Female polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*

G. BERNASCONI*† & L. KELLER†

*Institute of Ecology, Bâtiment de Biologie, University of Lausanne, 1015 Lausanne, Switzerland
†Abteilung Verhaltensökologie, Ethologische Station Hasli, University of Berne, 3032 Hinterkappelen, Switzerland

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Abstract

A potential benefit to females mating with multiple males is the increased probability that their sons will inherit traits enhancing their pre- or post-mating ability to obtain fertilizations. We allowed red flour beetle (*Tribolium castaneum*) females to mate on three consecutive days either repeatedly to the same male or to three different males. This procedure was carried out in 20 replicate lines, 10 established with wild-type, and 10 with the Chicago black morph, a partially dominant phenotypic marker. The paternity achieved by the sons of females from polyandrous vs. monandrous lines of contrasting morph was assessed in the F1, F2 and F3 generation by mating wild-type stock females to two experimental males and assigning the progeny to either sire based on phenotype. The sons of polyandrous wild-type females achieved significantly higher paternity when mating in the second male role than the sons of monandrous wild-type females. By contrast, when mating in the first male role, males produced by females from polyandrous lines tended to have lower paternity than males from monandrous lines. Both effects were independent of the number of mates of the black competitor's mother, and interacted significantly with the number of progeny laid by the female. These results provide the first evidence that manipulating the number of mates of a female can influence her sons' mating success and suggest a potential trade-off between offence and defence in this species.

Introduction

The relative male success in siring the progeny of multiply mated females varies greatly in insects (e.g. Prout & Bungaard, 1977; Lewis & Austad, 1990; Cooper *et al.*, 1996), the intraspecific standard deviation of P2 values (proportion of a female's progeny sired by the second male to mate, Boorman & Parker, 1976) across 52 species being on average 0.23 ± 0.14 (Simmons & Siva-Jothy, 1998). In some species, variation among males in their fertilization success has been shown to be repeatable (Lewis & Austad, 1990; Arnqvist & Danielsson, 1999) and heritable (Chapman *et al.*, 1995; Clark *et al.*, 1995; Rice, 1996; Radwan, 1998). The fertilization

Correspondence: Dr G. Bernasconi, Institut für Umweltwissenschaften, University of Zurich, Winterthurerstr.190, 8057 Zurich, Switzerland.
Tel.: +41 1 635 48 07; fax: +41 1 635 57 11;
e-mail: bernasco@uwinst.unizh.ch

success of males can be determined by premating factors such as the ability to obtain (repeated) copulations, or by post-mating mechanisms such as the quantity of sperm they produce and transfer during mating, the success of their sperm in reaching and fertilizing an egg (Birkhead *et al.*, 1999), their ability to displace the sperm that females stored during previous matings (Civetta, 1999; Price *et al.*, 1999), their ability to prevent any other male from subsequently introducing sperm (e.g. with mating plugs) or displacing their own sperm (Keller & Reeve, 1995). In fact, the diversity in mechanisms affecting paternity (Simmons & Siva-Jothy, 1998) testifies that the degree of competition among males for fertilization is a significant evolutionary force shaping species morphology and behaviour (Arnqvist, 1998; Presgraves *et al.*, 1999).

One critical factor that can affect the males' fertilization ability is the intensity of sperm competition

experienced by their male ancestors in previous generations. Females mating with multiple males should, on average, produce sons with higher fertilization success than females mating with a single male, when there are heritable differences among males in their sperm competition ability (Harvey & May, 1989; Curtsinger, 1991; Keller & Reeve, 1995), and/or in their attractiveness (Weatherhead & Robertson, 1979). The potential benefits of multiple mating may help to explain why females mate with multiple males in so many species (Keller & Reeve, 1995). Mating entails costs such as time and energy devoted to courtship and copulation, exposure to predation during mating, exposure to toxic seminal fluid products and risk of contracting diseases (Daly, 1978; Thornhill & Alcock, 1983; Arnqvist, 1989; Fowler & Partridge, 1989; Chapman *et al.*, 1993; Sheldon, 1993), yet in many species females actively promote mating with multiple males. For instance, in the polyandrous pseudoscorpion, *Cordylochernes scorpioides*, once-mated females actively discriminate against males from whom they had previously accepted sperm but not against new males (Zeh *et al.*, 1998), and female hide beetles, *Dermestes maculatus*, are more likely to remate with a novel male than with their first mate (Archer & Elgar, 1999).

In this study, we investigated the consequences of female multiple mating by presenting females of the red flour beetle *Tribolium castaneum* (HERBST) either to one or to three males and repeating this procedure for three generations. *T. castaneum* is a polyandrous species with frequent remating (Sokoloff, 1974; Lewis & Iannini, 1995) and long-term sperm storage (Bloch Qazi *et al.*, 1996; Lewis & Jutkiewicz, 1998). Sperm precedence varies considerably among males. For 11 pairs of rival males mated with replicate females second male of paternity varied between 40% and 86% (Lewis & Austad, 1990) and comparable variation is found also for per-copulation P2 values (Lewis & Jutkiewicz, 1998). We assessed the reproductive success of sons of the females in the mating regimes with and without sperm competition by mating wild-type stock females to two experimental males and using a partially dominant cuticular colour marker to assign paternity. We studied both offence ability (defined as paternity achieved in competition with a previous rival) and defence ability (paternity achieved against a future rival) by testing all combinations of morphs and mother's mating regimes with respect to mating order.

Methods

For all experiments beetles were kept in 10-mm × 100-mm vials (all with 2.5 g medium) in a dark, ventilated rearing chamber at 28–31 °C, 60–70% RH. Standard medium was prepared every 2–3 weeks with pretreated flour (65 °C/12 h, 95% by weight), dried yeast (–20 °C/12 h, 5%) and Fumadil B (Bicyclohexyl-ammonium Fumagillin, 0.03%; Wade & Goodnight, 1991).

We simultaneously established 10 lines in which females were presented a single male for mating (monandrous lines) and 10 lines in which they were sequentially presented three different males (polyandrous lines). Each line was established with 24 females and 24 males, and this number of individuals was kept constant throughout the experiment. Half of the lines in each treatment were established using the wild-type morph, the other half using the Chicago black morph. Chicago black is a partially dominant, autosomal phenotypic marker (Sokoloff *et al.*, 1960), with fertility and egg-to-adult survival comparable to the wildtype under optimal rearing conditions (Sinnock, 1969). Stock populations were obtained by interbreeding laboratory strains of different origin (1: Tribolium Stock Center; 2: Sara M. Lewis; 3: Carolina Biological Supply, Barlington NC; black morphs came from 1 and 2, wild-type morphs from 1, 2, 3) during four to five generations (Wade, 1980). The number of adult founders was 350 for the wild-type morph and 300 for the black morph (juveniles not counted). In the monandrous lines females were given the same male during three days, whereas in the polyandrous lines each female was mated to three different males in succession (24 h per male). This procedure ensured that in both monandrous and polyandrous lines both females and males were under similar conditions, the only difference being that they were with the same mate during three days in the monandrous regime and three different mates in the polyandrous regime. Matings were not directly observed. We used a randomised design to determine which male was presented to which female, with the restriction that maternal sib- or half-sib matings were avoided. Randomization of mating schemes was carried out independently within each line to ensure stochasticity in the levels of inbreeding with respect to line and mating regimes. During the 3-day mating period, males and females in the monandrous lines were sham-manipulated (taken out of the medium and then reintroduced to the same vial) to ensure the same level of disturbance in monandrous and polyandrous lines.

Immediately after the 3-day mating period, females were transferred sequentially in two oviposition vials containing fresh medium (three days per vial). This was done to ensure that, if there was no progeny in the first oviposition vial, we could use progeny from the second vial to continue the line. To obtain virgin individuals for the next generation, two males and two females were selected at the pupal stage (days 15–18 after mid-oviposition) and reared to adulthood. One adult individual of each sex was used to continue the lines. We also randomly selected one male offspring from each of eight females in each line for mating experiments to compare the fertilization success of males from monandrous and polyandrous regimes. Two males of contrasting cuticular morph and from different lines were allowed to mate to one wild-type stock virgin female. We conducted all

Table 1 Proportion of progeny sired by the second male to mate (P2-value) and female fecundity (number of offspring); n = number of replicate test matings (total n = 218 females). Values are given as mean ± SD.

Male 1	Male 2		F1	F2	F3
Black	*Wild-type*				
Polyandrous	Polyandrous	P2	0.91 ± 0.14	0.83 ± 0.19	0.97 ± 0.04
		Fecundity	41 ± 22, n = 8	71 ± 29, n = 10	60 ± 19, n = 10
Monandrous	Polyandrous	P2	0.96 ± 0.07	0.94 ± 0.11	0.97 ± 0.03
		Fecundity	53 ± 27, n = 8	86 ± 33, n = 10	53 ± 22, n = 10
Polyandrous	Monandrous	P2	0.75 ± 0.37	0.93 ± 0.08	0.94 ± 0.07
		Fecundity	53 ± 30, n = 9	76 ± 33, n = 9	42 ± 23, n = 10
Monandrous	Monandrous	P2	0.78 ± 0.23	0.82 ± 0.22	0.96 ± 0.06
		Fecundity	42 ± 22, n = 9	44 ± 37, n = 8	49 ± 19, n = 10
Wild-type	*Black*				
Polyandrous	Polyandrous	P2	0.69 ± 0.17	0.69 ± 0.20	0.67 ± 0.23
		Fecundity	58 ± 16, n = 8	76 ± 23, n = 9	55 ± 19, n = 9
Monandrous	Polyandrous	P2	0.59 ± 0.33	0.52 ± 0.26	0.62 ± 0.21
		Fecundity	41 ± 20, n = 8	74 ± 29, n = 9	65 ± 24, n = 7
Polyandrous	Monandrous	P2	0.65 ± 0.29	0.66 ± 0.21	0.68 ± 0.23
		Fecundity	41 ± 24, n = 10	80 ± 27, n = 9	59 ± 28, n = 9
Monandrous	Monandrous	P2	0.47 ± 0.27	0.58 ± 0.30	0.56 ± 0.36
		Fecundity	45 ± 29, n = 9	54 ± 27, n = 10	63 ± 27, n = 10

possible morph × mother's mating regime × mating order-combinations (Table 1), but tested only some fixed combinations of lines. In total we tested 240 females and 480 males. Each male was mated to only one female (i.e. used in only one test mating). Matings were conducted by placing a female with the first male for a 24-h mating period during which pairs were free to mate *ad libitum*. This protocol does not allow determining the fertilization success per copulation, since we did not control the number of copulations, and was chosen because not all copulations lead to fertilization (Sokoloff, 1974, p. 199). Copulation can fail to result in progeny production in as many as 48% of the cases (37 directly observed copulations, G. Bernasconi, unpublished data), most likely because of lack of sperm transfer (as observed through female dissection for 15% out of 130 copulations, here defined as including multiple intromission bouts, Bloch Qazi *et al.*, 1996). To ensure that the first mating was successful, females were placed in isolation during 24 h in a vial later scored for the presence of progeny. After being placed in a new vial with the second male during 24 h, females were transferred sequentially in two oviposition vials containing fresh medium (3 ± 1 days per vial). This transfer was done to minimize sib-cannibalism which occurs between developmental stages (Lewis & Austad, 1990). We collected eggs over two oviposition vials to ensure a sufficient number of offspring. Sperm competition success was estimated as the proportion of the female's offspring sired by the second male (P2 value). The adult offspring of each female were sieved out of the medium (45 days after mid-oviposition) and the cuticular colour examined under low magnification by comparison with a reference of 10 wild-type and 10 heterozygous individuals. In total,

we assigned paternity of 12 578 offspring produced by 218 test females. The average (± SD) number of offspring per female was 46.7 ± 23.9 in the F1 (n = 69), 70.5 ± 1.2 in the F2 (n = 74) and 55.2 ± 22.9 in the F3 (n = 75) generation (Table 1). Variations in offspring number across generations were possibly due to variation in the oviposition time among generations (3 ± 1 days), or to a 2-day drop in temperature to 20 °C that occurred during the development of the F2 generation (all lines were at the same developmental stage ± 3 days). To control for possible effects of variation in female productivity over generations, the number of offspring produced was used as a covariable in the analyses.

To discard cases where matings had been unsuccessful we did not consider in the analyses cases where P2 was equal to 0 and 1 (except cases where P2 was 1 but the first mating had been successful as indicated by female egg-laying during the 24 h following the mating with the first male). Overall, 22 such cases were discarded, the final sample size being 218 (black second male = 107; wild-type second male = 111). For statistical analyses the fertilization success of the second male was expressed as the adjusted ratio of second to first male progeny, $y = A/(B + 1)$, where A = number of progeny sired by second male and B = number of progeny sired by first male (Haldane, 1956). This adjusted ratio is recommended to overcome the fact that angularly transformed P2 values rarely fulfil normality and homoscedasticity of the residuals (Gilchrist & Partridge, 1997; Hugues, 1997). The response variable $y = A/(B + 1)$ was further Box-Cox transformed. These transformed variables did not significantly depart from a normal distribution (Shapiro-Wilk's test; wild-type first male: $\lambda = -0.06$; W = 0.99, n = 107, P = 0.57; wild-type second male: λ = 0.26,

W = 0.98, $n = 111$, $P = 0.26$). For ease of comparison with previous studies, we also report P2 values in the results.

Wild-type second males had higher P2 values than black second males (see Results & Sinnock, 1969; Lewis & Austad, 1990) and the standard deviation of P2 values differed significantly between these groups (variance ratio test: $F = 2.33$, $P < 0.0001$). We therefore analysed separately data for matings where the second male was a wild-type vs. black morph. Data were analysed with an analysis of covariance (ANCOVA), the initial model containing the mother's mating regime of the first male, mother's mating regime of the second male and generation number as factors as well as the number of offspring per female as a covariable. Generation was entered as a factor because at each generation we tested eight randomly selected males from each line. The two-way interactions between factors and factors-covariable were also entered in the analysis. Model selection was carried out backwards by stepwise elimination of the term with highest P-value (i.e. the least-significant) until the minimal adequate model retained only terms for which $P < 0.10$. After the model selection was done we performed residual diagnostics and excluded cases with high leverage (> 0.10). There were three such cases in the group with wild-type second males and two in the group with wild-type first males. Including these values did not change the outcome of the analyses. Data were analysed using S-Plus 4.5 (MathSoft Inc., Cambridge, MA, USA) and Stata 6.0 (Stata Corporation, College Station, TX, USA).

Because broods consisted of full sibs in the monandrous lines and full- and half-sibs in the polyandrous lines, the intensity of sib competition might have differed between polyandrous and monandrous lines (see Garcia & Toro, 1993). Because P2 values have been shown to depend on the relative size of males in one study (Lewis & Austad, 1990; but see Lewis & Austad, 1994) we compared the size of males in the monandrous and polyandrous lines in the mating experiments of the F3 generation ($n = 160$ males) to ensure that the mother's mating regime did not influence male size. We used elytral length (measured to the nearest micrometre with a measuring table under 16×10 magnification) as a measure of body size (Lewis & Austad, 1990).

Results

P2 values were significantly higher (mean = 0.90, median = 0.97, SD = 0.17, $n = 111$) when wild-type males were second to mate than when black males were second to mate (mean = 0.61; median = 0.67; SD = 0.26, $n = 107$; Mann Whitney U-test: $U = 1917.5$, $P < 0.001$; Fig. 1, Table 1). A strong effect of male body colour genotype on male fertilization success is in agreement with previous studies showing that the two morphs differ in their competitive ability as second males when mated with wild-type females (Sinnock, 1969; Lewis & Austad, 1990).

In mating experiments where wild-type beetles were second males, paternity shares of competing males (expressed as the adjusted ratio of second to first male progeny) were significantly affected by the mother's mating regime of the second (wild-type) male, the number of offspring produced and the interaction between these two factors (Table 2). Second wild-type males from polyandrous lines obtained higher paternity (offence) than second wild-type males from monandrous lines. Offence ability of both types of males increased
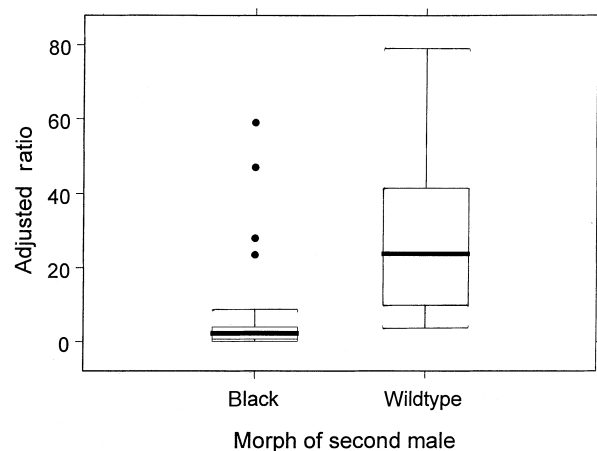

Fig. 1 Box plot of relative paternity obtained by the second male to mate for matings with black morph second males (left) and wild-type second males (right), given as the adjusted ratio of second to first male progeny (see Methods).
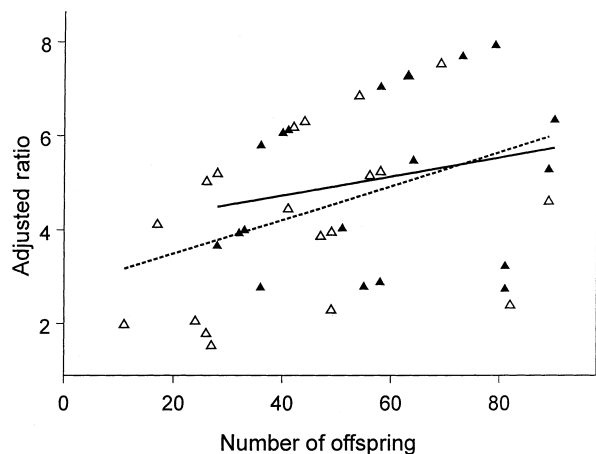
Table 2 ANCOVA table (minimal adequate model) for the Box-Cox transformed adjusted ratio of second to first male progeny, from matings with mating order: black male first, wild-type male second ($n = 108$, $R^2 = 0.26$).

Source	Partial SS	d.f.	MS	F	Prob > F
Model	165.0	5	33.0	7.15	< 0.0001
Number of offspring	80.2	1	80.2	17.38	0.0001
Generation	25.1	2	12.6	2.73	0.07
Mother's mating regime of second male	49.2	1	49.2	10.67	0.0015
Mother's mating regime of second male × Number of offspring	45.5	1	45.5	9.86	0.002
Residual	470.5	102	4.6		
Total	635.5	107	5.9		

Fig. 2 Effect plot for the mother's mating regime of wild-type *second* males (shown for the F3 generation). Filled symbols and line: second male from polyandrous regime. Empty symbols and dotted line: second males from monandrous regime. Y-axis: Box-Cox transformed adjusted ratio of second to first male progeny.

with number of offspring. However, this increase was slower for males from the polyandrous lines (Fig. 2; Table 2). Hence, the difference in offence ability of the two types of males was the greatest for females with smaller number of offspring.

The minimal adequate model explained 26% (R^2) of the observed variance. The effect of generation only showed a trend ($P = 0.07$). Neither the mother's mating regime of the first (black) male nor the interaction between mother's mating regime of first and second male were retained in the minimal adequate model. Hence, there was no evidence that the mother's mating regime influenced the ability of first (black) males to withstand sperm competition from a subsequent male (defence ability). Differences in paternity shares were due only to differences in offence associated with the mother's mating regime of the second (wild-type) male.

In mating experiments where wild-type males were first to mate, paternity shares of competing males were significantly affected only by the interaction between the mother's mating regime of the first (wild-type) male and the number of offspring produced (Table 3). There was a tendency for paternity shares to be affected by the

mother's mating regime of the first (wild-type) male, with first (wild-type) males from monandrous lines tending to have higher defence ability than wild-type males from polyandrous lines. Higher numbers of offspring were associated with greater differences in defence ability between wild-type males of both mother's mating regimes (Fig. 3). In fact, the defence ability of males from the polyandrous regime decreased with larger number of offspring, whereas defence ability of males from the monandrous regime increased with larger number of offspring. The minimal adequate model explained 14% (R^2) of observed variance. Again neither the mother's mating regime of the second (black) male, nor generation or other interactions were significant.

The mother's mating regime did not significantly affect male size ($F_{1,156} = 1.15$, $P = 0.32$) whereas morphs differed significantly ($F_{1,156} = 283.7$, $P < 0.001$), with wild-type males being larger (overall: 2.45 ± 0.12 mm; polyandrous: 2.46 ± 0.12 mm, $n = 40$; monandrous: 2.43 ± 0.12 mm, $n = 40$) than black males (overall: 2.15 ± 0.10 mm; polyandrous: 2.14 ± 0.11 mm, $n = 40$; monandrous: 2.16 ± 0.10 mm, $n = 40$). This suggests that there was no difference in pre-adult competition in
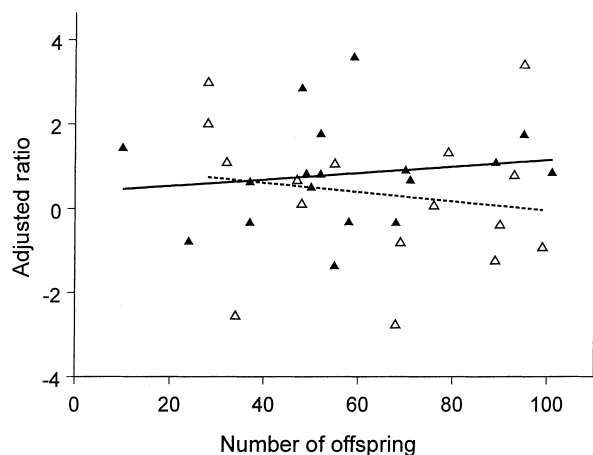

Fig. 3 Effect plot for the mother's mating regime of wild-type *first* males (shown for the F3 generation). Filled symbols and line: first male from polyandrous regime. Empty symbols and dotted line: first males from monandrous regime. Y-axis: Box-Cox transformed adjusted ratio of second to first male progeny.

Source	Partial SS	d.f.	MS	F	Prob > F
Model	25.3	3	8.4	5.60	0.0014
Number of offspring	0.603	1	0.603	0.40	0.53
Mother's mating regime of first male	4.46	1	4.46	2.96	0.088
Mother's mating regime of first male × Number of offspring	14.27	1	14.27	9.47	0.0027
Residual	152.26	101	1.51		
Total	177.59	104	1.71		

Table 3 ANCOVA table (minimal adequate model) for the Box-Cox transformed adjusted ratio of second to first male progeny, from matings with mating order: wild-type male first, black male second ($n = 105$, $R^2 = 0.14$).

broods of the two mother's mating regimes or that differences in competition did not translate into significant size differences. Wild-type males (2.45 ± 0.12 mm) were significantly larger than black males (2.15 ± 0.10 mm; $F_{1,156} = 283.7$, $P < 0.001$). There was no significant association between paternity shares and size ratio of second to first male (Spearman rank correlation, wild-type male mated second: $r_s = 0.28$, $n = 40$, $P = 0.08$; wild-type males mated first: $r_s = -0.11$, $n = 35$, $P = 0.54$). First males to mate had similar size as second males (2.30 ± 0.19 mm and 2.30 ± 0.19 mm, respectively; $F_{1,156} = 0.001$, $P = 0.97$), indicating that randomization produced unbiased assignment of males with respect to size.

Discussion

The share of paternity obtained by wild-type *T. castaneum* sons when competing against a female's previous mate was influenced by the mating regime to which we exposed their mothers, by the number of offspring produced after the competitive double mating and the interaction between these two terms. In the experiments where wild-type sons were second to mate, wild-type males produced by females that mated to three different males fathered a greater proportion of offspring than males produced by females that mated to a single male. Because females used in experiments comparing the fertilization success of males were taken from the unselected stock population, differences between males from the two mother's mating regimes cannot merely be accounted for by differences in female reproductive behaviour. There are three possible explanations for the finding that wild-type second males produced by females that mated to three different males fathered a greater proportion of offspring than males produced by females that mated to a single male.

First, this may result from direct benefits of mating with multiple males. However, this hypothesis is unlikely because all mothers had access to males during three days and there were no differences in the mating history of males between the monandrous and polyandrous regimes (our experiments, however, do not rule out the possibility that females mated more frequently when encountering new males). Moreover, our data show that sons of wild-type polyandrous females obtained a smaller share of paternity when mating in the first mating role (see below). It is thus difficult to conceive how direct benefits could increase the sons' share of paternity when mating in the second but not the first male mating role. Finally, the sons of polyandrous and monandrous mothers did not differ significantly in adult body size, as would be predicted if polyandrous females obtained more direct benefits.

The second hypothesis is that the mating regime affected the attractiveness of males, with males being selected to become more attractive in the polyandrous treatment (e.g. olfactory attractiveness, Lewis & Austad, 1994). Males from the polyandrous regime may thus obtain greater paternity if they obtain more matings when having access to females, or if they are better at performing copulatory courtship behaviour (Edvardsson & Arnqvist, 2000). However, this hypothesis also fails to explain the lower paternity of males from the polyandrous regime when mating in the first mating role.

Finally, the third hypothesis is that the mating regime affected the fertilization efficiency of males, for instance through differences in the quality or quantity of sperm and accessory substances transferred during mating or via other components affecting male offence and defence ability (Keller & Reeve, 1995). Depending on how the mating regime affects offence and defence of males, it is possible that a polyandrous regime increased only offence but not defence mechanisms of males (see below). As pointed out by Yasuii (1997), it is also possible that the fertilization efficiency of males is correlated with the viability of their offspring. Our experiments do not allow testing for such an association as we only assessed paternity based on adult progeny. To test for this association would require additional measurements of juvenile survival and/or of the viability of daughters of polyandrous or monandrous females, as has been recently suggested (Jennions & Petrie, 2000). Although our experiments do not allow determining exactly why wild-type second males from the polyandrous mother's mating regime had greater paternity, our study provides the first evidence that experimental manipulation of female mating frequency can influence the mating or fertilization success of their sons.

A surprising finding of this study was that, when mating in the first male role, wild-type males from polyandrous lines tended to sire a lower proportion of the offspring. If this difference is not merely due to variation in the number of copulations obtained, this suggests that males from polyandrous lines had lower defence ability than males from monandrous lines, and that traits enhancing fertilization when mating as second male (offence) may trade-off with traits that enhance success when mating as a first male (defence). The benefits conferred by higher offence ability may be greater under high levels of polyandry than the costs due to lower defence ability. *T. castaneum* is a highly promiscuous species (i.e. many males compete for the same set of ova) with long-term sperm storage (Bloch Qazi *et al.*, 1996). Last-male sperm precedence is unaffected by the number of prior males (doubly vs. triply mated females, Lewis & Jutkiewicz, 1998). Hence, sperm competition intensity (Parker *et al.*, 1996) is high and selection is presumably the strongest on the last male to mate because these males ultimately achieve greater reproductive success than males that mated in the first role. In a study examining fertilization success in *Drosophila melanogaster*, Clark *et al.* (1995) found genetic variation for both offence and defence but no significant correlation between these two processes (Clark *et al.*, 1995). This,

and another study showing that selection for delayed senescence leads to a correlated response in defence, but none in offence (Service & Fales, 1993), suggest that in *D. melanogaster* offence and defence might be achieved by distinct, possibly uncorrelated, mechanisms (Simmons & Siva-Jothy, 1998). In the waterstrider *Gerris lateralis*, offence and defence ability are associated with different components of genital morphology, suggesting selection for both types of adaptations in this species (Arnqvist & Danielsson, 1999).

Our analyses show that offence ability of wild-type males increased with the number of offspring produced by females. A positive covariance between female fecundity and sperm precedence has been reported for a few species without nuptial gifts, suggesting that successful males obtain a two-fold advantage (Arnqvist & Danielsson, 1999, and references therein). In our study, both the *higher* offence and the *lower* defence of wild-type males from the polyandrous regime compared to males from the monandrous regime interacted significantly with the number of offspring produced by the female. A possible explanation for this pattern is that phenotypic differences associated with variation in female fecundities influence female control over parentage of their offspring. Significant genetic variation among females affecting sperm displacement has been reported in *Drosophila melanogaster* (Clark & Begun, 1998) and *Callosbruchus* beetles (Wilson *et al.*, 1997). Individual differences in P2 values have also been documented for *T. castaneum* females mated with the same pair of males (Lewis & Austad, 1990). Alternatively, fecundity could also be influenced by males, e.g. via active components of the seminal fluid, and these be in turn correlated with traits influencing offence and defence ability.

In contrast to wild-type males, mother's mating regime did not significantly affect offence and defence ability of black males. A possible explanation is that our black stock – obtained from only two source laboratories – had only limited genetic variation. The low fertilization success of these males would be consistent with this hypothesis, but only a comparative study of the genetic diversity of both strains would allow to test whether the black morph went through a significant bottleneck. Alternatively, the lack of response of the black morph to mother's mating regime may stem from the lack of long-term coevolution prior to our mating experiments between males of this morph and females from the wild-type stock (that were used for testing fertilization success). Evidence suggests that there is rapid coevolution between males and females in the arms race about control of insemination and egg fertilization (Chapman *et al.*, 1995; Rice, 1996; Stockley, 1997) and it is possible that the lack of long-term coevolution of black males and wild-type females resulted in lower fertilization success and a lack of treatment effect for these males. Consistent with this idea, black second males achieve high P2 values when mated to black females (Sinnock, 1969; Lewis & Austad, 1990).

The size ratio of competing males was not significantly correlated with paternity shares. In two previous studies male body size was not significantly correlated either to the number of sperm transferred per mating, or with the number of sperm stored by females (singly mated females, male body mass, Bloch Qazi *et al.*, 1996), or with paternity patterns (Lewis & Austad, 1994; but see Lewis & Austad, 1990). Moreover, in our experiment neither the size of wild-type nor the size of black morph males were affected by the mating regime of their mother. Similarly, a study in which female crickets (*Gryllus bimaculatus*) were mated repeatedly to the same or to different males found no significant difference in offspring adult body mass (Tregenza & Wedell, 1998).

In conclusion, this study provides the first experimental evidence that manipulation of the number of mates of females can affect their sons' reproductive success. That the effect of mothers' mating regime differently affected the sons' reproductive success depending on their mating order relative to the competing male also suggests a potential trade-off between male 'offence' and 'defence' ability in a species with last male advantage. Overall, the evidence that the reproductive success of males is influenced by the number of mates of their mothers is important because it may help to explain why females mate with multiple males in this and other animal species, particularly if differences in male reproductive success are due to post-mating differences.

Acknowledgments

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