

ORIGINAL ARTICLE

No genetic correlation between the sexes in mating frequency in the bean beetle, *Callosobruchus chinensis*

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Female multiple mating, which is common in animals, may have evolved not in response to fitness advantages to females but as a genetic corollary to selection on males to mate frequently. This nonadaptive hypothesis assumes a genetic correlation between females and males in mating frequency, which has received a few empirical investigations. We tested this hypothesis by observing the correlated response in male mating frequency in the adzuki bean beetle, *Callosobruchus chinensis* to artificial selection on female propensity to remate. Compared to control females, females from lines selected for increased or decreased female propensity to remate had, respectively, higher or lower mating frequency measured by the number of mating within a given period. This indicates that female receptivity to

remating is genetically correlated with female mating frequency, and thus the artificial selection for female propensity to remate influenced female mating frequency. In contrast, males from the selected lines that diverged in female mating frequency did not vary significantly in their mating frequency. These results indicate that there is no genetic correlation between the sexes in mating frequency in *C. chinensis*. This study shows that the reason why females in *C. chinensis* remate despite suffering fitness costs cannot be explained by indirect selection resulting from selection on males to mate multiple times.

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Introduction

By definition, males invest less and females invest more in each gamete; so they typically differ in the relationship between their mating frequency and fitness. In general, male reproductive success is limited by the number of mates, whereas female reproductive success is limited by resources invested in reproduction (Bateman, 1948; Trivers, 1972). Thus, the fitness advantages of multiple mating are clear for males, but they are less clear for females. Superfluous mating may, moreover, reduce female fitness, because mating involves various costs to females (Thornhill and Alcock, 1983; Arnqvist and Nilsson, 2000). However, female multiple mating, or polyandry, is common in most animal species (Thornhill and Alcock, 1983; Ridley, 1988; Birkhead and Møller, 1998; Birkhead, 2000). This fact has produced a variety of adaptive explanations for female multiple mating (Thornhill and Alcock, 1983). These hypothesize that multiple mating may increase female fitness: directly through offering sufficient sperm to fertilize eggs or materials that contribute to female survival and/or reproduction (Arnqvist and Nilsson, 2000), or indirectly

through offering genes that improve offspring fitness or increase genetic diversity in offspring (Yasui, 1998; Zeh and Zeh, 2003).

On the other hand, nonadaptive explanations for female multiple mating do not assume that it has some fitness advantages for females. Instead, one of the explanations hypothesizes that female multiple mating has evolved as a genetic corollary to selection on males to mate multiple times (Halliday and Arnold, 1987; Arnold and Halliday, 1988, 1992). Mating frequency in general should be subjected to sex-specific selection, because males have a higher optimal mating frequency than females (Bateman, 1948; Trivers, 1972; Arnqvist and Nilsson, 2000; Arnqvist and Rowe, 2005). If a trait is determined by genes at the same locus or loci in males and females and thus shows a genetic correlation between the sexes, then sex-specific selection for the trait results in intralocus sexual conflict, which will constrain the adaptive evolution in both sexes (Parker and Partridge, 1998; Rice and Chippindale, 2001; Chapman *et al.*, 2003; Arnqvist and Rowe, 2005). Homologous traits of males and females typically exhibit high genetic correlation (Lande, 1980; Lynch and Walsh, 1998). When there is a genetic correlation between the sexes in mating frequency, selection for mating frequency of one sex would cause indirect selection for mating frequency of the other sex. Thus, females may evolve to mate more frequently than their optima under stronger selection for mating frequency in males than in females (Halliday and Arnold, 1987; Arnold and Halliday, 1988, 1992). This

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nonadaptive hypothesis is controversial (Sherman and Westneat, 1988; Cheng and Siegel, 1990; Gromko, 1992; Schwartz and Boake, 1992) and has received only a little empirical attention (Grant *et al.*, 2005), in contrast to the adaptive hypotheses that have been investigated extensively (see, for example, Arnqvist and Nilsson, 2000).

A genetic correlation between the sexes in mating frequency has been examined by observing a correlated response in one sex to artificial selection on the other sex. There was no correlated response in female mating frequency to selection for male mating frequency in the domestic chicken, *Gallus domesticus* (Dunnington and Siegel, 1983; Cheng and Siegel, 1990). In *Drosophila melanogaster*, some studies have addressed a genetic correlation between the sexes in traits that is considered to be associated with mating frequency. In this fly, a genetic correlation between the sexes was found in the time to first mating (Manning, 1963; Stamenkovic-Radak *et al.*, 1992), although this trait is not necessarily related to mating frequency (Sgrò *et al.*, 1998). The time interval between the first mating and remating was proved to be genetically associated with mating frequency in *D. melanogaster* (Sgrò *et al.*, 1998). For this trait, Gromko and Newport (1988) reported that artificial selection on females produced a correlated response in males in one population, but it produced no correlated response in another, and Sgrò *et al.* (1998) reported that artificial selection on females produced no correlated response in males. Thus, the results of artificial selection experiments are mixed and provide no clear evidence for a genetic correlation between the sexes in mating frequency in *D. melanogaster*. In the stalk-eyed fly, *Cyrtodiopsis dalmanni*, artificial selection for male mating frequency produced no correlated response in female mating frequency (Grant *et al.*, 2005). So far, only these data are available to determine a genetic correlation between the sexes in mating frequency. Moreover, some of these studies had no replication of selection lines and no control line comparable with selection lines. Arnold and Halliday (1992), Sgrò *et al.* (1998) and Grant *et al.* (2005) pointed out the problem that such studies do not consider the effects of random genetic drift and inbreeding. Given this problem, artificial selection designed rigorously is restricted to only the two Dipteran studies: *D. melanogaster* (Sgrò *et al.*, 1998) and *C. dalmanni* (Grant *et al.*, 2005). Therefore, empirical evidences in other species are required to draw a general conclusion about whether there is a genetic correlation between the sexes in mating frequency.

The nonadaptive hypothesis for female multiple mating holds on the assumption that the direction of selection for mating frequency differs between the sexes. Remating reduces female fecundity in the adzuki bean beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae) (Harano *et al.*, 2006a). This implies that in this beetle, direct selection acts on males to mate as many times as possible, but it acts on females to mate once. If a trait has no genetic variation, then a genetic correlation between this trait and any other trait cannot be detected. Genetic variation is found in female propensity to remate in *C. chinensis* (Miyatake and Matsumura, 2004; Harano and Miyatake, 2005, 2007). Thus, artificial selection on the trait is reasonable to test whether this trait is genetically correlated with the other trait. In *C. chinensis*, artificial selection for increased and decreased female propensity

to remate produced direct responses and established lines of females that have genetically high or low receptivity to remating (Harano and Miyatake, submitted). In the present study, we used these lines to test whether a genetic correlation between the sexes in mating frequency may account for the evolution of female multiple mating in *C. chinensis*. We first confirmed that female receptivity to remating is correlated with female mating frequency. Second, we investigated a correlated response in male mating frequency to artificial selection on female propensity to remate.

Materials and methods

Culture and selection lines

Two replicate lines selected for increased female propensity to remate (high selection regime), two replicate lines selected for decreased female propensity to remate (low selection regime) and two unselected replicate lines (control regime) were established from the base population that was constructed through crossing four strains of *C. chinensis* that have different origins. At each generation in each replicate line of high and low selection regimes, usually, 50 virgin females were paired individually with virgin males chosen randomly from their own line and kept until either they had mated once or 1 h had passed. Among the females, the mated females were allowed to lay eggs on adzuki beans, *Vigna angularis*, and then they individually received opportunities to remate with virgin tester males that were collected from the stock culture of a single strain (isC: originating from Ishigaki City, Japan, in 1997) for 1 h on days 1, 3 and 5 after the first mating. The 10 females that remated most readily were selected in each replicate line of the high selection regime, and 10 females out of those that did not remate at all were selected in each replicate line of the low selection regime. The eggs that the selected females had laid before their opportunity to remate were used to establish the next generation. The selection was repeated until generation 17, except for generations 5, 15 and 16. Each of the two control lines was maintained by propagating 10 females that were randomly chosen and mated individually with virgin males chosen randomly from their own line at every generation.

After more than 10 generations from the commencement of artificial selection, the female propensity to remate responded to selection in both directions and diverged between high and low selection regimes. During this artificial selection, the selected females and the males that were mated first with the selected females contributed to the next generation in each line, and thus the experimental design selected jointly for female receptivity to remating and male ability to inhibit female remating, both of which are associated with the female propensity to remate. To test whether the female receptivity to remating responded to selection, the selection line females were mated first with and received opportunities to remate with tester males. In this case, the propensity of females to remate differed significantly between the selection regimes, indicating that the female receptivity to remating responded to selection. To test whether the male ability to inhibit female remating responded to selection, tester females were mated first with the selection line males. The propensity of the tester

females to remate with tester males did not differ significantly between the first males from different selection regimes, indicating that the male ability to inhibit female remating did not respond to selection. The observed response to selection for female propensity to remate was therefore attributable only to female receptivity to remating.

All selection and control lines were maintained without selection after generation 18 of selection. Then, to equalize the number of mates for females in all replicate lines, approximately 20 virgin females were paired individually with virgin males chosen randomly within each line at every generation, and these pairs were used to establish the next generation. All rearing and experiments were performed in a chamber maintained at 25°C and 50% relative humidity under a photoperiod cycle of 14:10 h light/dark.

Female mating frequency

We assayed mating frequency for females of all selection and control lines by using the tester males as mates for all the females. The tester males were randomly collected as virgins from a stock culture of single strain (isC), and they were kept in groups of up to 10 beetles in plastic cups (2.8 cm high, 7 cm diameter) and given water and adult food (1:2 yeast extract/sugar) *ad libitum* until they were 2–5 days old. At generation 27 after artificial selection had commenced, we collected virgin females emerging from each replicate line. These females were kept in plastic cups in groups of up to 10 beetles and given water and adult food *ad libitum*. When she was 2–5 days old, we placed a virgin female in a glass vial (4.4 cm high, 1.7 cm diameter) with a virgin tester male and recorded the number of matings for 30 min. If mating was observed, a tester male was replaced with another virgin male immediately after copulation was completed to continuously present a virgin tester male to the female, because it is unknown whether males change their mating behaviour after mating. After the observation, females were kept individually in a plastic cup containing 10 adzuki beans as the oviposition substrate, and given water and adult food *ad libitum*. The provision of

oviposition substrate for females, which increases female receptivity to remating (Harano *et al.*, 2006b), followed the condition that females were given oviposition substrate before their opportunity to remate during artificial selection. This procedure was repeated after 1 and 2 days so that females had opportunities to mate on 3 consecutive days.

Male mating frequency

At generation 28 after artificial selection commenced, we collected virgin males emerging from each replicate line of all selection and control regimes. For these males, we recorded daily the number of matings with virgin tester females derived from a single strain (isC) in 30 min periods for 3 consecutive days in the same way female mating frequency was tested, as described above, with the exception that the males were kept without beans between the observation periods. If mating was observed, a tester female was replaced with another virgin female immediately after copulation was completed to present continuously a virgin tester female to a focal male, because female receptivity decreases after mating (Miyatake and Matsumura, 2004; Harano and Miyatake, 2005, 2007).

Statistical analysis

To compare the number of matings between selection regimes, we applied a generalized linear model with selection regime and replicate lines nested within the selection regime as explanatory variables, using Poisson distribution, a log-link function and overdispersion test, because the data are counts. If there was a significant effect of the selection regime, then pairwise comparisons were performed between selection regimes correcting the significance level for multiple comparison by the sequential Bonferroni method (Rice, 1989). All statistical analyses were carried out using JMP version 6 (SAS Institute Inc., 2005).

Results

For females from each replicate line of high selection, control and low selection regimes, the number of matings

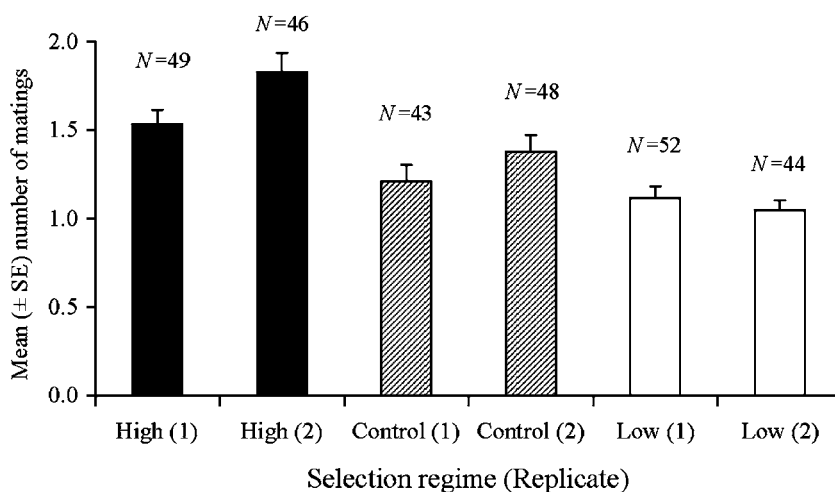


Figure 1 The number of matings observed in 30-min periods on 3 consecutive days, for females from the lines artificially selected for female propensity to remate. All females were continuously presented with virgin tester males as mates during the periods.

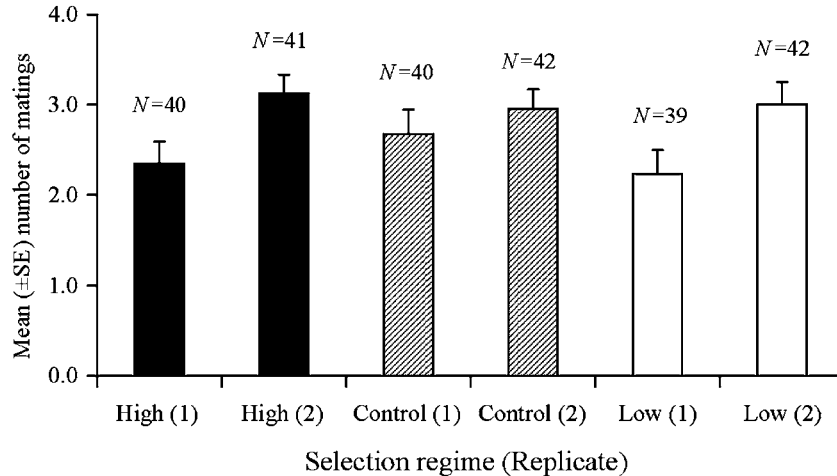


Figure 2 The number of matings observed in 30-min periods on 3 consecutive days, for males from the lines artificially selected for female propensity to remate. All males were continuously presented with virgin tester females as mates during the periods.

with virgin tester males that was observed over the 3-day period is shown in Figure 1. Selection regime had a significant effect on the number of matings of females ($\chi^2 = 50.98$, d.f. = 2, $P < 0.001$), and there was marginal but no significant difference between replicate lines nested within the selection regime ($\chi^2 = 7.43$, d.f. = 3, $P = 0.059$). After we corrected for multiple comparison, the number of matings of females was significantly larger in the high selection regime than in the control (selection regime: $\chi^2 = 16.00$, d.f. = 1, $P < 0.001$; replicate (selection regime): $\chi^2 = 6.01$, d.f. = 2, $P = 0.050$) and low selection (selection regime: $\chi^2 = 57.19$, d.f. = 1, $P < 0.001$; replicate (selection regime): $\chi^2 = 6.31$, d.f. = 2, $P = 0.043$) regimes, and it was significantly smaller in the low selection regime than in the control (selection regime: $\chi^2 = 7.20$, d.f. = 1, $P < 0.01$; replicate (selection regime): $\chi^2 = 2.46$, d.f. = 2, $P = 0.291$).

For males from each replicate line, the number of matings with virgin tester females that was observed over the 3-day period is shown in Figure 2. In our observation, males commonly mated more than once in all replicate lines. Selection regime had no significant effect on the number of matings of males ($\chi^2 = 0.83$, d.f. = 2, $P = 0.660$), but there was a significant difference between replicate lines nested within the selection regime ($\chi^2 = 10.63$, d.f. = 3, $P = 0.014$).

Discussion

Females from the *C. chinensis* lines selected artificially on female propensity to remate varied in their mating frequency within a given period; females with genetically higher and lower receptivity to remating mated significantly more and less frequently, respectively, than control females did (Figure 1). This indicates that female receptivity to remating is genetically correlated with female mating frequency and thus the artificial selection for female propensity to remate influenced female mating frequency. On the other hand, males from lines of different selection regimes that diverged in female mating frequency did not vary significantly in their mating frequency (Figure 2). Thus, for mating frequency, artificial selection on females produced no correlated

response in males. There were also some evidence for variation in female mating frequency (Figure 1) and significant variation in male mating frequency (Figure 2) between replicate lines within the selection regime, suggesting the effects of unidentified random factors on the mating frequencies of females and males. In this study, the base population of artificial selection was founded by progeny from crosses between four different strains, and thus a genetic correlation can be attributable to the pleiotropic action of genes as well as to linkage disequilibrium if particular sets of linked genes had been built up within an ancestral strain. In any case, our results indicate that there is no genetic correlation between the sexes in mating frequency in *C. chinensis*, which is consistent with findings in *D. melanogaster* (Sgrò *et al.*, 1998) and in the stalk-eyed fly, *C. dalmanni* (Grant *et al.*, 2005).

The strains of *C. chinensis* that include those used to construct the base population of artificial selection differ in male traits associated with mating: interruption behaviour on the mating pair (Miyatake and Matsumura, 2004), ability to inhibit female remating, ability to persuade nonvirgin females to remate (Harano and Miyatake, 2007), and modulation of the number of sperm ejaculated in response to larval density, which may correlate with risk of sperm competition (Yamane and Miyatake, 2005). Thus, detecting no genetic correlation between the sexes in mating frequency was unlikely to be due to the lack of genetic variation in the base population. A correlated response in mating frequency of one sex to artificial selection for that of the other sex might be obstructed by inadvertent selection resulting from disassortative mating, such that females with high mating frequency mate preferentially with males with low mating frequency (Butlin, 1993; Grant *et al.*, 2005). This problem should not apply in this study because random pairing was enforced on virgin females and virgin males within each line during artificial selection.

Sexually antagonistic selection for mating frequency would favour modifiers that make sex-limited expression of genes controlling mating frequency if the modifiers have arisen (Sherman and Westneat, 1988). In *C. chinensis*, the sex-limited expression of genes may

eliminate the genetic correlation between the sexes in mating frequency. On the other hand, the mating frequency of females and males may be determined by different physiological mechanisms that are controlled by originally separate genes, and thereby the trait may have no genetic correlation between the sexes in *C. chinensis*. The physiological factors involved in mating frequency differ between females and males in *D. melanogaster* (Gromko, 1992; Sgrò *et al.*, 1998), although they are little known in *C. chinensis*.

In the present study, we tested a correlated response in males to artificial selection on females, which is in the reverse order to the scenario described by the nonadaptive hypothesis for female multiple mating (Halliday and Arnold, 1987; Arnold and Halliday, 1988, 1992). Our method is reasonable, because a genetic correlation between two traits estimated from a correlated response in one trait when the other trait is selected is expected to be equal to that estimated when the reverse is done (Falconer and Mackay, 1996). However, reciprocal test should provide more strong evidence for a genetic correlation. We performed artificial selection for female propensity to remate, based on *a priori* prediction that this trait is genetically associated with female mating frequency. The present study verified this prediction, although the effect of selection for the propensity to remate on mating frequency may possibly differ from that of selection for mating frequency itself. In future, observing no correlated response in female mating frequency to artificial selection on male mating frequency would reinforce our finding.

We conclude that a genetic correlation between the sexes would not constrain the evolution of mating frequency of each sex in *C. chinensis*. This contradicts the nonadaptive hypothesis that the female multiple mating has evolved as a genetic corollary to selection on males to mate as many times as possible (Halliday and Arnold, 1987; Arnold and Halliday, 1988, 1992). Therefore, although males generally mate multiple times (Figure 2), the nonadaptive hypothesis cannot explain the question why females in *C. chinensis* mate multiple times despite suffering fitness costs (Harano *et al.*, 2006a). Testing other potential explanations such as genetic benefits of polyandry (Yasui, 1998) and convenience polyandry (Thornhill and Alcock, 1983) is required to account for female multiple mating in this beetle.

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