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Fitness consequences of female preference for male pheromones in *Tenebrio molitor*

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ABSTRACT

Hypothesis: Sexual selection is maintained by 'good genes' effects of mate choice on females, and by mating with males with preferable pheromones females increase their fitness.

Organism: Cultured mealworm beetles (*Tenebrio molitor*).

Methods: Male mealworm beetles were paired by mass and age, their pheromones were collected by confining them on filter paper, and the two halves of the filter paper disks were presented to two mass-matched females. Females and males were then allowed to mate in three ways: (1) Among females preferring pheromones of the same male, one female mated with the male who had the preferred pheromones and the other female with the male who had the non-preferred pheromones. Among females preferring separate disks, both females mated either with (2) the male with the preferred pheromones or (3) the male with the non-preferred pheromones.

Results: Females made a clear choice between the pheromone disks, but preference for male pheromones did not translate into the number of live or dead offspring. However, females who preferred the disk of the same male and mated with the male with the preferred pheromones lived longer than the females who mated with the male with the non-preferred pheromones.

Keywords: fitness, good genes, handicap, lifespan, sexual conflict.

INTRODUCTION

Sexual selection refers to mating bias that is based on an individual's preference for a trait in the opposite sex (Andersson, 1994; Kokko *et al.*, 2003). Such preferences may evolve if the choosiness improves the fitness of individuals performing mate discrimination. Fitness consequences can be direct – that is, avoidance of horizontally transmitted parasites, avoidance of sexually harassing males, or acquisition of resources. Indirect fitness benefits can occur if preference for highly ornamented or dominant individuals enhances the genetic composition of descendants. According to the theory of sexual conflict (reviewed by Chapman *et al.*, 2003),

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the genes favourable to one sex may be deleterious to the other sex, which in turn may lead to mate discrimination but not necessarily 'good genes' sexual selection.

The offspring of highly ornamented males may have genes for attractiveness (Fisher, 1930), for good parasite resistance (Hamilton and Zuk, 1982; Folstad and Karter, 1992), or generally for the current environment as predicted by several handicap models (e.g. Pomiankowski, 1987; Iwasa and Pomiankowski, 1999). Any genetic fitness benefits of sexual selection require that sexual signals are honest indicators of viability, at least on average (Johnstone and Grafen, 1993). A positive correlation between a male's signalling and his qualities such as immune function or condition has been established (e.g. Andersson, 1986; David *et al.*, 2000; Ditchkoff *et al.*, 2001; Rantala *et al.*, 2000, 2002, 2003a; Johnsen *et al.*, 2003; Rantala and Kortet, 2003; Ahtiainen *et al.*, 2004). Honesty of sexual signalling may result from a quality-dependent cost of signal production (Zahavi, 1975; Pomiankowski, 1987; Folstad and Karter, 1992; Iwasa and Pomiankowski, 1999; Eshel *et al.*, 2002).

There are no direct data on the costs of pheromone production in insects. However, Rantala *et al.* (2003a) suggested that pheromones are condition-dependent signals, whose attractiveness decreases due to food deprivation. Wedekind (1994) showed theoretically that good genes signalling could evolve through direct revelation of quality by odours with no handicapping effect (MHC in vertebrates). Thus, females that prefer males who produce attractive pheromones should improve their fitness compared with females showing no or only a slight preference for male odours. However, the benefits of sexual selection could be small if the cost of female choice is also small (Alatalo *et al.*, 1998). In contrast to predictions, previous work on pheromone-based sexual selection in the flour beetle (*Tribolium castaneum*) did not find any relationship between progeny fitness and male attractiveness (Boake, 1985).

Other studies that have explored the effects of mate choice on offspring viability have yielded mixed results. Offspring of highly ornamented males of peacocks (*Pavo cristatus*) grew and survived better than the offspring of less ornamented males (Petrie, 1994). In guppies (*Poecilia reticulata*), a negative relationship between male sexual attractiveness and offspring survival has been reported (Brooks, 2000), but the opposite was observed in a later study by Watt *et al.* (2001), where the offspring of larger and preferred males survived better than the offspring of smaller and less preferred males. Kortet *et al.* (2004) found that male roach (*Rutilus rutilus*) with more dead than live *Ripidocotyle campanula* parasites produced offspring that were less viable at early ages than males that had smaller proportions of dead gill parasites. However, viability differences were absent in relation to male ornamentation. Thus, it would appear that the good genes effects of mate selection may exist in some species, but the average effects might be minor (Møller and Alatalo, 1999). In addition, some recent studies exploring the fitness effects of sexual selection in the context of sexual conflict (Chapman and Partridge, 1996) have suggested that the relationship between the sexual signalling of males and female fitness could even be negative (e.g. Droney, 2003).

The mealworm beetle, *Tenebrio molitor* L. (Coleoptera, Tenebrionidae), is a cosmopolitan pest of stored grains, which naturally lives 1–2 years as a larva before maturing into an adult. There is no obvious sexual dimorphism in this species, but each sex produces distinct pheromones that attract members of the opposite sex (Happ, 1969; August, 1971; Tanaka *et al.*, 1986; Carazo *et al.*, 2004). It has been found that female *T. molitor* prefer pheromones of males that have good immunocompetence (Rantala *et al.*, 2002). The pheromone of the male mealworm beetle stimulates the female's mobility, promotes aggregation of females to the vicinity of the male, and enhances copulatory behaviour (August, 1971; Tanaka *et al.*, 1986; Hurd and Parry, 1991).

Using a paired design of two mass-matched males and two mass-matched females, our aim in this study was to determine whether female mealworm beetles produce more, or more viable, offspring with males whose pheromones are preferred than with males whose pheromones are not preferred. Using dyadic female preference tests, we assigned the male pairs to two categories: (1) preferred by both mass-matched females, or (2) preferred by one of the females only. The males whose pheromones were preferred by both females were randomized between the females so that one female mated with the male with the preferred pheromone disk and the other female with the male with the non-preferred disk. The males whose pheromones were preferred by only one of the two females mated both either with the preferring female or with the non-preferring female. We hypothesized that the females with a preference for the same disk would produce more offspring with the male who had the preferred pheromones than with the male who had the non-preferred pheromones. In addition, we hypothesized that the females with a preference to separate disks would produce more offspring with a male who had the preferred pheromones than with the male who had the non-preferred pheromones. To highlight possible trade-offs between reproductive effort and lifespan, we followed the longevity of both males and females. To ensure that the results were either resource-dependent or genetically dependent, we performed additional trials to confirm that copulatory success was similar between males despite the females' preference for their pheromones.

MATERIALS AND METHODS

Beetles

The beetles were from a laboratory stock population purchased from a local commercial supplier and maintained at the University of Jyväskylä by the authors (12 : 12 h light/dark cycle, 27°C). We collected pupae twice in a week from a large laboratory stock housed in a 30-litre tub filled with 5 litres of wheat flour and fed *ad libitum* with cabbage. We determined the sex of pupae by examining the developing genitalia on the eighth abdominal segment (Bhattacharya *et al.*, 1970). The pupae were held in single-sex groups, and newly emerged adults were checked every other weekday and placed individually in plastic film roll canisters to ensure the virginity of both sexes. Beetles were offered a piece of fresh apple (*ad libitum*) every other weekday until they were used in experiments. We excluded individuals that had visible developmental abnormalities (less than 10% of the beetles).

Pheromone preference tests

Twelve days (12 ± 1 day) after emergence, we confined the experimental males ($n > 300$) individually on a filter paper disk (diameter 37 mm) placed in a circular petri dish provided with a lid for 48 h to acquire a sample of their pheromones (Rantala *et al.*, 2002). The most attractive sex pheromone of the male mealworm beetle has been reported to be long-chained and thus not a very volatile alcohol, 4-methyl-1-nonanol (Tanaka *et al.*, 1986). After removal of the males, we weighed them to the nearest 0.1 mg and assigned them to pairs ($n = 126$) that were at least 95% similar in mass (the mass of the lighter one was at least 95% of that of the larger one). Two-week-old females were paired using a similar procedure, but due to the limited number of females available, we formed 11 female pairs that did not fit into the 95% similarity requirement (mean difference 14.6%). These pairs were used in the

analysis of total preferences for males and for their lifespan, but were excluded from the other analyses (see below). The mean body mass (± standard deviation) of the males and females was 89.7 ± 21.7 mg and 91.9 ± 20.5 mg, respectively.

The filter paper disks for the two mass-matched males were cut in two and presented to two mass-matched females in two simultaneous dyadic female preference tests lasting 10 min (Rantala *et al.*, 2002, 2003a, 2003b). The masses of males and females were not matched, and consequently they were used in the statistical analyses as covariates. All the disks were used within 8 h of the removal of males from them. The two arenas for female choice trials both consisted of a 20-cm diameter glass dish inverted over a filter paper. The two halves of a disk containing male scent were placed at opposite ends of the arena, both about 1.5 cm from the boundary. Each half of a disk covered 1.7% of the total area of the arena. In a trial, a virgin female beetle was placed under a small petri dish in the centre of the circular arena to calm down for 8 min before release. During each pair of trials, the times the focal females spent on the filter paper disk halves were recorded using computer-aided timing (AV Bio-Statistics, by A. Vainikka, available at http://www.cc.jyu.fi/~ansvain/avbs).

Female preference was defined as the total time that a focal female spent on each piece of filter paper, and the disk on which the female spent more time was denoted the 'preferred' disk (for details, see the Results section). Preference tests had two possible outcomes: (1) both females preferred the half disk from the same male, or (2) the two females preferred separate halves of the disks. At random, we paired one of the two females preferring the same disk with the male whose disk the preferred disk was and the other female with the male whose disk was not preferred. There were two ways in which to assign the pairs in trials in which females preferred separate disks: (1) both females with a male whose disk was preferred by the particular female, or (2) both females with the male whose disk was not preferred by the particular female.

Tests of reproductive success

After the preference tests, males were identified by a small spot of silver drawing ink applied to their elytra and then placed in a plastic container ($6 \times 8 \times 8$ cm) together with the specified female (see above). The floor of each plastic container was covered with filter paper to enhance mobility of the beetles. In addition, wheat flour (14 ± 1 g) was added to one end of the container to act as egg-laying material and substrate for larvae. We gave males and females fresh apple *ad libitum* on days 1 and 3 and left them to mate for 3 days in plastic containers. After mating, we removed the males and placed them individually in film roll canisters with a piece of tissue paper. The paper was moistened every other day for as long as the male lived. Eighteen days after removal of the male, we performed a similar procedure for females. The females took 3 weeks to lay all of their fertilized eggs (see Worden *et al.*, 2000). We checked mortalities daily until all the beetles were dead. If the male or the female died before it was removed from the container, the pair of males was excluded from the analysis of offspring production. This was necessary because in such cases mating could have been interrupted or females might not have laid all of their eggs.

Viable eggs hatch normally in 4–14 days (Day, 1989). We counted the total numbers of live and dead larvae 21 days after the female was removed. We did not count the eggs because the number of hatched larvae was thought to represent a biologically more meaningful measure of fitness. Due to the mortality of either males or females during reproduction, 77 of 115 (67%) mass-matched pairs could be used in the analysis of reproductive success.

Since a few individuals escaped from the film roll canisters, the total number of males used for the analysis of longevity was 223. The number of larvae from those pairs that had live offspring 21 days after the female was removed was counted again 113–140 days after the female was removed.

Tests of copulation success

To determine whether the males who had a preferred pheromone disk differed in copulatory success from the males who had a non-preferred disk, we performed additional trials where we recorded time from the introduction of a male to copulation. We performed the 30-min tests in similar containers to those used for the determination of offspring production. We marked males with silver ink and placed females individually in the containers. After a few minutes to calm down, we introduced the males to the females. Nineteen pairs of males whose pheromones were preferred by both females and 24 males who had pheromones preferred by one female were used to compare the copulatory success between males. In all other respects, we treated these males in the same way as the males that we paired with the females without observation, and used these males for the analysis of offspring production.

Statistical analyses

The normal distribution of the parameters examined was tested using a Kolmogorov-Smirnov test, and a natural logarithm transformation was applied on the numbers of live and dead offspring and on the times until copulation. The chi-square test was used to assess the proportion of trials in which females preferred the same disk versus the proportion of trials in which females preferred separate disks. To determine whether the visiting times of females or the mass of males differed between the males whose disks were preferred either by one or two females, we used independent samples *t*-tests. To determine whether the males whose pheromones were preferred by both females copulated faster with the preferring female than with the non-preferring female, we used paired-samples *t*-tests. The paired design was broken when analysing differences in the times until copulation among males whose pheromones were preferred by one of the two females only. For this reason, analysis of covariance (ANCOVA) with both male and female mass as covariates was used to determine whether the males placed with a preferring female copulated faster than the males placed with a non-preferring female.

To determine whether the total (dead + live) number of offspring was associated with the mass or the lifespan of a male or a female, we performed Pearson's correlation analysis. We used a chi-square test to determine whether the proportion of successfully reproducing males was higher among males mated with a preferring female than among males mated with a non-preferring female.

Between-group differences in offspring production (at 21 days) were analysed using ANCOVA for repeated measures (RM-ANCOVA) excluding all the pairs with no offspring. To examine differences among the females with a preference for the same disk, within-subject factors (offspring: dead vs. live; preference: preferred vs. non-preferred) were entered. To examine differences among the females with a preference for different disks, offspring only was entered as a within-subject factor, and because the paired design was broken, preference was entered as a between-subject factor. Lifespan (days) and body mass of both males and females were used as covariates in the analyses. In the case of mass-

matched pairs, only average mass of males and females was entered. To study the effect of female preference for male pheromones on the number of live offspring 4 months after copulation, we used analyses of covariance with male and female mass, and the day of counting, as covariates. Pearson's correlation analyses were used to explain the effects of covariates.

Survival of males and females between mating groups was compared using a Kaplan-Meier survival analysis. Furthermore, average lifespan was analysed using a *t*-test for dependent samples. All statistical analyses were performed using SPSS 11.0.1 (SPSS Inc., USA).

RESULTS

Pheromone preference tests

In 63.5% of trials both females preferred the pheromones of the same male, whereas in 35.7% of trials females preferred different males (80 of 126 trials had agreeing females, chi-square test: $\chi^2_1 = 9.18$, $P = 0.002$). In one trial pair, one focal female did not enter either of the disks, and thus this trial was removed from the subsequent analyses. Among the females preferring the same disk, females spent on average 192 ± 153 s on the preferred disk and 41 ± 43 s on the non-preferred disk (mean time on the preferred disk as a proportion of total time on the disks, $78.5 \pm 16.9\%$). Among the females that preferred different disks, females spent on average 164 ± 148 s on the preferred disk and 40 ± 40 s on the non-preferred disk (mean time on the preferred disk as a proportion of total time on the disks, $75.4 \pm 18.1\%$), indicating that both groups of females made a clear choice between the disks containing a sample of male pheromones. In a random situation, focal females should have spent only 10.2 s on each disk.

Those male pheromone disks that were preferred by both females did not receive on average more preference time (time that a focal female spent on it during a 10-min dyadic trial) than the disks that were preferred by one female only (independent samples *t*-test, $t_{248} = -1.10$, $P = 0.271$). Similarly, those pheromone disks that were not preferred by either female did not receive less preference time than those that were not preferred by one female only (independent samples *t*-test, $t_{248} = -0.06$, $P = 0.956$). This suggests that disks preferred or not preferred by both females were not generally more attractive or repulsive than the disks preferred or not preferred by one female only. Male mass did not affect the outcome of two simultaneous preference tests: male body mass (averaged between males within a pair, and then averaged across all pairs) was not significantly different between the males that had pheromones preferred either by one or two females (independent samples *t*-test, $t_{168} = 0.24$, $P = 0.812$).

Copulation tests

Altogether, 92.5% (35 of 38) of males whose disks were either preferred or not preferred by both of the females copulated during the 30-min trials. Of those three males that did not copulate, two had preferred pheromones and one non-preferred pheromones. Moreover, males that had a preferred pheromone disk did not copulate faster with the focal female than the males with non-preferred pheromones: 309 ± 349 s and 220 ± 275 s, respectively (paired samples *t*-test, $t_{15} = 0.89$, $P = 0.390$; Fig. 1). However, female preference time and

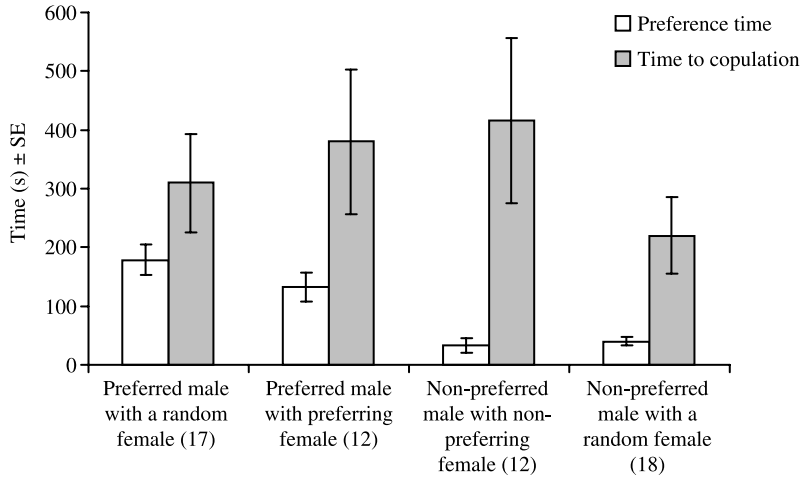

Fig. 1. The time that a focal female spent on a disk containing male pheromones during a 10-min dyadic trial (preference time) compared with the time until copulation between the male and the focal female by female preference groups. Preferred male refers to a male whose pheromone disk was preferred in the female preference test. In the first and the last group, both focal females preferred the same disk: in the first group, the male was paired with the preferring female and in the last group with the non-preferring female. In the two middle groups, females preferred the disks of separate males. Thus, the focal females in these groups were paired either with the male having the preferred disk or the male having the non-preferred disk. Since we used a paired design, only the first and last groups, and the two middle groups, are directly comparable with each other. None of these differences between males was statistically significant (for details, see the Results section). The number of pairs is shown in parentheses.

time until copulation were strongly negatively correlated among males who were paired with one of the two preferring females (Pearson's $r = -0.69$, $n = 17$, $P = 0.002$) but not among males who were paired with a female that did not prefer a male's pheromones (Pearson's $r = -0.08$, $n = 18$, $P = 0.756$).

All 24 males whose pheromones were preferred by one of the two females copulated with the female in the 30-min trial despite the particular preference of the focal female. The time until copulation did not differ between males whose pheromones were either preferred or not preferred by the particular female (ANCOVA: $F_{1, 20} = 0.02$, $P = 0.889$; Fig. 1). Mass of neither male nor female affected the time until copulation (ANCOVA: mass of the male $F_{1, 20} = 1.40$, $P = 0.250$; mass of the female, $F_{1, 20} = 0.23$, $P = 0.640$).

Offspring production

In the data combining all preference groups and pairs with no offspring, the total number of offspring (both live and dead larvae) produced was positively correlated with female mass (Pearson's $r = 0.27$, $n = 154$, $P = 0.001$) but not with male mass (Pearson's $r = -0.02$, $n = 154$, $P = 0.814$). The total number of offspring was not correlated with the lifespan of a female (Pearson's $r = -0.05$, $n = 150$, $P = 0.561$) or with the lifespan of a male (Pearson's $r = -0.03$, $n = 149$, $P = 0.640$).

Among the males whose pheromones were preferred by both of the mass-matched females, 57.1% (28/49) paired with the preferring female produced offspring, whereas 69.4% (34/49) paired with the non-preferring female produced offspring. This difference in ratios was not statistically significant ($\chi^2_1 = 1.58$, $P = 0.209$). Among the males whose pheromones were preferred by one of the mass-matched females only, 50.0% (11/22) paired with the preferring female produced offspring, whereas 41.2% (14/34) paired with the non-preferring female produced offspring. This difference in ratios was not statistically significant ($\chi^2_1 = 0.16$, $P = 0.690$). For subsequent analyses, only the pairs with either dead or live larvae were included.

The females that both preferred the disk from the same male and were paired with the male who had the preferred pheromones did not produce more live or dead offspring than the females who were paired with the males who had the non-preferred pheromones (Table 1, Fig. 2). Neither was there a difference in the number of live offspring between the females in their choice for pheromones after 4 months (RM-ANCOVA: preference,

Table 1. Results of RM-ANCOVA on the offspring production (live vs. dead, within-subject) by preference (preferred vs. non-preferred, within-subject), by female and male mass, and by male and female lifespan within the pairs, where both females preferred the same half disk

Source	*F*	d.f.	*P*
Preference	2.68	1	0.121
Preference * lifespan of preferred male	3.10	1	0.098
Preference * lifespan of non-preferred male	0.20	1	0.658
Preference * lifespan of the female with preferred male	0.18	1	0.674
Preference * lifespan of the female with non-preferred male	1.88	1	0.189
Preference * male mass	0.01	1	0.939
Preference * female mass	5.52	1	0.032
Error (Preference)		16	
Offspring	0.15	1	0.706
Offspring * lifespan of preferred male	4.20	1	0.057
Offspring * lifespan of non-preferred male	6.75	1	0.019
Offspring * lifespan of the female with preferred male	0.38	1	0.546
Offspring * lifespan of the female with non-preferred male	0.27	1	0.609
Offspring * male mass	0.15	1	0.706
Offspring * female mass	0.71	1	0.411
Error (Offspring)		16	
Preference * offspring	4.08	1	0.060
Preference * offspring * lifespan of preferred male	4.15	1	0.058
Preference * offspring * lifespan of non-preferred male	11.62	1	0.004
Preference * offspring * lifespan of the female with preferred male	1.19	1	0.291
Preference * offspring * lifespan of the female with non-preferred male	0.71	1	0.413
Preference * offspring * male mass	1.46	1	0.245
Preference * offspring * female mass	7.49	1	0.015
Error (preference * offspring)		16	
Lifespan of the preferred male	4.77	1,16	0.044
All other between-subject effects		1,16	>0.123

Note: All between-subject effects of covariates except the lifespan of preferred male were insignificant ($P > 0.05$). Only pairs where both males produced offspring are included.

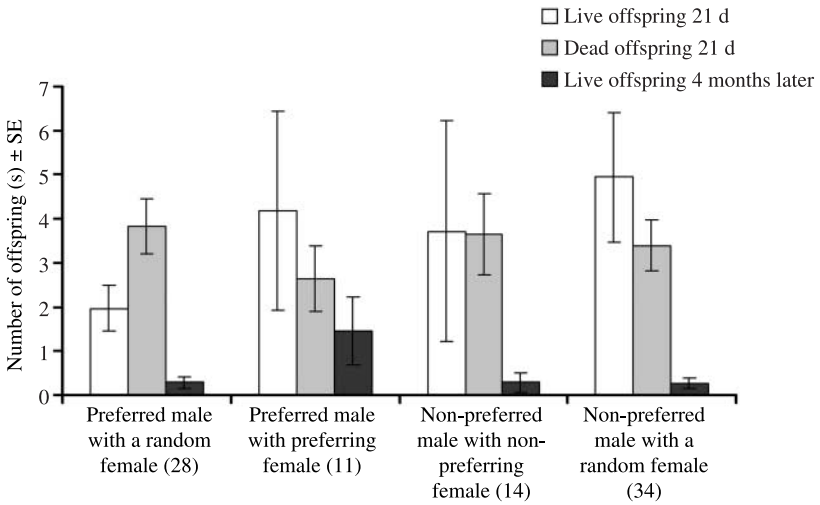

Fig. 2. Reproductive success of pairs according to the female preference for male pheromones. For description of groups, see legend to Fig. 1. Due to the paired design, only the first and the last groups, and the two middle groups, are directly comparable with each other. None of these differences between males were statistically significant (see the Results section). The number of pairs is shown in parentheses.

$F_{1,16} = 3.00$, $P = 0.103$, all covariates $P \geq 0.079$) (Fig. 2). The significant interaction between offspring and lifespan of the non-preferred male (Table 1) suggests that some trait correlated with male lifespan affects his offspring production. Indeed, there was a negative correlation between the number of dead offspring and lifespan of the males whose pheromones were not preferred (Pearson's $r = -0.61$, $n = 24$, $P = 0.002$; preferred males: Pearson's $r = -0.11$, $n = 23$, $P = 0.626$). However, lifespan of males did not correlate with the number of live offspring produced (males with preferred disks: Pearson's $r = -0.22$, $n = 23$, $P = 0.307$; males with non-preferred disks: Pearson's $r = 0.35$, $n = 24$, $P = 0.097$).

The significant interaction between preference (preferred vs. non-preferred), offspring (dead vs. live), and lifespan of males with a non-preferred disk (Table 1) suggests that some trait correlated with the lifespan of a male with a non-preferred disk had an effect on female choice and the resulting reproductive success. However, lifespan of males with preferred and non-preferred disks did not differ when tested separately using a paired-samples *t*-test ($t_{22} = 0.93$, $P = 0.365$). This conclusion did not change when all the mass-matched males with no offspring were included ($t_{51} = 1.43$, $P = 0.158$).

The significant interaction between female mass and her preference (Table 1) suggests that females choose differentially as a function of their body mass. Moreover, a significant interaction between female preference, female mass, and the number of offspring suggests that a female's life-history decisions change together with body size (Table 1). Female mass correlated negatively with the number of live offspring with males who had the preferred pheromones (Pearson's $r = -0.42$, $n = 24$, $P = 0.039$) but not with the number of dead offspring with males who had the preferred pheromones (Pearson's $r = 0.29$, $n = 24$, $P = 0.177$). In contrast, female mass correlated positively with the number of live offspring with males who had the non-preferred pheromones (Pearson's $r = 0.44$, $n = 24$, $P = 0.030$)

but again not with the number of dead offspring (Pearson's $r = 0.05$, $n = 24$, $P = 0.829$). However, the overall difference in preference times between males who had the preferred or non-preferred pheromones did not correlate with the difference in the total number of offspring between these males (Pearson's $r = -0.21$, $n = 24$, $P = 0.330$).

Among the females who preferred the disks of different males, females paired with the preferred male did not produce more live or dead offspring than the females paired with the male who had the non-preferred pheromones (Table 2, Fig. 2). Only female mass explained the variation in the number of offspring produced (Table 2). There was no difference in the number of live offspring 4 months after copulations (for preference: ANCOVA, $F_{1, 18} = 2.53$, $P = 0.129$; all covariates: $P \geq 120$) (Fig. 2).

Longevity

Longevity of males whose pheromones were preferred by one, two, or none of the two females did not differ (Kaplan-Meier survival analysis, log rank = 2.59, $n = 223$, $P = 0.274$) (Fig. 3). Males whose pheromones were preferred by both of the females ($n = 71$) lived 32.1 ± 1.4 days (mean ± standard error) and the males whose pheromones were not preferred ($n = 74$) lived 29.1 ± 1.5 days. Males whose pheromones were preferred by one of the two mass-matched females ($n = 78$) lived 31.5 ± 1.5 days.

The females that both preferred the disk from the same male and mated with the male who had the preferred pheromones lived longer (54.2 ± 2.7 days; mean ± standard error) than the females who mated with the males who had the non-preferred pheromones (46.5 ± 2.5 days) (Kaplan-Meier survival analysis, log rank = 6.10, $n = 97$, $P = 0.014$).

Table 2. Results of RM-ANCOVA on the offspring production of pairs, where the focal females preferred separate pheromone disks, and females produced larvae

Source	*F*	d.f.	*P*
W-S: Offspring	0.1	1	0.759
W-S: Offspring * male mass	1.6	1	0.222
W-S: Offspring * female mass	2.06	1	0.169
W-S: Offspring * male lifespan	0.15	1	0.704
W-S: Offspring * female lifespan	0.01	1	0.911
W-S: Offspring * preference	0.78	1	0.387
W-S: Error (offspring)		18	
B-S: Male mass	0.58	1	0.457
B-S: Female mass	6.08	1	0.024
B-S: Male lifespan	3.04	1	0.098
B-S: Female lifespan	1.17	1	0.293
B-S: Preference	0.13	1	0.727
B-S: Error		18	

Note: The between-subject (B-S) term 'preference' tests whether there is a difference in the number of offspring between females that were paired with males who had either the preferred or non-preferred pheromones. The within-subject (W-S) term 'offspring' includes live and dead larvae. Both mass and lifespan of males and females are included as covariates.

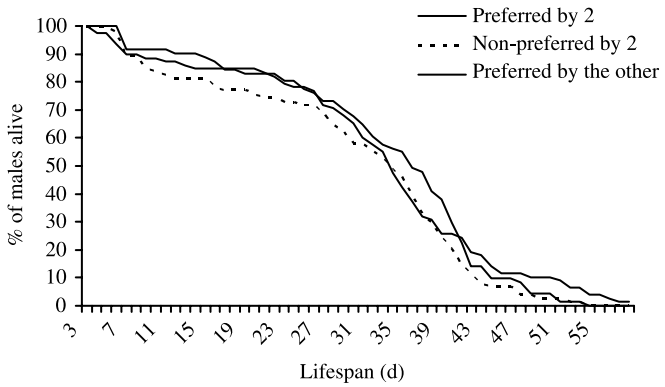

Fig. 3. Lifespan of the males whose pheromones were preferred by one, two, or none of the focal females. Only males that lived more than 3 days (were mated with females) after the preference tests are included.

However, females that had a preference for different pheromone disks did not differ in their lifespan whether they mated with the male who had the preferred or non-preferred pheromones (Kaplan-Meier survival analysis, log rank = 0.01, $n = 53$, $P = 0.935$).

DISCUSSION

Females were able to differentiate males based on their pheromones, because two-thirds of females preferred the pheromones from the same male, and the preferred disks received on average approximately 75% of total visiting time (see also Rantala *et al.*, 2002, 2003a, 2003b). However, females that mated with males who had the preferred pheromones did not produce more offspring or more viable offspring than females that mated with males who had the non-preferred pheromones. Thus, our results do not lend support to good genes models of sexual selection. Our results are in line with those of Boake (1985), who did not find a relationship between progeny fitness and attractiveness of male pheromones in *Tribolium castaneum*. Together these two studies suggest that male pheromones do not signal qualities that could increase female reproductive success in one generation.

In a previous study, the males whose pheromones were preferred by females were found to possess better immunocompetence than the males with less attractive pheromones (Rantala *et al.*, 2002). If immunocompetence is heritable as shown by Cotter and Wilson (2002), females should gain fitness benefits by favouring immunocompetent males (Hamilton and Zuk, 1982). In sticklebacks (*Gasterosteus aculeatus*), the offspring of bright males grew more slowly but resisted parasitic infection better than the offspring of dull males (Barber *et al.*, 2001). In white-fish (*Coregonus* sp.), eggs sired by highly ornamented males survived better from a bacterial infection than the offspring sired by less ornamented males (Wedekind *et al.*, 2001). In a study of *Tenebrio molitor* by Rantala *et al.* (2003), immune function and attractiveness of male pheromones were found to be condition-dependent. Thus, phenotypic variation in condition and in condition-dependent signalling may hide the genetic qualities of a male that would increase the fitness of offspring. This is especially possible in the laboratory, where the pathogenic environment may not represent natural threats. Thus, we cannot exclude the possibility that the offspring of males who had the preferred pheromones would

have had better immune system genes, or better genes for attractiveness, than the offspring of males who had the non-preferred pheromones (see also Siva-Jothy and Skarstein, 1998).

Kortet *et al.* (2004) found that the early viability of offspring from male fish (*Rutilus rutilus*) was negatively correlated with his ability to kill parasites. This kind of trade-off between early development and immune function at maturity would explain our neutral results in mealworm beetle as well, if good parasite resistance in adults is traded off against larval viability. Indeed, our results suggest that if any fitness effects exist due to mate choice in *T. molitor* they are negative, as indicated by the non-significant difference in the number of live offspring between preferred and non-preferred males (preferred: average 2.13 live offspring; non-preferred: average 5.17 live offspring). However, we studied only the effect of pheromonal cues in mate selection. Other cues might determine the final mating decisions as well, but pheromones of male mealworm beetles have been shown to play a major role in these sexually monomorphic animals (Happ, 1969; August, 1971; Tanaka *et al.*, 1986; Carazo *et al.*, 2004).

Males whose pheromones were preferred by females had similar copulatory success to those whose pheromones were not preferred; however, among the males with preferred pheromones, the time until copulation was negatively correlated with the time females spent on the disk. This result, however, was not observed among the males who had the non-preferred pheromones or between males with the preferred and non-preferred pheromones. This suggests that not only do attractive pheromones increase male copulatory success in the field, but also less attractive males are accepted as mates when the number of available males is limited. However, our correlations were affected by the time the female spent on the disk of the other male within the pair. Thus, this issue should be clarified using sequential mate choice tests.

Although we did not find a difference in offspring production according to females' preference for male pheromones, pheromones of males might have an effect on females' total fitness, because females that mated with the males that had the preferred pheromones lived longer than the females that mated with the males that had the non-preferred pheromones. It is unclear whether this result was due to females' life-history decisions or due to male manipulation. However, a similar observation was reported by Moore *et al.* (2003) in cockroaches (*Nauphoeta cinerea*), where it was thought to result from females' avoidance of male manipulation.

It has been shown that both the level of promiscuity and number of males available affect the extent of sexual conflict (Crudgington *et al.*, 2005). Because *T. molitor* females usually mate multiply (Worden and Parker 2001), it is possible that a conflict arises between manipulative males and choosy females, when forced into monogamy as in our study. Indeed, it is interesting that female mealworm beetles produce more offspring when they are allowed to mate with multiple males (Worden and Parker, 2001). Thus, genetic incompatibility between assigned pairs might have hidden at least some of the possible positive effects of free mate choice (see the model of Colegrave *et al.*, 2002). However, we did not observe differences in reproductive success between males whose pheromones were preferred only by one of the two females, and paired either with the preferring female or with the other female, suggesting a more important role for sexual conflict than for genetic mismatch.

We detected a negative correlation between the numbers of live offspring and female mass among females who preferred the same disk and mated with males who had the preferred pheromones. The same correlation was positive among females who mated with males who had the non-preferred pheromones. This could indicate that the larger the female the better it might be to resist male manipulation by pheromones, or that females use less resources on

'good-smelling' and manipulative males than on 'not-so-good-smelling' males (see also Droney, 2003). This careful use of resources on good smelling but manipulative males could explain why females did not produce more offspring but lived longer with the males who had preferred pheromones. To address this it would be essential to measure the amount and qualities of pheromones produced by males.

Female mass correlated positively with her offspring production in the pooled data, but interactions between female body size, her offspring, and mate preference could indicate that large females choose their mates more carefully, although this should be studied further together with possible male preferences for large females. Also, male mealworm beetles use pheromones to detect the reproductive status of potential mates (Carazo *et al.*, 2004). However, all the females we used were of similar age and the copulation tests did not indicate differences between males who had the preferred or non-preferred pheromones. Males' preferences for females might explain why not all males copulated during the copulation tests.

Neither male nor female lifespan was correlated with the reproductive success of the pairs. This is a rather surprising result, since several studies have demonstrated that reproduction is costly and reduces the lifespan of the mother or both parents (e.g. Hunt *et al.*, 2002; Sarma *et al.*, 2002; Koivula *et al.*, 2003). The lack of a trade-off between reproduction and survival could mean that the beetles did not invest all possible effort in reproduction, or that the beetles with no larvae used substantial resources without reproductive success. The suggestion that some trait correlated with the lifespan of males (condition?) could relate to the number of his offspring. In any case, these results should be interpreted within the experimental constraints of this study.

In this system, it is not costly for females to be choosy over mates or even to copulate with several males. Thus, for *T. molitor* females, it would appear that it is optimal to mate with several good-smelling males (Worden and Parker, 2001). This strategy might lead to a better total fitness through a longer lifespan and possible sexy son effects in comparison to mating with non-preferred males. The question of whether offspring qualities differ between preferred and non-preferred males remains to be examined further in multi-generational studies.

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REFERENCES

Ahtiainen, J.J., Alatalo, R.V., Kortet, R. and Rantala, M.J. 2004. Sexual advertisement and immune function in an arachnid species (Lycosideae). *Behav. Ecol.*, **15**: 602–606.

Alatalo, R.V., Kotiaho, J., Mappes, J. and Parri, S. 1998. Mate choice for offspring performance: major benefits or minor costs? *Proc. R. Soc. Lond. B*, **265**: 2297–2301.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution*, **40**: 804–816.

Andersson, M. 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.

August, C.J. 1971. The role of male and female pheromones in the mating behaviour of *Tenebrio molitor*. *J. Insect Physiol.*, **17**: 739–751.

Barber, I., Arnott, S.A., Braithwaite, V.A., Andrew, J. and Huntingword, F.A. 2001. Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc. R. Soc. Lond. B*, **268**: 71–76.

Bhattacharya, A.K., Ameel, J.J. and Waldbaer, G.P. 1970. A method for sexing living pupal and adult yellow mealworms. *Ann. Entomol. Soc. Am.*, **63**: 1783.

Boake, C.R.B. 1985. Genetic consequences of mate choice: a quantitative genetic method for testing sexual selection theory. *Science*, **227**: 1061–1063.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature*, **406**: 67–70.

Carazo, P., Sanchez, C.P. and Desfilis, E. 2004. Chemosensory cues allow male *Tenebrio molitor* beetles to assess the reproductive status of potential mates. *Anim. Behav.*, **68**: 123–129.

Chapman, T. and Partridge, L. 1996. Sexual conflict as fuel for evolution. *Nature*, **381**: 189–190.

Chapman, T., Arnqvist, G., Bangham, J. and Rowe, L. 2003. Sexual conflict. *TREE*, **18**: 41–47.

Colegrave, N., Kotiaho, J.S. and Tomkins, J.L. 2002. Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. *Evol. Ecol. Res.*, **4**: 911–917.

Cotter, S.C. and Wilson, K. 2002. Heritability of immune function in the caterpillar *Spodoptera littoralis. Heredity*, **88**: 229–234.

Crudgington, H.S., Beckerman, A.P., Brüstle, L., Green, K. and Snook, R.R. 2005. Experimental removal and evolution of sexual selection: does sexual selection generate manipulative males and resistant females? *Am. Nat.*, **165**: S72–S87.

David, P., Bjorksten, T., Fowler, K. and Pomiankowski, A. 2000. Condition-dependent signaling of genetic variation in stalk-eyed flies. *Nature*, **406**: 186–188.

Day, E. 1989. *Darkling Beetle Factsheet*. Blacksburg, VA: Virginia Cooperative Extension.

Ditchkoff, S.S., Lochmiller, R.L., Masters, R.E., Hoofer, S.R. and Van Den Bussche R.A. 2001. Major-histocompatibility-complex-associated variation in secondary sexual traits of white-tailed deer (*Odocoileus virginianus*): evidence for good-genes advertisement. *Evolution*, **55**: 616–625.

Droney, D.C. 2003. Females lay fewer eggs for males with greater courtship success in a lekking *Drosophila*. *Anim. Behav.*, **65**: 371–378.

Eshel, I., Sansone, E. and Jacobs, F. 2002. A long-term genetic model for the evolution of sexual preference: the theories of Fisher and Zahavi re-examined. *J. Math. Biol.*, **45**: 1–21.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Oxford University Press.

Folstad, I. and Karter, A.J. 1992. Parasites, bright males and the immunocompetence handicap. *Am. Nat.*, **139**: 603–622.

Hamilton, W.D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, **218**: 384–387.

Happ, G.M. 1969. Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor L. Nature*, **222**: 180–181.

Hunt, J., Simmons, L.W. and Kotiaho, J.S. 2002. A cost of maternal care in the dung beetle *Onthophagus taurus*? *J. Evol. Biol.*, **15**: 57–64.

Hurd, H. and Parry, G. 1991. Metacestode-induced depression of the production, and responses to, sex pheromone in the intermediate host *Tenebrio molitor*. *J. Inverteb. Pathol.*, **58**: 82–87.

Iwasa, Y. and Pomiankowski, A. 1999. Good parent and good genes models of handicap evolution. *J. Theor. Biol.*, **200**: 97–109.

Johnsen, A., Delhey, K., Andersson, S. and Kempenaers, B. 2003. Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. *Proc. R. Soc. Lond. B*, **270**: 1263–1270.

Johnstone, R.A. and Grafen, A. 1993. Dishonesty and the handicap principle. *Anim. Behav.*, **46**: 759–764.

Koivula, M., Koskela, E., Mappes, T. and Oksanen, T.A. 2003. Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology*, **84**: 398–405.

Kokko, H., Brooks, R., Jennions, M.D. and Morley, J. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B*, **270**: 653–664.

Kortet, R., Vainikka, A., Rantala, M.J., Myntti, J. and Taskinen, J. 2004. *In vitro* embryo survival and early viability of larvae in relation to male sexual ornaments and parasite resistance in roach, *Rutilus rutilus* L. *J. Evol. Biol.*, **17**: 1337–1344.

Møller, A.P. and Alatalo, R.V. 1999. Good-genes effects in sexual selection. *Proc. R. Soc. Lond. B*, **266**: 85–91.

Moore, A.J., Gowaty, P.A. and Moore, P.J. 2003. Females avoid manipulative males and live longer. *J. Evol. Biol.*, **16**: 523–530.

Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate traits. *Nature*, **371**: 598–599.

Pomiankowski, A. 1987. Sexual selection: the handicap principle does work – sometimes. *Proc. R. Soc. Lond. B*, **231**: 123–145.

Rantala, M.J. and Kortet, R. 2003. Courtship song and immune function in the field cricket *Gryllys bimaculatus*. *Biol. J. Linn. Soc.*, **79**: 503–510.

Rantala, M.J., Koskimäki, J., Suhonen, J., Taskinen, J. and Tynkkynen, K. 2000. Immunocompetence, developmental stability and wing spot size in *Calopteryx splendens*. *Proc. R. Soc. Lond. B*, **267**: 2453–2457.

Rantala, M.J., Jokinen, I., Kortet, R., Vainikka, A. and Suhonen, J. 2002. Do pheromones reveal male immunocompetence? *Proc. R. Soc. Lond. B*, **269**: 1681–1685.

Rantala, M.J., Kortet, R., Kotiaho, J.S., Vainikka, A. and Suhonen, J. 2003a. Condition dependence of pheromones and immune function in the grain beetle *Tenebrio molitor*. *Funct. Ecol.*, **17**: 534–540.

Rantala, M.J., Vainikka, A. and Kortet, R. 2003b. The role of juvenile hormone in immune function and pheromone production trade-offs: a test of the immunocompetence handicap principle. *Proc. R. Soc. Lond. B*, **270**: 2257–2261.

Sarma, S.S.S., Nandini, S. and Gulati, R.D. 2002. Cost of reproduction in selected species of zooplankton (rotifers and gladocerans). *Hydrobiologia*, **481**: 89–99.

Siva-Jothy, M.T. and Skarstein, F. 1998. Towards a functional understanding of 'good genes'. *Ecol. Lett.*, **1**: 178–185.

Tanaka, Y., Honda, H., Ohsawa, K. and Yamamoto, I. 1986. A sex attractant of the yellow mealworm, *Tenebrio molitor* L., and its role in the mating behaviour. *J. Pest. Sci.*, **11**: 49–55.

Watt, P.J., Shohet, A.J. and Renshaw, K. 2001. Female choice for good genes and sex-biased broods in guppies. *J. Fish Biol.*, **59**: 843–850.

Wedekind, C. 1994. Handicaps not obligatory in sexual selection for resistance genes. *J. Theor. Biol.*, **170**: 57–62.

Wedekind, C., Müller, R. and Spicher, H. 2001. Potential genetic benefits of mate selection in whitewish. *J. Evol. Biol.*, **14**: 980–986.

Worden, B.D. and Parker, P.G. 2001. Polyandry in grain beetles, *Tenebrio molitor*, leads to greater reproductive success: material or genetic benefits? *Behav. Ecol.*, **12**: 761–767.

Worden, B.D., Parker, P.G. and Pappas, P.W. 2000. Parasites reduce attractiveness and reproductive success in male grain beetles. *Anim. Behav.*, **59**: 543–550.

Zahavi, A. 1975. Mate selection – a selection for a handicap. *J. Theor. Biol.*, **53**: 205–214.