

# No detectable genetic correlation between male and female mating frequency in the stalk-eyed fly *Cyrtodiopsis dalmanni*

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There is much interest in explaining why female insects mate multiply. Females of the stalk-eyed fly *Cyrtodiopsis dalmanni* can mate several times each day in a lifetime which may span several months. There are many adaptive explanations, but one hypothesis that has received little rigorous empirical attention is that female multiple mating has evolved for non-adaptive reasons as a correlated response to selection for high male mating frequency rather than because of direct or indirect benefits accruing to females. We tested this hypothesis in stalk-eyed flies by measuring the mating frequency of females from lines that exhibited a direct

response in males to artificial selection for increased ('high') and decreased ('low') male mating frequency. We found that the mating frequency of high-line females did not differ from that of low-line females. Hence, there was no support for a genetic correlation between male and female mating frequency in this species. Our study suggests that the genes which influence remating may not be the same in the sexes, and that females remate frequently in this species to gain as yet unidentified benefits.

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## Introduction

In general, males are expected to have a higher optimal mating frequency than females (Arnqvist and Nilsson, 2000; Gavrillets *et al.*, 2001). This is because males are assumed to be able to produce sperm in unlimited quantity and so increase their reproductive success with successive matings in contrast to the situation for females (Bateman, 1948). In females, reproductive traits such as egg production are assumed to be limiting, so each extra mating beyond an optimum value may provide diminishing benefits (Bateman, 1948; Parker, 1979). However, multiple mating is in fact common in female insects (Thornhill and Alcock, 1983; Ridley, 1988) and it is therefore important to evaluate the rival explanations for this phenomenon.

Females may benefit directly from multiple mating by obtaining sufficient sperm to fertilise available eggs (Ridley, 1988; Arnqvist and Nilsson, 2000). Also female fitness may be enhanced by the receipt of nutrients, for example, prey items (Thornhill and Alcock, 1983) which may increase fecundity (Boggs and Gilbert, 1979; Butlin *et al.*, 1987). Female multiple mating may also yield indirect, genetic benefits. For example, genes enhancing offspring fitness can be acquired through the adaptive consequences of sperm competition (Curtsinger, 1991; Keller and Reeve, 1995; Yasui, 1997). Similarly, multiple mating may reduce genetic incompatibilities and the

costs of inbreeding (eg Zeh and Zeh, 1997; Yasui, 1998; Tregenza and Wedell, 2002).

A contrasting and relatively unexplored nonadaptive hypothesis is that high female mating frequency has evolved due to a strong genetic correlation between the sexes in mating rate (Halliday and Arnold, 1987; Arnold and Halliday, 1988, 1992). If mating frequency is controlled by the same genes in males and females, then selection on males for higher remating would result in high remating frequency in females. This hypothesis assumes that selection on males to mate frequently is stronger than on females to mate at their lower optimal mating frequency (Halliday and Arnold, 1987).

The available data on whether there is a genetic correlation between the sexes in mating frequency are inadequate. Previous studies have used artificial selection experiments with *Drosophila melanogaster* (Manning, 1963; Gromko and Newport, 1988; Stamenkovic-Radak *et al.*, 1992; Sgrò *et al.*, 1998). However, genetic drift (Arnold and Halliday, 1988, 1992) or assortative mating (Butlin, 1993; see also Stamenkovic-Radak *et al.*, 1993) are plausible explanations for the correlated responses reported in some of the *Drosophila* studies (Manning, 1963; Gromko and Newport, 1988; Stamenkovic-Radak *et al.*, 1992). The most rigorously designed experiment shows no genetic correlation between the sexes (Sgrò *et al.*, 1998). Taken together, these data provide mixed results and little evidence of a genetic correlation between the sexes for remating frequency in *D. melanogaster*. A major problem is that none of the previous studies practised artificial selection on mating frequency *per se*. Instead, they focused selection on the time to first mating (Manning, 1963; Stamenkovic-Radak *et al.*, 1992)

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or on the length of time between the first and the second matings (Gromko and Newport, 1988; Sgrò *et al*, 1998). Genetic correlations between the sexes in related traits are of interest, but may be misleading since their sign and/or magnitude may differ markedly from that between male and female mating frequency itself. Hence, a rigorous test for the presence of a genetic correlation between the sexes directly on mating frequency rather than related traits is needed to evaluate the correlational hypothesis.

Bi-directional artificial selection for male mating frequency may be difficult to achieve logistically in species such as *D. melanogaster* which may mate typically only once every 1 or 2 days. This is because it is difficult to actually observe the required number of matings to impose selection and so traits related to mating frequency, such as time to first mating, have instead been the focus of selection in previous studies. However, the sexually dimorphic stalk-eyed fly, *Cyrtodiopsis dalmanni* presents an ideal opportunity to more readily practise selection on mating frequency, because mating rates are extremely high (Wilkinson and Dodson, 1997). In the laboratory, females can mate more than 10 times in a morning (Reguera *et al*, 2004). A second and important motivation for this study is that it is crucially important to examine the reasons for multiple mating in species other than *D. melanogaster* in order to establish generality. Stalk-eyed flies are of further interest when testing for the potential benefits of remating, because they represent a rare opportunity among insects to study determinants of female choice. They exhibit dramatic lateral extension of the head capsule with the eyes displaced onto the ends of stalks. In *C. dalmanni*, eyespan is greater in males than in females of comparable body size (Burkhardt and de la Motte, 1988) and females prefer to roost and mate with males bearing the largest eyespan (Wilkinson and Reillo, 1994; Hingle *et al*, 2001).

Here we test the hypothesis that the high frequency of female multiple mating in *C. dalmanni* results from a genetic correlation for mating frequency between the sexes. We used lines that had undergone eight generations of artificial selection for increased ('high' lines) and decreased ('low' lines) male mating frequency (Rogers *et al*, 2005) and showed direct responses in males to selection. The lines were founded from a wild-type base population exhibiting significant phenotypic and genetic variation in diverse morphological and life history traits in both sexes (David *et al*, 1998, 2000). We tested the genetic correlation between the sexes in mating frequency by making a direct measure of mating frequency (number of matings observed) for females from replicate high, low and control lines.

## Materials and methods

### Fly stocks and culturing

The base stock was a laboratory population founded in 1993 from individuals captured by AP in Gombak, Malaysia. The stock was maintained at high numbers in population cages (typically 10 cages with >200 individuals per cage). The cages (height = 20 cm, width = 20 cm, length = 30 cm) had a base of damp cotton to maintain cage humidity, at an approximately 1:1 sex ratio. Flies were kept at 25°C on a 12:12 h

dark:light cycle with a 30-min period of reduced lighting to simulate dawn and dusk. Flies were fed puréed sweet corn, to which a mould inhibitor (10% w/v Nipagin in 100% ethanol) was added. Flies for the experiment were obtained by collecting eggs from population cages on petri dishes and raising emerging larvae to adulthood. To obtain virgins, flies were segregated by sex 14–21 days after eclosion, using ice anaesthesia. Virgin flies were then placed in single sex population cages.

### Artificial selection lines

Full details of the artificial selection protocol are given in Rogers *et al* (2005). In brief, two replicate lines were selected for increased male mating frequency ('high' selection regime), two replicate lines were selected for decreased male mating frequency ('low' selection regime) and there were two unselected 'control' lines. All lines were derived from the wild-type laboratory population described above. Mating frequency was measured as the total number of matings achieved in a 1.5-h period commencing at artificial dawn on each of two consecutive days. In each generation, the mating frequencies of 24 males from each selection line were assayed with base stock females. The eight males with the highest or lowest mating frequencies were mated to females of their own line to produce the next generation of the high or low lines, respectively. Within each control line, the mating frequency of 12 males was measured and eight males were randomly chosen. There was a direct response to selection for male mating frequency. High-line and low-line males mated significantly more and less frequently, respectively, than did control males (Rogers *et al*, 2005). For example, at generation 7 of selection: mean  $\pm$  SD for replicate one, high-mating males =  $14.53 \pm 1.32$  matings, low-mating males =  $8.98 \pm 0.43$ ; for replicate two, high-mating males =  $13.43 \pm 1.06$ , low-mating males =  $10.23 \pm 0.70$ .

### Correlated response in female mating frequency

Female mating frequency was assayed using females from the eighth generation of selection, when there was significant divergence between high and low lines in male mating frequency (Rogers *et al*, 2005). For the assay, we replicated the conditions under which male mating frequency had been assessed during artificial selection and provided females from the selection lines with unselected base stock males.

At 2 days prior to behavioural observations, sets of five females from each replicate line (1 or 2) of each selection regime (high, control or low) were placed in assay pots (400 ml: height = 95 mm, diameter = 75 mm) containing a damp cotton wool base and 2 g of puréed sweetcorn. On the morning of observation, a randomly chosen single male was added to each set of females. On each of three consecutive days, observation began at the start of artificial dawn and lasted 1.5 h. The total number of matings over 40 s by each set of females ('number of matings') was recorded.

During the study, we obtained measures for a series of eight groups. Each group was comprised of four assay pots for each replicate line of each selection regime, yielding a total sample size over the entire experiment of 32 sets of females per line. To control for variation in male mating frequency within the 3-day assays, indivi-

dual males were moved between selection line females after each consecutive day; one day with high-line females, one day with low-line females and one day with control-line females. The sequence of exposure to females from each selection regime was randomly assigned.

Statistical analysis was performed using JMP statistical software (version 5, SAS Institute Inc.) for the Apple Macintosh.

## Results

The number of matings for females from each selection regime, defined as the total number of matings by all five females in each assay pot, are summarised in Table 1. The number of matings was normally distributed (Shapiro–Wilk tests), while the experimental group did not have a significant effect on the number of matings (one-way ANOVA  $F_{7,184} = 0.87$ ,  $P > 0.5$ ). Therefore, data were pooled across experimental groups for further analysis.

The number of matings was analysed using a fully balanced nested ANOVA with selection regime as a fixed effect and replicate line nested within selection regime as a random effect (Table 2). There was no difference in the mean number of matings of females from the high, low or control selection regimes ( $F_{2,3} = 0.24$ ,  $P > 0.75$ ). There were no significant differences between replicates within selection regimes ( $F_{3,186} = 1.05$ ,  $P > 0.25$ ).

## Discussion

There was no evidence for a genetic correlation between male and female mating frequency in the stalk-eyed fly *C. dalmanni*. Artificial selection for increased and decreased male mating frequency (number of matings) produced a direct response (Rogers *et al*, 2005). We found no significant differences in the mating frequency of females, estimated directly as the number of matings, from the high, low or control selection lines using the same assay conditions as those used to test male flies from these lines. Remating rates in our assays were lower than reported elsewhere (eg Reguera *et al*, 2004), possibly because the female biased ratio used in our assays could have reduced the mating opportunities for each female.

If mating frequency was controlled by the same genes in males and females, females might mate beyond their optimal frequency if there was stronger selection on males to mate at a high frequency than on females to mate just once or a few times (Halliday and Arnold, 1987). Strong selection on females to reduce the mating frequency could lead to the evolution of modifying

elements to limit the expression of genes which increase the mating frequency (Sherman and Westneat, 1988). The lack of genetic correlation between the sexes for mating frequency in *C. dalmanni* implies that mating frequency is under the control of different or sex-limited genes in males and females. Alternatively, it is possible that the selection pressures on mating frequency in males and female stalk-eyed flies are not divergent as generally predicted (Halliday and Arnold, 1987), and this is an interesting topic for future study. The potential existence of sex-limited genes determining the mating frequency in males and females suggests that constraints on selection on mating frequency in males and females may be minimised.

Relatively little is known about the determinants of female remating in *C. dalmanni*. Given a pairwise choice, females mate more frequently with males with large eyespan than those with small eyespan (Wilkinson *et al*, 1998; Hingle *et al*, 2001). Large females mate more than small females, while large females have significantly more mature eggs in their ovaries and may therefore mate at a higher frequency than small females to maintain high fertility (Grant, 2003). It is possible that the difference in mating frequency between large and small eyespan females reflects a male preference for large, fecund females. However, previous studies found no significant differences between selection regimes in eyespan, a measure highly correlated with body size (Rogers *et al*, 2005) or female fecundity (Segar *et al*, unpublished data). Remating in male and female *C. dalmanni* could be influenced by other factors, including environmental influences via physiological limitations such as ejaculate size or sperm storage capacity, availability of oviposition sites or chemical cues such as male accessory gland proteins. Future evaluation of the selection pressures on female mating frequency will need to assess the strength of the potential costs to remating in females (Reguera *et al*, 2004) relative to the likely benefits such as sperm replenishment.

The lack of a genetic correlation between the sexes for mating frequency in *C. dalmanni* is consistent with evidence from *D. melanogaster* (Sgrò *et al*, 1998). The factors involved in determining remating in *D. melanogaster* are well characterised and differ between the sexes. Female *D. melanogaster* are stimulated to remate by nutritional status (Harshman *et al*, 1988), availability of oviposition sites (Trevitt *et al*, 1988), the number of sperm in storage (Letsinger and Gromko, 1985) and accessory gland proteins transferred by mating males (Chen *et al*, 1988; Wolfner, 1997, 2002; Chapman, 2001; Chapman *et al*, 2003; Liu and Kubli, 2003). Males are stimulated to

**Table 1** Mean  $\pm$  standard error for the total number of matings observed in 1.5-h periods on 3 consecutive days, for sets of five females from the high, low and control lines artificially selected for male mating frequency

Selection regime	Replicate	Number of matings	Number of sets assayed
High	1	13.22 $\pm$ 0.78	32
High	2	11.97 $\pm$ 0.74	32
Control	1	12.50 $\pm$ 0.60	32
Control	2	13.62 $\pm$ 0.68	32
Low	1	12.78 $\pm$ 0.72	32
Low	2	13.09 $\pm$ 0.55	32

**Table 2** ANOVA of number of matings of females from the high, low and control lines artificially selected for male mating frequency, with selection regime (fixed effect) and replicate line nested within selection regime (random effect)

Source of variation	df	MS	F	P
Selection regime	2	3.77	0.24	>0.75
Replicate line within selection regime <sup>a</sup>	3	15.60	1.05	>0.25
Error <sup>b</sup>	186	14.83		

<sup>a</sup>Error term for MS<sup>selection regime</sup>.

<sup>b</sup>Error term for MS<sup>replicate line within selection regime</sup>.

remate by visual stimuli (Willmund and Ewing, 1982), female cuticular hydrocarbons (Scott, 1986) and the number of recent matings obtained (Markow *et al*, 1978). If, as these findings suggest, different factors influence mating in males and females, it is unlikely that selection on the mating frequency in one sex will significantly affect the mating frequency of the unselected sex.

If the ancestral population for the selection lines exhibited low levels of genetic variation, then a genetic correlation between the sexes in *C. dalmanni* might be underestimated. However, the base population is maintained at relatively large population size and shows significant genetic variation in a variety of traits (including eyespan, thorax length and wing size parameters) in *both* sexes (David *et al*, 1998, 2000). There is no indication therefore that adaptation to the laboratory environment has removed genetic variance in general or genetic variance for female mating frequency in particular.

A genetic correlation between the sexes might also be obscured if there was inadvertent selection on female mating frequency within the lines (Butlin, 1993), for example, disassortative mating, where male *C. dalmanni* from 'high' lines preferentially mate with females with low mating frequency. Ideally, individual males and females should be paired randomly (eg Sgrò *et al*, 1998). Here, this was partially achieved by placing selected males each generation with five randomly chosen selection line females (Rogers *et al*, 2005). The imposition of equal family size can also reduce variation between lines due to disassortative mating (Sgrò *et al*, 1998). The procedure for these lines minimised, but did not eliminate, variation in family size. The contribution of selected males was equalised across regimes by taking an equal number of progeny from each male every generation, but it remains possible that the contribution by females varied across regimes.

The present study provided no evidence for a genetic correlation between males and females for mating frequency in the stalk-eyed fly, *C. dalmanni*. The hypothesis that female mating frequency has evolved as a correlated response to selection on male mating frequency is not supported. Our experiments therefore suggest that the high rate of female remating may be adaptive in this species and future work should focus on identifying potential explanations.

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