

# Remating in *Drosophila melanogaster*: an examination of the trading-up and intrinsic male-quality hypotheses

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## Abstract

Female *Drosophila melanogaster* remate more frequently than necessary to ensure fertilization. We tested whether polyandrous females gain genetic benefits for their offspring by (1) selecting secondary sires of higher genetic quality than original partners or (2) because post-copulatory mechanisms bias fertilizations towards genetically superior males. We screened 119 hemiclones of males for lifetime fitness then selected eight hemiclones (four of extreme high fitness and four of extreme low fitness) and mated them to virgin females. Females were then given the opportunity to remate with males of benchmark-genetic quality and their propensity to remate (fidelity) and sperm displacement scored. A female's fidelity and her level of sperm displacement varied depending on which hemiclone she mated first, but not on male-genetic quality. These findings indicate that female remating and sperm displacement are strongly influenced by male genotype, but provide no evidence that these traits contribute to adaptive female choice to obtain superior genes for offspring.

## Introduction

In many species females mate with multiple males despite receiving no material benefits and incurring substantial fitness costs (Singh *et al.*, 2002; Chapman *et al.*, 2003). In these situations polyandry may be the outcome of males coercing females to mate suboptimally in the context of sexual conflict (Dawkins, 1976; Parker, 1979; Holland & Rice, 1998; Arnqvist & Rowe, 2002). Alternatively, polyandry may provide females with genetic benefits that enhance the success of their offspring and outweigh the costs of mating. A variety of hypotheses have been proposed to explain how polyandrous females might secure genetic benefits for their offspring (Andersson, 1994) and most of these fall into two categories (i) polyandry reduces the probability that genetically incompatible or closely related genomes combine (Stockley *et al.*, 1993; Zeh & Zeh, 1996, 1997) and (ii) polyandry increases the likelihood that females gain access to 'high-quality' genes for their offspring

(summarized in Yasui, 1998). While there is a growing body of evidence to suggest that polyandrous females can benefit by avoiding genetic incompatibility and inbreeding (e.g. see Jennions *et al.*, 2004), there exists comparatively little evidence that polyandrous females benefit by securing 'high-quality genes' for their offspring (Jennions & Petrie, 2000).

Mechanisms through which females bias fertilizations towards males of higher genetic quality potentially operate either before or after copulation. If females can accurately assess the genetic quality of sires prior to mating, then they can secure sperm with higher genetic quality by remating with males of successively higher genetic quality (trading-up hypothesis; Thornhill & Alcock, 1983; Watson, 1991). This hypothesis assumes that females can use cues associated with male courtship or nonbehavioural attributes such as appearance or smell to recognize potential sires carrying superior genes, and that patterns of environmental change are predictable so that fitter genes for the next generation can be predicted (Yasui, 1998). There is currently only limited data supporting the trading-up hypothesis (Kempnaers *et al.*, 1992; Gabor & Halliday, 1997; Jennions & Petrie, 2000).

Post-copulatory mechanisms also may operate to bias fertilizations towards the sperm of genetically superior

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males (intrinsic male-quality hypothesis; Madsen *et al.*, 1992; Birkhead, 2000). One way this form of female choice could be achieved is through active sperm selection (sperm-selection hypothesis; Sivinski, 1980, 1984; Eberhard, 1996). Morphological analysis of the female reproductive tract in various groups has shown that there is substantial potential for females to preferentially store and use the sperm of different mates. However, technical difficulties associated with investigating sperm selection within the female reproductive tract have made the collection of the requisite quantitative data difficult to obtain (Eberhard, 1996; Telford & Jennions, 1998).

Even when females are unable to preferentially use the sperm from different males the potential still exists for superior genes to be obtained through sperm competition. Making the controversial assumption that the genetic quality of males correlates with the competitive ability of their sperm, males of higher genetic quality would gain a greater share of paternity in females inseminated by multiple males (good-sperm hypothesis; Harvey & May, 1989; Birkhead *et al.*, 1993; Yasui, 1997). In support of the 'good-sperm' hypothesis associations have been detected between male sperm competitiveness and elements of offspring fitness, including growth rate (Hosken *et al.*, 2003), the attractiveness of sons (Pai & Yan, 2002) and the fecundity of daughters (Konier *et al.*, 2001; Kozielska *et al.*, 2004). Links have also been found between pre and post-copulatory sexual selection. Recently, Evans *et al.* (2003) documented that in guppies a male's sperm competitive success was correlated with the degree of orange colouration.

In this study we investigated the 'trading-up' and 'intrinsic male-quality' hypotheses in a laboratory population of the fruit fly *Drosophila melanogaster*. Females in this species remate at frequencies beyond that necessary to ensure fertilization (see for review, Singh *et al.*, 2002) and mating does not provide any material (direct) benefits (Chapman *et al.*, 1994; Pitnick *et al.*, 1997). Conversely, remating has negative impacts upon female fitness, mainly because male seminal fluid contains substances that reduce female survival (Partridge *et al.*, 1987; Fowler & Partridge, 1989; Chapman *et al.*, 1993, 1995; Rice, 1996; Civetta & Clark, 2000; Sawby & Hughes, 2001), fecundity (Pyle & Gromko, 1978; Chapman *et al.*, 1993; Pitnick & García-González, 2002) and fertility (Prout & Clark, 2000). These costs of remating will generate sexual conflict over remating rate, unless females recoup the cost by obtaining genetic benefits for their offspring (Singh *et al.*, 2002, Brown *et al.*, 2004).

Here, we quantify the relationship between the genetic quality of a female's first mate and her propensity to remate with other males and use sperm from these secondary males. This was achieved by taking advantage of the fact that a set of 119 hemiclones of male *D. melanogaster* had been screened for lifetime fitness in two previous studies (Rice & Chippindale, 2001a,b). A hemiclone is a group of individuals that all share the

same genome-wide haplotype (the X chromosome and the two major autosomes, but excluding the dot fourth chromosome), each expressed in a different random genetic background. A hemiclone is produced through specialized cytogenetic techniques (Chippindale *et al.*, 2001) but it can be conceptualized as a group of individuals produced by randomly selecting a group of eggs from a population and then fertilizing each egg with the same sperm genotype. From this screen we selected four hemiclones that consistently showed high fitness (top 10%) and four hemiclones that consistently showed low fitness (bottom 10%). In the time since these hemiclones were isolated, they have been preserved through repeated cytogenetic cloning. By using this set of eight hyper-dispersed low- and high-fitness hemiclones, rather than random hemiclones, we greatly increase the power of detecting whether females can use pre or post-copulatory mechanisms to enhance the net quality of their offspring. If females remate to secure superior genes two findings were predicted: (1) females would remate at a lower rate when first mated to a male from a high-fitness hemiclone and (2) paternity of offspring would be biased towards males from hemiclones of superior heritable-genetic quality.

## Materials and methods

### Base populations

Flies were obtained from two laboratory populations (LH<sub>M</sub>) and (LH<sub>M</sub>-*bw*). LH<sub>M</sub> is a large (adults  $n > 1700$  per generation) outbred population that was founded in 1991 from central California (for details see Rice *et al.*, in press). This stock expresses the wild-type red-eye colour. The LH<sub>M</sub>-*bw* stock is a replica of the LH<sub>M</sub> population that carries a brown eye color (*bw*) recessive marker that has been introgressed through 13 backcross generations into the LH<sub>M</sub> genetic background. Culture conditions for both stocks were 12 h light: 12 h dark cycle at 25 °C. Larvae were reared at 150–200 per 10-dram vial provisioned with 10 mL of cornmeal/molasses medium, seeded with approximately 10 mg of live yeast. At the time of the experiment the LH<sub>M</sub> and the LH<sub>M</sub>-*bw* populations had been maintained under these conditions for over 300 generations.

### Acquiring hemiclones of low and high genetic quality

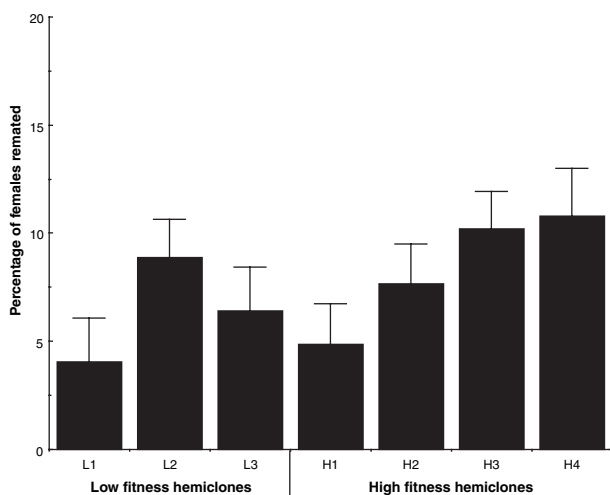
For this study we used males from four 'low-fitness' hemiclones (hereafter referred to as L1-4) and four 'high-fitness' hemiclones (hereafter referred to as H1-4). To obtain the four hemiclones of high, and four of low, lifetime fitness, 119 hemiclones were screened in a multi-generation fitness assay for lifetime fitness as described in Rice & Chippindale (2001a,b). Because the assay traced the frequency of male hemiclones across multiple generations, all fitness components were included in

out metric of net fitness. Next the distribution of life-time fitness was plotted and four hemiclones were selected from the top 10% of the fitness distribution and four from the lower 10%, as illustrated in Fig. 1 of Lew & Rice (2005).

The general procedure to produce a hemiclone has been described in detail elsewhere (Chippindale *et al.*, 2001; Rice *et al.*, in press). Briefly, to produce the 25 males that constituted each of the eight hemiclones used in each replicate, we placed eight males (carrying the target genomic haplotype) with eight 'clone-generator' females (wild type except that they had C (1) DX, *y*, *f*, *sb* substituted for their two X chromosomes, and they carried a Y chromosome from the LH<sub>M</sub> base population) in a standard culture vial and permitted mating to occur. The mated females were allowed to oviposit for 18 h before being cleared from the vial. In order to control larval density, eggs in excess of 150 were removed from the culture medium. Remaining eggs were then cultured under conditions used to maintain the LH<sub>M</sub> base population. This culture procedure was repeated 90 times for each of the eight-hemiclone lines. The entire procedure was replicated over three consecutive days (blocks). Independent parental flies were used each day. After 12 culture days post-egg deposition, adult males of each hemiclone were collected under brief (<30 s) CO<sub>2</sub> anaesthesia and stored at 25 °C in culture vials containing 25 males per vial. Males were not collected as virgins so as to give them courtship experience.

#### Technique for constructing tester males of benchmark genetic quality

To obtain 'tester males' that were of uniform genetic quality we crossed two inbred LH<sub>M</sub>-*bw* populations (I-1



**Fig. 1** Mean ( $\pm$ SE) percentage of females that remated with tester males after they were mated first to males from seven hemiclones of either high or low quality.

and I-2) to produce males that were that were genetically uniform, but which still had normal levels of heterozygosity. We crossed four males from I-1 with four females from I-2. We made 70 replicates of this cross. In each cross females were permitted to lay eggs for 18 h. After this time adults were discarded and eggs in excess of 150 per vial removed. The remaining eggs were then cultured under the same conditions used to maintain the LH<sub>M</sub> and LH<sub>M</sub>-*bw* base populations. This process was repeated over three consecutive days. Independent parental flies were used each day. After 12 culture days post-egg deposition tester males (*bw/bw*) were collected under brief CO<sub>2</sub> anaesthesia and stored at 25 °C in culture vials containing 15 males per vial. Males were not collected as virgins to give them courtship experience.

The expected fitness of these tester males is the average fitness of the LH<sub>M</sub> population. Compared to our sample of four high-fitness and four low-fitness hemiclones, and under the assumptions of either additive or multiplicative fitness models, we can be 99% certain that the tester males were not the lowest fitness males ([prob of one haplotype in tester males being in lower 10% of fitness distribution]  $\times$  [probability that the second haplotype of tester males has less than average fitness]  $\times$  [probability of being the worst of five in the lower fitness group] =  $0.1 \times 0.5 \times 1/5 = 0.01$ ), and by the same logic, we can be 99% confident that the tester males were not, on average, the highest fitness males.

#### Acquiring experimental females

To acquire females we established 80 culture vials, each containing 16 pairs taken directly from the LH<sub>M</sub>-*bw* base population. Mated females were permitted to lay eggs for 18 h. After this time they were discarded and eggs in excess of 150 per vial removed. The remaining eggs were then cultured under conditions used to maintain the LH<sub>M</sub> base population. This procedure was repeated over three consecutive days using independent parental flies each day. Within 5 h of emergence females were collected under brief (<30 s) CO<sub>2</sub> anaesthesia and stored at 25 °C in culture vials containing 15 females per vial. After 2 days female virginity was assured by checking storage vials for eggs. Any vials containing eggs were discarded. On the night before experimentation virgin females were provided with 10 mg of live yeast to prime them for mating and induce them to lay eggs.

#### Experimental procedure

For each hemiclone we transferred 25 males (primary males) into a standard culture vial containing 15 LH<sub>M</sub>-*bw* virgin females taken from the outbred base population (see above). We used an approximate 50% excess of males compared to females to increase the probability that females were mated. Flies were left undisturbed for 2 h then males and females were separated under brief

(<30 s) CO<sub>2</sub> anaesthesia and females transferred to a standard culture vial where they were left to recover for 2 h. This procedure was repeated for each of the eight hemiclones (four low fitness and four high fitness) to produce females that were mated to males of either low or high quality. Following primary insemination we provided females with the opportunity to remate by transferring them without anaesthesia into a culture vial containing 15 LH<sub>M</sub>-*bw* tester males (see above). We used 15 males because this approximates the number of flies used in standard LH<sub>M</sub> and LH<sub>M</sub>-*bw* cultures, which is the breeding environment to which the flies have adapted. Flies were left together for 8 h. Remating trials were run within 10 h of primary mating because Scott (1987) reported that female receptivity begins to decrease 6–8 h after mating, and is strongly suppressed after 12 h. Hemiclone males were presented to females first and tester males second because males from the different hemiclones differ in their level of persistent courtship (unpublished data). Therefore, if hemiclone males were mated second we could not rule out the possibility that variation in female remating was related to variation in antagonistic male persistence, rather than female choice.

Following treatment females were separated from tester males under brief (<30 s) CO<sub>2</sub> anaesthesia and placed individually in test tubes containing cornmeal/molasses medium and a small amount of yeast to induce oviposition. After 48 h females were removed from the laying vials. The progeny were then reared under standard culture conditions and following maturity we used eye colour (red-eyed offspring [*bw*<sup>+</sup>/*bw*] were derived from the primary sires and brown-eyed [*bw*/*bw*] from secondary sires) to score female remating frequency in relation to the genetic quality of the primary mate. Viability of *bw*/*bw* and *bw*/*bw*<sup>+</sup> offspring is statistically indistinguishable (Chippindale *et al.*, 2001). To test whether females can bias paternity towards genetically superior males, and/or whether genetically superior males were more successful in sperm competition, we scored the proportion of offspring sired by primary males (P<sub>1</sub>).

In addition to experimental matings we ran control matings to test the fertilization efficiency of hemiclones. These matings followed the same protocol as experimental matings, except that immediately after primary insemination females were individually placed in small culture tubes containing standard cornmeal/molasses medium, seeded with live yeast. After 48 h females were removed from the tubes that were then incubated under standard conditions. After 14 days females were scored as mated or unmated based on the presence or absence of offspring.

All adult flies used in the experiment were approximately 3 days old. Three replicated blocks of the experiment were made over three consecutive days. For each hemiclone we made a minimum of four and a maximum of 10 replicates on each day the experiment

was repeated. This was with the exception of a single hemiclone (L4), for which we made a minimum of two and a maximum of five replicates each day. For each hemiclone, between one and two control trials were run in parallel on each experimental day.

### Data collection and analysis

The proportion of females that remated was scored for individual experimental vials of 15 females. Comparison of remating among hemiclones was made using a least squares model with hemiclone and trial day (blocks 1, 2 or 3) as random effects and proportion of females remating (arcsine-transformed, and weighted by number of females assayed) as the dependant variable. Comparison between hemiclone quality and remating was made using a least-squares model with quality category (low vs. high) as a fixed factor, trial day (blocks 1, 2 or 3) as a random factor, hemiclone as a random factor nested within quality, and percentage of females remating (arcsine-transformed, and weighted by number of females assayed) as the dependant variable.

For females that remated we also scored for P<sub>1</sub>, the proportions of progeny with red eyes (*bw*<sup>+</sup>/*bw*, sired by the primary males) vs. brown eyes (*bw*/*bw*, sired by the tester males). Comparison of P<sub>1</sub> among hemiclones was made using a least squares model with hemiclone and trial day (blocks 1, 2 or 3) as random effects, and the proportion of progeny with red eyes (P<sub>1</sub>) within individual female families (arcsine-transformed and weighted by family sizes) as the dependant variable. To test for the effect of hemiclone quality on P<sub>1</sub> we used a least-squares model with quality category (low vs. high) and trial day (blocks 1, 2 or 3) as random factors, hemiclone as a random factor nested within quality and P<sub>1</sub> (arcsine-transformed and weighted by number of females assayed) as the dependant variable. Six of the 170 total families analysed were deleted from analyses because they contained a single brown-eyed female and so were likely to have been contaminated when a brown-eyed mother was inadvertently retained in the culture vial along with her offspring. All analyses were made using JMP statistical software (Version 5.1.1).

## Results

### Insemination controls

The insemination controls revealed that there was a significant difference between hemiclones in their ability to primarily inseminate virgin females, (two-way ANOVA:  $F_{7,32} = 3.1$ ,  $P = 0.018$ ), and this was unaffected by trial day (two-way ANOVA:  $F_{2,32} = 0.39$ , NS,  $P = 0.68$ ). The reason for this heterogeneity was that the percentage of females successfully inseminated was markedly lower for hemiclone L4 than for any of the other hemiclones (Table 1). When this hemiclone was excluded from

**Table 1** Results from mating controls that tested the ability of 25 male *D. melanogaster* from eight hemiclones, of varying genetic quality (low vs. high), to primarily inseminate 15 virgin females from the LH<sub>M</sub>-*bw* base population.

Hemiclone	Quality	Mean	SE	<i>n</i>
L1	Low	98.33	1.84	4
L2	Low	98.57	1.65	5
L3	Low	96.42	1.84	4
L4	Low	88.57	2.13	3
H1	High	100	1.84	4
H2	High	98.07	1.84	4
H3	High	98.66	1.65	5
H4	High	100	1.84	4

*n*: the number of control replicates (vials containing 25 males and 15 virgin females).

analysis there was no significant difference between hemiclones in insemination capability (two-way ANOVA: hemiclone;  $F_{6,29} = 0.41$ , NS,  $P = 0.86$ , trial day;  $F_{2,29} = 0.07$ , NS,  $P = 0.93$ ). Because hemiclone L4 did not reliably mate the experimental virgin females this caused a substantial proportion of females to mate for the first time with tester males, thereby generating overestimates of remating rates and underestimates of  $P_1$ . Therefore, we excluded hemiclone L4 from all further analyses.

#### Effect of male hemiclone on female remating

The percentage of females that remated was significantly affected by male hemiclone ( $F_{6,129} = 2.21$ ,  $P = 0.046$ ) but not trial day ( $F_{2,129} = 2.46$ , NS,  $P = 0.09$ ). Remating rates ranged between  $4.06 \pm 2.03$  and  $10.798 \pm 2.195\%$  (Fig. 1). These data demonstrate that our analysis had sufficient power to detect variation among hemiclones in their influence on female remating rate.

#### Effect of male quality on female remating

Despite the significant heterogeneity in the propensity of females to remate after primary insemination by the different hemiclones, this heterogeneity was not associated with genetic quality (low vs. high) of the primary males ( $F_{1,129} = 1.05$ , NS,  $P = 0.35$ ), and this pattern was consistent across trial days ( $F_{2,129} = 2.46$ , NS,  $P = 0.09$ ). Average ( $\pm$ SE) female remating rate was  $8.86 \pm 1.04\%$  when females' first mated with males of high quality and  $6.59 \pm 1.13\%$  when females mated first with males of low quality. Confidence intervals (95%), based on the sample means and variances of the seven hemiclones are (5.27, 12.46) and (2.26, 10.89) for the high- and low-fitness primary sires, respectively.

#### Effect of male hemiclone on $P_1$

The proportion of progeny sired by the primary males ( $P_1$ ) was significantly affected by male hemiclone, but

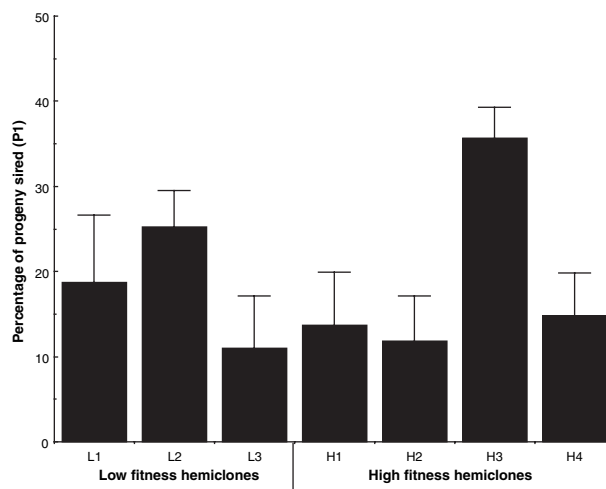
not trial day (hemiclone;  $F_{6,155} = 4.77$ ,  $P = 0.0002$ , trial day;  $F_{2,155} = 1.41$ , NS,  $P = 0.25$ ). Mean ( $\pm$ SE)  $P_1$  ranged between  $10.98 \pm 6.17$  and  $35.67 \pm 3.60\%$  (Fig. 2). These data demonstrate that our analysis had sufficient power to detect variation among hemiclones in their influence on sperm displacement in females.

#### Effect of male quality on $P_1$

Despite the significant heterogeneity in  $P_1$  among the different hemiclones, this heterogeneity was not associated with genetic quality (low vs. high) of the primary males (male quality;  $F_{1,155} = 0.05$ , NS,  $P = 0.83$ ) and this pattern was consistent across replicated blocks of the experiment (two-way ANOVA:  $F_{2,155} = 1.41$ , NS,  $P = 0.25$ ). High-quality males sired an average of  $22.99 \pm 2.62\%$  of progeny while low quality males sired an average of  $20.25 \pm 3.04\%$  of progeny. Confidence intervals (95%), based on the sample means and variances of the seven hemiclones, are (9.32, 36.65) and (1.47, 39.04) for the high- and low-fitness primary sires, respectively.

#### Discussion

The results of our study indicate that female *D. melanogaster* are no more likely to remate after having mated with males of low heritable quality compared to males of high heritable quality. This pattern does not support the trading-up hypothesis of adaptive female remating. Because the sample of male hemiclones that we used was a hyper-dispersed subset from a much larger sample of 119 hemiclones that were screened for lifetime fitness, the variance in genetic quality among alternative mates was high and the resolving power of the experiment was



**Fig. 2** Mean ( $\pm$ SE) proportion of total progeny sired by the first two males to mate following female remating ( $P_1$ ). First males were from one of four hemiclones of high quality or one of three hemiclones of low quality. Second males to mate were tester males.

expected to be substantial. The ample statistical power of our experimental design is supported by the fact that we detected variation among hemiclones for remating rate. The contrasting findings of significant variation among male hemiclones, but not among male fitness categories, indicate that any advantage to trading-up, if present, is likely to be small relative to the influence of other male attributes influencing female remating, which could contribute to sexual conflict.

We also found that males of higher quality were no more successful in sperm competition ( $P_1$ ) than males of low quality when competing against 'tester males'. These data provide no evidence to support the hypothesis that females secure superior genes for their offspring, and increase their own fitness, by selecting the sperm of genetically superior males to fertilize their eggs (sperm-selection hypothesis) (Eberhard, 1996; Tregenza & Wedell, 2002). Furthermore, the observed lack of association between male quality and paternity success provide no support for the hypotheses that females are remating to secure genes from genetically superior males through sperm competition because the sperm of superior males have an advantage in sperm competition (good-sperm hypothesis) (Madsen *et al.*, 1992). As in the case of our test of the trading-up hypothesis, the simultaneous finding of statistically significant heterogeneity in  $P_1$  among hemiclones indicates that statistical power was sufficient to resolve differences among genetic categories. The contrasting findings of significant variation among male hemiclones, but not among male fitness categories, indicate that if sperm derived from high-fitness males are used preferentially or are more successful in sperm competition, that these associations are likely to be weak relative to the influence of other male attributes influencing fertilization success.

Our results suggest that hypotheses proposing that *D. melanogaster* females remate as an adaptive strategy to secure genetic benefits are not the primary factors controlling remating in female *D. melanogaster*, at least in the context of large outbred laboratory populations. The results corroborate a previous study of our LH<sub>M</sub> base population that found that the operation of sexual selection did not speed the rate of adaptive evolution when the flies were exposed to a new environment (Holland, 2002). They are also consistent with a recent study which found that female *D. melanogaster* mated to multiple males gained no fitness advantages over females mated repeatedly to the same males and that the paternity of a female's progeny showed no bias towards males that were favoured in precopulatory mate choice (Brown *et al.*, 2004). It is yet to be tested whether females can procure any benefits through increased genetic diversity of offspring (Baer & Schmid-Hempel, 1999). However, it must be considered that if any benefits are provided from increased heterogeneity of offspring they would have to be substantial to offset the extensive costs

that are associated with remating (Chapman *et al.*, 1995; Wolfner, 1997).

Our finding that male genotype, but not genetic quality, affects female remating indicates that males can influence whether females remate, irrespective of whether or not remating increases female fitness. Although we did not directly measure the fitness of offspring, we did experimentally control the heritable variation of the primary sires. This result, finding an effect of male genotype but not heritable fitness category, is more consistent with sexual conflict, rather than traditional models of sexual selection based on adaptive female choice. This finding also adds to a growing body of evidence that indicates that at least some of the multiple mating observed in *D. melanogaster* is sexually antagonistic. Several findings from past experiments suggest that female *D. melanogaster* are mating beyond their optimum in our LH<sub>M</sub> base population. First, when males were permitted to evolve, but females were prevented from counter-evolving, males rapidly responded by increasing mating rate and toxicity of seminal fluid (Rice, 1996). Second, when monogamy was experimentally enforced, males evolved to become less harmful to their mate and concurrently females evolved to become less resistant to male imposed harm (Holland & Rice, 1999). Third, experimentally imposed monogamy has been shown to favour the evolution of males whose ejaculates are less effective at inducing female nonreceptivity, and favour the evolution of females that remate less frequently, presumably reflecting reduced female resistance to male ejaculatory manipulation (Pitnick *et al.*, 2001).

In conclusion, this study provides an assessment of the contribution of the 'trading-up', and the 'good-sperm' hypotheses for the evolution of polyandry in the *D. melanogaster* laboratory model system. We found no evidence to support them, but because the statistical power of any experiment is limited, our results do not disprove these hypotheses. Our findings do, however, indicate that any influence of genetic benefits on female remating rate are small relative to other genetic factors that can potentially contribute to sexual conflict.

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