, and all mating partners were black beetles from the stock population. Females were allowed to lay eggs for 48 h, and the number of eggs was counted. The eggs were then transferred to tubes with 5 g of fresh flour medium and allowed to grow for eight weeks. The number of adult progeny was counted and the proportion of eggs that survived to adulthood was determined. We conducted 20 replicates for each treatment for each sex.

(h) Data analysis

For the experiments on F_1 offspring production and sex ratio from singly and multiply mated females, analysis of variance (ANOVA) with trial and number of mates (mating treatment) as factors was used; the sex ratio was tested against the null hypothesis that there was an equal proportion of male and female progenies, using the χ^2 test. To compare relative fitness of F_1 males and females from singly and multiply mated mothers, ANOVA with trial, sex and mating treatment as factors was used. In ANOVA, sex and mating treatment were treated as fixed factors, whereas the trial was treated as a random factor. The fitness correlates of F_1 were analysed using an ANOVA model with mating treatment and sex as fixed factors to determine the effects of mating treatment on the fitness correlates. The Tukey–Kramer Honestly Significant Difference (HSD) test was used to determine statistical differences among the five mating treatments within each sex and trial. All analyses were conducted using the JMP computer program (SAS 1995).

3. RESULTS

(a) F_1 offspring production and sex ratio from singly and multiply mated females

There was no significant difference in the number of F_1 adult offspring produced by females that had single and multiple partners during the 10 day mating period, however there was significant variation in offspring production between trials, presumably due to differences in female age (table 1). χ^2 tests found that the sex ratio was not different from 1 : 1 for offspring from singly or multiply mated females ($p > 0.05$ for all five treatments).

(b) F_1 male fitness assay

Five vials (*ca.* 1%) contained no offspring from the focal males, probably due to male sterility, and thus were excluded from the analysis. Fitness assays from the three

Table 2. Summary of Tukey–Kramer HSD test results comparing relative fitness of F_1 offspring from multiply and singly mated females. Treatments not connected by lines are different at least at the significance level $p < 0.05$.

experiment	number of mates of mother	mean relative fitness (s.e.)
F_1 males		
trial 1	16	1.900 (0.122)
trial 1	8	1.644 (0.115)
trial 1	4	1.579 (0.120)
trial 1	1	1.392 (0.107)
trial 1	2	1.069 (0.090)
trial 2	16	2.460 (0.123)
trial 2	8	1.921 (0.096)
trial 2	4	1.502 (0.115)
trial 2	1	1.483 (0.143)
trial 2	2	1.283 (0.123)
trial 3	2	1.849 (0.171)
trial 3	16	1.610 (0.116)
trial 3	8	1.583 (0.083)
trial 3	4	1.469 (0.131)
trial 3	1	1.247 (0.135)
F_1 females		
trial 1	1	1.774 (0.108)
trial 1	4	1.686 (0.089)
trial 1	8	1.564 (0.099)
trial 1	2	1.546 (0.100)
trial 1	16	1.345 (0.092)
trial 2	1	1.633 (0.211)
trial 2	4	0.711 (0.108)
trial 2	2	0.632 (0.117)
trial 2	8	0.558 (0.090)
trial 2	16	0.459 (0.087)

trials were analysed separately because variance among trials was significant (table 1). The results between trials 1 and 2 were remarkably similar. The relative fitness of F_1 males from mothers with more mates was generally higher than that from mothers with fewer mates. In trial 1, sons of females exposed to 4, 8 or 16 partners had higher relative fitness than sons of females exposed to two partners (table 2). Also, sons of females from the treatment with 16 males had higher fitness than sons of singly mated females (table 2). In trial 2, male progeny of females with 8 or 16 partners had significantly higher relative fitness than the other three treatments (table 2). However, in trial 3 there was no clear relationship between F_1 male fitness and number of mates that their mother had (table 2).

(c) F_1 female fitness assay

Data from the two trials of female fitness assay were not pooled due to significant between-trial variance (table 1). Although the relative fitness estimation for F_1 females differed between the two trials, the general pattern in the relationship between F_1 female fitness and polyandry of their mothers was consistent. In particular, F_1 females from singly mated mothers showed higher relative fitness in both trials than F_1 females from females exposed to 16 males (table 2). There was no significant difference in the relative fitness of F_1 females from mothers with 2, 4, 8 and 16 males (table 2).

(d) F_1 offspring fitness correlates assays

(i) Mating behaviour of F_1 offspring and insemination capacity of F_1 males and females

F_1 male and female offspring differed significantly in copulation frequency and average copulation duration (table 3). In particular, F_1 males spent more time *in copula* than F_1 females, but F_1 females exhibited higher mating frequencies than F_1 males (figure 1). There was no significant difference between F_1 males from singly and multiply mated mothers in copulation frequency (figure 1a), the time to first copulation, average copulation duration, or total time spent on mating activities (figure 1b; table 3). Insemination capacity of F_1 males differed significantly among treatments (figure 1c; table 3). F_1 males from mothers exposed to 16 males inseminated significantly more females compared with sons from all other treatments (Tukey–Kramer HSD test, $p < 0.05$). However, the insemination capacity of sons from mothers exposed to one, two, four and eight males was not significantly different. Likewise, there was no difference between F_1 females from singly and multiply mated mothers in copulation frequency (figure 1a), the time to first copulation, average copulation duration, or total time spent on mating activities (figure 1d; table 3).

(ii) F_1 male-partner fecundity, F_2 adult production and F_2 egg-to-adult viability

Females used in the assay were chosen randomly, thus any variation in offspring production between mates of F_1 males from monandrous and polyandrous mothers was due to fertilization difference. There was no significant variation in egg production by the partners of F_1 males from various mating treatments, however F_2 adult production and egg-to-adult viability varied significantly among the five mating treatments (table 3; figure 2a). Specifically, F_1 males from mothers exposed to one or two males produced fewer F_2 adults than those from mothers with four or more males (ANOVA orthogonal contrast, $t = 2.35$, d.f. = 1, $p = 0.02$). The egg-to-adult viability of F_2 sired by sons from mothers with four or more mates was higher in comparison with F_2 eggs sired by sons from mothers with one or two mates (ANOVA orthogonal contrast, $t = 3.08$, d.f. = 1, $p = 0.002$).

(iii) F_1 female fecundity, F_2 adult production and F_2 egg-to-adult viability

F_1 females from monandrous and polyandrous mothers produced similar numbers of eggs but a higher number of offspring (table 3; figure 2b). This resulted from a difference in egg-to-adult viability among treatments (table 3). F_1 females from mothers with one or two partners produced significantly fewer offspring than F_1 females from mothers with four or more partners (ANOVA orthogonal contrast, $t = 2.11$, d.f. = 1, $p = 0.03$). F_1 females from mothers with 8 or 16 mates had significantly higher egg-to-adult viability of F_2 than those from mothers with one, two and four males (Tukey–Kramer HSD test, $p < 0.05$; figure 2b). The egg-to-adult viability of F_2 eggs produced by F_1 females from mothers with one, two and four mates was reduced by more than 50% in comparison with those produced by F_1 females from mothers with 8 and 16 mates. This result suggests that polyandry may confer a

Table 3. Summary of ANOVA results comparing fitness correlates of F_1 offspring from multiply and singly mated females.

experiment	source of variation	d.f.	<i>F</i>	<i>p</i>
<i>(a)</i> mating behaviour				
<i>(i)</i> copulation frequency	sex	1	9.42	0.002
	mating treatment	4	0.43	0.79
	sex × mating treatment	4	3.10	0.02
	error	119	—	—
<i>(ii)</i> time to first copulation	sex	1	0.03	0.85
	mating treatment	4	1.25	0.29
	sex × mating treatment	4	2.51	0.04
	error	119	—	—
<i>(iii)</i> average copulation duration	sex	1	9.58	0.002
	mating treatment	4	0.12	0.97
	sex × mating treatment	4	1.29	0.27
	error	119	—	—
<i>(iv)</i> total time spent on copulation activities	sex	1	0.03	0.85
	mating treatment	4	1.87	0.12
	sex × mating treatment	4	0.72	0.57
	error	127	—	—
<i>(b)</i> F_2 male insemination capacity	mating treatment	4	8.72	< 0.001
	error	77	—	—
<i>(c)</i> F_2 egg and adult production by F_1 males and females				
<i>(i)</i> F_2 egg production	sex	1	46.92	< 0.001
	mating treatment	4	1.93	0.10
	sex × mating treatment	4	0.93	0.44
	error	177	—	—
<i>(ii)</i> F_2 adult production	sex	1	1.61	0.20
	mating treatment	4	4.09	0.004
	sex × mating treatment	4	1.70	0.15
	error	121	—	—
<i>(iii)</i> egg-to-adult viability	sex	1	59.28	< 0.001
	mating treatment	4	7.50	< 0.001
	sex × mating treatment	4	3.21	0.02
	error	121	—	—

fitness advantage to F_1 females under conditions of low intraspecific competition.

4. DISCUSSION

This study demonstrated that in *T. castaneum* beetles, male offspring from mothers with multiple mates had higher fitness (the proportion of offspring in a test population) than those from mothers with single mates, but multiply mated mothers produced female offspring with reduced fitness under the condition of high intraspecific competition. The male offspring produced by polyandrous females had increased insemination capacity compared with those produced by monandrous mothers. Although there was among-trial variation in F_1 male fitness from monandrous and polyandrous mothers, our results are consistent with the findings of Bernasconi & Keller (2001), who also showed that polyandry enhanced the reproductive success of sons. We also demonstrated that effects of polyandry on F_1 female fitness depend on the level of intraspecific competition. Under low intraspecific competition conditions, the egg-to-adult viability produced by F_1 females from mothers with more mates was higher than those with fewer mates. Considering present data together with our previous findings (A. Pai, unpublished data), we conclude that (i) polyandrous female beetles do not gain direct fitness benefits to

themselves from rapid multiple mating; (ii) polyandry confers a fitness benefit to F_1 males but a fitness cost to F_1 females in a competitive environment; and (iii) the effect of polyandry on F_1 female fitness is contingent on environmental conditions such as intraspecific competition. To our knowledge, this is the first empirical demonstration that female polyandry produces sons with increased fitness and daughters with reduced fitness under the condition of high intraspecific competition.

Recent studies suggest that females may gain indirect benefits through female multiple mating and mate preference (Reynolds & Gross 1992; Zeh & Zeh 1996; Wilkinson *et al.* 1998; Baer & Schmid-Hempel 1999). Several hypotheses have been proposed to account for indirect benefits associated with female multiple mating. The first is the 'good genes' model which predicts both male and female offspring from multiply mated females have higher fitness than those from singly mated females, because 'good genes' and 'compatible genes' are genes that enhance the fitness of all offspring (Yasui 1998). Such a benefit from multiple mating to both male and female offspring was demonstrated in guppies (Evans & Magurran 2000). The second is the 'sexy sperm' model, which postulates that multiple mating produces sexy sons if 'sexy sperm' fertilize most of the female's ova (Yasui 1998). Hence, sexy sons are those with a reproductive success higher than average individuals (Yasui 1998). Because

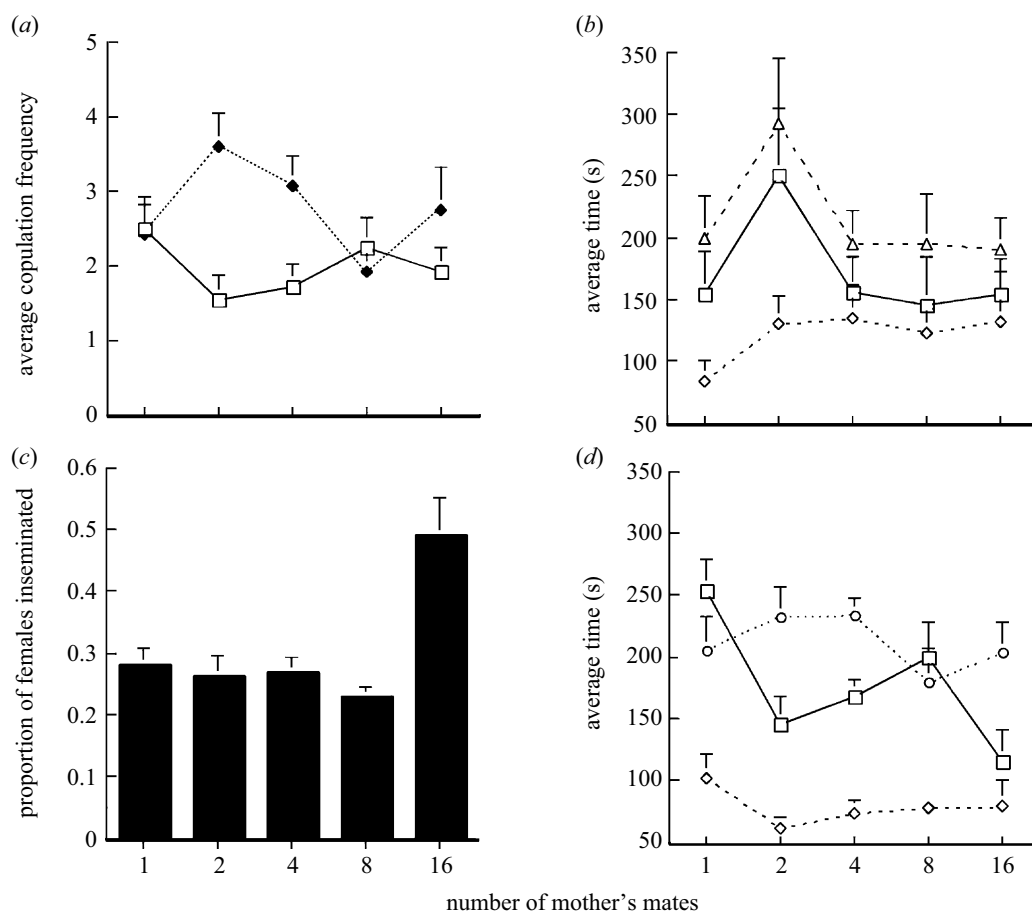


Figure 1. Mating behaviour of F₁ progeny: (a) copulation frequency (sons, squares; daughters, circles); (b) sons' mating behaviour (time to first copulation, squares; copulation duration, circles; total time on copulation activity, triangles); (c) sons' insemination capacity; and (d) daughters' mating behaviour (time to first copulation, squares; copulation duration, circles; total time on copulation activity, triangles). Means and standard errors are shown.

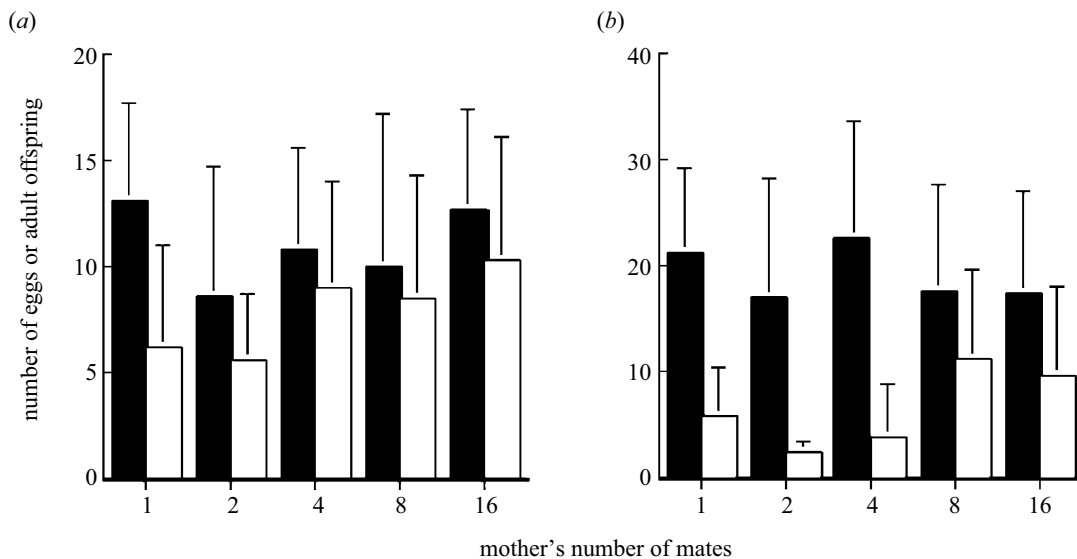


Figure 2. Offspring production of F₁ progeny. (a) F₂ eggs (black bars) and adult offspring (white bars) sired by F₁ males, and (b) F₂ eggs (black bars) and adult offspring (white bars) production by F₁ females. Means and standard deviations are shown.

'sexy sperm' confers a benefit to sons only, only sons, not daughters, produced by polyandrous females would be expected to have higher fitness than those produced by monandrous females. The third hypothesis is that females may acquire both 'sexy sons' and 'good genes' benefits from post-copulatory paternity-biasing processes.

Our results support the hypothesis that females acquire both 'sexy sons' and 'good genes' benefits from multiple mating because the sons from polyandrous females had increased ability to inseminate females (sexiness) and the F₂ individuals derived from polyandrous grandmothers showed increased egg-to-adult viability (good genes). Our

male insemination assays allowed only one male to mate with five different virgin females consecutively. Although male insemination success in our assays may be affected by the ability to attract, locate, guard or mount mates, produce and pass sperm, and other factors, we found no difference in the frequency or duration of copulation, time to first copulation or number of mating attempts for F_1 males from singly and multiply mated females. Thus, a higher insemination capacity of sons from females that had 16 mates probably reflected the consequences of increased ability to produce and transfer sperm. Male offspring from other multiple mating treatments (two, four or eight partners) did not differ from sons of singly mated females in this characteristic, probably due to insufficient male–male competition intensity before copulation or sperm competition after copulation in these treatments.

However, the ‘sexy sperm’ and ‘good genes’ hypotheses cannot explain why daughters from multiply mated females had significantly lower fitness than those from singly mated females. Cannibalism rates in the treatments of a female with more males (e.g. 8 or 16 males) may be higher than in the treatments of a female with fewer males (e.g. one or two males) (Park *et al.* 1965). Higher cannibalism rates in the treatments with multiple males should not affect F_1 male and female fitness differently because there was no evidence that male and female eggs were cannibalized differentially by the adult beetles. We suggest three possibilities to account for the observed opposite fitness effects between F_1 males and F_1 females from females with multiple potential mates: (i) reduced non-genetic investment by one or both parents on daughters of multiply mated females; (ii) differential paternity of sons and daughters; and (iii) sexually antagonistic genetic variation in traits for fitness enhancement (Chippindale *et al.* 2001).

The first hypothesis postulates that lower fitness of daughters of polyandrous mothers results from reduced non-genetic investment (such as nutrients) in female progeny by one or both parents. Non-genetic contributions from parents may be very important for offspring (e.g. monarch butterflies (Oberhauser 1989, 1997); stink-bugs (McLain 1998); crickets (Weigensberg *et al.* 1998)) and may even affect F_2 quality (seed beetles (Fox & Savalli 1998)). Many studies document differential maternal investment based on mate quality and its importance for offspring fitness. For example, offspring of more attractive sires receive more maternal investment as indicated by greater egg mass in a butterfly species (Wedell 1996) and in the mallard (Cunningham & Russell 2000) compared with offspring from less attractive sires. The differential maternal-investment hypothesis predicts a difference between male and female offspring from multiply mated females in life-history traits, such as pupal weight, survival and growth rate of male and female larvae.

The latter two hypotheses invoke a genetic mechanism. The differential paternity hypothesis relies on the premise that sperm from more fit males are more likely to sire sons, whereas sperm from less fit males are more likely to sire daughters. Detailed analysis on paternity of sons and daughters from polyandrous females is needed to refute this hypothesis. The sexually antagonistic variation hypothesis states that there may be a reduction in daughters’ fitness with an increase in sons’ fitness due to

negative correlation of the genes that determine sons’ and daughters’ fitness (Chippindale *et al.* 2001). Conflict of interest between the sexes may cause certain traits that are important for reproductive success to be selected for in opposite directions in males and females (Chippindale *et al.* 2001). If such traits are not sex limited (found in both sexes), then it would result in negative correlation in the fitness of males and females (Chippindale *et al.* 2001). Antagonistic variation may result from differences in the reproductive roles of adults. Because the conflict of interests between sexes exists only in the adult (reproductive) stages and not prior to that, the negative correlation would not be seen in pre-adult stages. Our finding that both sons and daughters of multiply mated females had increased F_2 egg-to-adult viability (a trait affecting pre-reproductive fitness) supports this prediction. Similar results were observed in *Drosophila*, in which a study of males and females sharing the same haploid genome showed that the adult fitness of the two sexes was negatively correlated, whereas juvenile fitness of males and females was positively correlated (Chippindale *et al.* 2001).

Three types of data are needed to determine which of the above mechanisms lead to the opposite fitness pattern of sons and daughters from polyandrous mothers. First, information on offspring paternity is required. Sperm precedence studies in *T. castaneum* show that the last male to mate sires the majority of the offspring (71.4% in the first 48 h (Lewis & Jutkiewicz 1998)). Therefore, either precopulatory male–male competition or female choice (Lewis & Austad 1994; Edvardsson & Arnqvist 2000) could have an important influence on the offspring paternity. The intermating interval for females is as short as a few minutes (A. Pai, unpublished data) and is much shorter than the time taken for sperm transfer from bursa to spermathecae (usually more than 30 min (Bloch Qazi *et al.* 1996)). Because females remate even before sperm from the initial copulation has been transferred to storage sites, sperm competition via sperm displacement by rival’s sperm (Haubruge *et al.* 1999) will probably take place. Thus, females may not use the sperm from all their mates. Paternity analysis of offspring from multiply mated females can reveal the number of different males that have sired a female’s offspring, and this is the focus of our ongoing research. Second, the exact fitness correlates that cause the fitness of F_1 females from polyandrous mothers to reduce in competitive environments need to be determined. Finally, more sensitive tests for differential parental investment in male and female offspring need to be conducted.

In summary, this paper demonstrated that in *Tribolium* beetles, rapid female multiple mating conferred fitness benefits on F_1 males but reduced the fitness of F_1 females under the condition of high intraspecific competition. Under the condition of low intraspecific competition, F_1 females from highly polyandrous mothers also gained indirect fitness benefits because their egg-to-adult viability was increased. The extent of female multiple mating may depend on environmental conditions such as levels of intraspecific competition. The cost of multiple mating suggests a conflict of interest between the sexes. The trade-off between genetic benefits and costs should determine the extent of female multiple mating.

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REFERENCES

- Baer, B. & Schmid-Hempel, P. 1999 Experimental variation in polyandry affects parasite loads and fitness in a bumblebee. *Nature* **397**, 151–154.
- Bernasconi, G. & Keller, L. 2001 Female polyandry affects son's reproductive success in the red flour beetle *Tribolium castaneum*. *J. Evol. Biol.* **14**, 186–193.
- Birkhead, T. R., Fletcher, F., Pellatt, E. J. & Staples, A. 1995 Ejaculate quality and the success of extra-pair copulation in the zebra finch. *Nature* **377**, 422–424.
- Bloch Qazi, M. C., Herbeck, J. T. & Lewis, S. M. 1996 Mechanisms of sperm transfer and storage in the red flour beetle (Coleoptera: Tenebrionidae). *Ann. Entomol. Soc. Am.* **89**, 892–897.
- Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. 1995 Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**, 241–244.
- Chippindale, A., Gibson, J. & Rice, W. 2001 Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 1671–1675.
- Cunningham, E. J. A. & Russell, A. F. 2000 Egg investment is influenced by male attractiveness in the mallard. *Nature* **404**, 74–77.
- Drummond, B. 1984 Multiple mating and sperm competition in the Lepidoptera. In *Sperm competition and the evolution of animal mating strategies* (ed. R. L. Smith), pp. 291–370. Orlando, FL: Academic Press.
- Edvardsson, M. & Arnqvist, G. 2000 Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. *Proc. R. Soc. Lond. B* **267**, 559–563.
- Evans, J. P. & Magurran, A. E. 2000 Multiple benefits of multiple mating in guppies. *Proc. Natl Acad. Sci. USA* **97**, 10 074–10 076.
- Fox, C. W. & Savalli, U. M. 1998 Inheritance of environmental variation in body size: superparasitism of seeds affects progeny and grandprogeny body size via a nongenetic maternal effect. *Evolution* **52**, 172–182.
- Haubruege, E., Arnaud, L., Mignon, J. & Gage, M. J. G. 1999 Fertilization by proxy: rival sperm removal and translocation in a beetle. *Proc. R. Soc. Lond. B* **266**, 1183–1187.
- Jennions, M. & Petrie, M. 2000 Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**, 21–64.
- Keller, L. & Reeve, H. 1995 Why do females mate with multiple males? The sexually selected sperm hypothesis. *Adv. Study Behav.* **24**, 291–315.
- Lewis, S. M. & Austad, S. N. 1994 Sexual selection in flour beetles: the relationship between sperm precedence and male olfactory attractiveness. *Behav. Ecol.* **5**, 219–224.
- Lewis, S. M. & Jutkiewicz, E. 1998 Sperm precedence and sperm storage in multiply mated red flour beetles. *Behav. Ecol. Sociobiol.* **43**, 365–369.
- McLain, D. 1998 Non-genetic benefits of mate choice: fecundity enhancement and sexy sons. *Anim. Behav.* **55**, 1191–1201.
- Oberhauser, K. 1989 Effects of spermatophores on male and female monarch butterfly reproductive success. *Behav. Ecol. Sociobiol.* **25**, 237–246.
- Oberhauser, K. 1997 Fecundity, lifespan, and egg mass in butterflies: effects of male derived nutrients and female size. *Funct. Ecol.* **11**, 166–175.
- Otronen, M. 1994 Repeated copulations as a strategy to maximize fertilization in the fly, *Dryomyza anilis* (Dryomyzidae). *Behav. Ecol.* **5**, 51–56.
- Park, T., Mertz, D. B., Grodzinski, W. & Prus, T. 1965 Cannibalistic predation in populations of flour beetles. *Physiol. Zool.* **38**, 289–321.
- Parker, G. 1984 Sperm competition and the evolution of animal mating strategies. In *Sperm competition and the evolution of animal mating strategies* (ed. R. L. Smith), pp. 2–60. Orlando, FL: Academic Press.
- Reynolds, J. D. & Gross, M. R. 1992 Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **250**, 57–62.
- Ridley, M. 1988 Mating frequency and fecundity in insects. *Biol. Rev. Camb. Phil. Soc.* **63**, 509–549.
- SAS 1995 *JMP statistics and graphics guide*. Cary, NC: SAS Institute Inc.
- Simmons, L. & Siva-Jothy, M. 1998 Sperm competition in insects: mechanisms and the potential for selection. In *Sperm competition and sexual selection* (ed. T. Birkhead & A. Moller), pp. 341–434. San Diego, CA and London: Academic Press.
- Sokoloff, A. 1974 *The biology of Tribolium*. Oxford University Press.
- Wade, M. 1977 An experimental study of group selection. *Evolution* **31**, 134–153.
- Watson, P. J., Arnqvist, G. & Stallmann, R. R. 1998 Sexual conflict and the energetic costs of mating and mate choice in water striders. *Am. Nat.* **151**, 46–58.
- Wedell, N. 1996 Mate quality affects reproductive effort in a paternally investing species. *Am. Nat.* **148**, 1075–1088.
- Weigensberg, I., Carriere, Y. & Roff, D. A. 1998 Effects of male genetic contribution and paternal investment to egg and hatchling size in the cricket, *Gryllus firmus*. *J. Evol. Biol.* **11**, 135–146.
- Wilkinson, G. S., Presgraves, D. C. & Crymes, L. 1998 Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. *Nature* **391**, 276–279.
- Yan, G. & Stevens, L. 1995 Selection by parasites on components of fitness in *Tribolium* beetles: the effect of intraspecific competition. *Am. Nat.* **146**, 795–813.
- Yasui, Y. 1997 A 'good-sperm' model can explain the evolution of costly multiple mating by females. *Am. Nat.* **149**, 573–584.
- Yasui, Y. 1998 The 'genetic benefits' of female multiple mating reconsidered. *Trends Ecol. Evol.* **13**, 246–250.
- Zeh, J. A. & Zeh, D. W. 1996 The evolution of polyandry. I. Intra-genomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. B* **263**, 1711–1717.

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