doi:10.1111/j.1420-9101.2006.01186.x

The cost of mating rises nonlinearly with copulation frequency in a laboratory population of *Drosophila melanogaster*

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Keywords:

experimental evolution;
mating costs;
remating;
sexual conflict;
sexually antagonistic coevolution.

Abstract

Previous studies of *Drosophila melanogaster* have demonstrated a cost to females from male courtship and mating, but two critically important parameters remain unresolved: (i) the degree to which harm from multiple-mating reduces lifetime fitness and (ii) how harm from mating might change with successive matings (rematings). Here we use 'laboratory island analysis' to quantify the costs that females incur with each remating, in the currency of lifetime fitness and under conditions that closely match those to which the flies have adapted for hundreds of generations. We experimentally manipulated the number of female matings by varying the order of daily 2-h exposures of females to either sperm-less males (XO) or intact males (XY). Females that mated more often had substantially reduced lifetime fecundity, and importantly, the fitness cost from remating rapidly accelerated.

Introduction

Demonstrating that there is a cost to remating (i.e. mating more than once), in the currency of lifetime fitness, is critically important in order to infer genetic conflict between the sexes over this shared trait. Moreover, quantifying the cost of remating is important because it is this cost that must be weighed against any direct or indirect benefits that females may gain through sexual selection (Chapman *et al.*, 2003a; Pizzari & Snook, 2003; Arnqvist & Rowe, 2005; Orteiza *et al.*, 2005; Stewart *et al.*, 2005). However, measuring the fitness cost of remating is difficult, because for it to be of true utility, it must be measured under conditions that closely match those to which the population is adapted (Sgro & Partridge, 2000; Rice *et al.*, 2005, 2006).

The importance of measuring costs and benefits of remating in the appropriate environmental context is illustrated by a recent controversy concerning genetic conflict in the *Drosophila melanogaster* model system. The interpretation of a long series of experiments that have been one of the empirical pillars of the process of sexually antagonistic coevolution has recently come into question (Cordero & Eberhard, 2003). The primary concerns were that the observed male-induced harm to their mates was measured under simplified laboratory conditions, and that this harm was only manifest under higher than normal nutrition (Chapman & Partridge, 1996). There are two ways to address the generality of these concerns: (i) *in situ* analysis: measure male-induced harm under field conditions in wild populations, or (ii) laboratory island analysis: measure this same parameter in large, outbred laboratory populations that have adapted to a competitive environment for hundreds of generations, so that the laboratory approximates their new, native environment (Rice *et al.*, 2005, 2006). At present, the first approach cannot be feasibly accomplished, at least with the technology currently available. Here we use the second approach by extending prior work of L. Partridge, K. Fowler, T. Chapman and colleagues (Partridge *et al.*, 1986, 1987; Fowler & Partridge, 1989; Partridge & Fowler, 1990; Trevitt & Partridge, 1991; Chapman, 1992; Chapman *et al.*, 1993, 1994, 1995) by measuring the cost of remating in a large, outbred laboratory-adapted population under the environmental conditions that closely match those to which it had adapted.

Partridge and collaborators demonstrated that the presence of males is harmful to females by showing that: (i) the act of mating, itself, is costly, in part due to the

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transfer of seminal proteins and (ii) there is a behavioural cost to females when interacting with males, even when mating does not occur. It was also shown that this harm was manifest primarily as a reduction in long-term survival, rather than daily fecundity (although their experiments were not specifically designed to test for fecundity effects). The sensitivity of measures of male-induced harm to environmental context is exemplified by the study by Chapman & Partridge (1996), in which male-induced harm was observed only under the highest of five nutrient conditions supplied to females (i.e. in the treatment that provided a 50% increase in the food level to which the flies had adapted).

As *in situ* analysis of male-induced harm is not feasible in *D. melanogaster*, we have used laboratory island analysis. Our base population of *D. melanogaster* is LH_M, an outbred (*c.* 1800 breeding adults per generation) population that has adapted to laboratory conditions for over 350 generations (see Rice *et al.*, 2005 for details). Key features of the laboratory environment include (i) high resource competition among females due to restricted availability of the limiting resource for female reproduction [i.e. live yeast (Stewart *et al.*, 2005)], (ii) high levels of sexual selection among males (Chippindale *et al.*, 2001; Friberg *et al.*, 2005), (iii) a nonoverlapping life cycle of only 14 days, during which adult mortality is negligible (Chippindale *et al.*, 2001), and most importantly, (iv) the capacity to measure lifetime fitness under nearly identical conditions as those to which the population has adapted (Rice *et al.*, 2005, 2006).

One potential complication with the use of the LH_M base population is the previous finding, albeit in a different population, that most male-induced harm to females is attributable only to reductions in their long-term adult survival (Partridge *et al.*, 1986, 1987; Fowler & Partridge, 1989; Partridge & Fowler, 1990; Trevitt & Partridge, 1991; Chapman, 1992; Chapman *et al.*, 1993, 1994, 1995) – a trait that would not be manifest in our short-lived LH_M base population. Moreover, the LH_M population is reared under restricted food conditions, which Chapman & Partridge (1996) found to reduce all measurable male-induced harm. However, recent work with the LH_M-population has shown that male-induced harm is, in fact, substantial in this population [persistent male courtship and remating cause females to lose an average of 21.6% of their potential lifetime fecundity (Rice *et al.*, 2006)], despite the limiting resources and short adult lifespan. In addition, it has been established that in the LH_M population there is a negative selection gradient on remating (some or none) in females (Linder & Rice, 2005) and a positive selection gradient on this trait in males (Friberg *et al.*, 2005), indicating that there is intersexual conflict over this parameter.

The work of Partridge, Fowler, Chapman and coworkers (see list above) has demonstrated that remating contributes to the female cost of male–female interactions, at least under high nutrient conditions. Here we extend these past studies by using laboratory island analysis to quantify the cost to females for each sequential remating when costs are measured under the environmental conditions that closely match those to which the population has adapted for over 350 generations.

Although there is evidence that *D. melanogaster* rapidly adapts to laboratory conditions, with reports of a two-fold increase in fitness after only 1 year of adaptation (eight generations) (Frankham & Loebel, 1992) and a three-fold increase after 2 years (Briscoe *et al.*, 1992), there is no expectation that 350 (or many thousands of) generations of adaptation will fully adapt our base population to the laboratory environment. However, most life history traits are polygenic quantitative characters (Prasad & Joshi, 2003) and selection over a period of several hundred generations is ample time to move most such traits many standard deviations (Falconer & Mackay, 1996). Nonetheless, all environments are expected to be changing due to climate change and the red queen process, so there is no expectation that any natural population will ever be at equilibrium, with respect to its environment – only that it will not experience a radically novel environment across a small number of generations.

To measure the cost of each of a series of rematings, we provided all females across treatments with equal total exposure (2 h day^{-1} over a 5-day period) to sperm-less (XO) males (6 h) and to intact (XY) males (4 h), that produce normal amounts of sperm. By varying the sequence in which females experienced intact and sperm-less males, we were able to experimentally control the number of times that females remated (see below), and then measure the corresponding lifetime fecundity of these females.

Materials and methods

Base populations

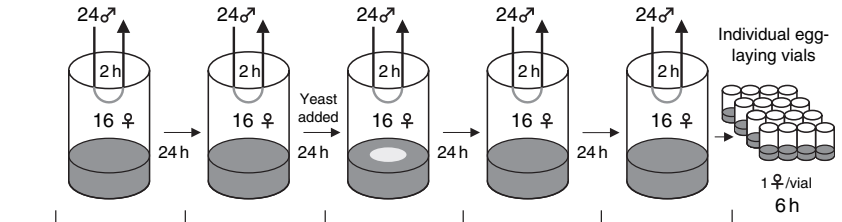
The outbred LH_M-population has adapted to laboratory conditions for over 350 generations. It is propagated on a 2-week cycle with nonoverlapping generations (for a detailed description, see Rice *et al.*, 2005). Flies are transferred to three consecutive vials each generation. On day 0/14, eggs are laid in a first set of 56 'juvenile competition' vials. The offspring remain in these vials during the larval, pupal and early adult stages, at a density of 150–200 per vial. By day 12, nearly all flies are sexually mature and most females (*c.* 99%, G. Rice unpublished data) are nonvirgin. On day 12, adult flies are mixed among vials. Next, 1792 randomly selected flies are transferred to a second set of 56 'adult competition' vials (16 pairs/vial) for 2 days (days 12.25–14.25). During the adult competition stage, females compete for a limited supply (10 mg) of live-yeast, which strongly influences their fecundity (Orteiza *et al.*, 2005; Stewart *et al.*, 2005), and males compete to fertilize females. During the last 18 h before the end of the 2-week life

cycle (the last 3/4 of day 14), the adult flies are placed in 'oviposition-vials', without live yeast. Eggs produced during the oviposition phase of the lifecycle are reduced to 150–200 per vial and used to begin the next generation.

XO males used in our study (see Experimental design section below) were obtained from crossing virgin LH_M females and C(1;Y) y^1B^1/O males. These C(1;Y) y^1B^1/O males were maintained by crossing them with C(1)RM y^1 v^1/O females and reared on the same lifecycle as the LH_M base population. The population of C(1;Y) males and C(1)RM females was backcrossed three times into the LH_M-population (by A. Chippindale, Queens University, and kindly provided to us), to reduce differences in genetic background between XO and intact LH_M males. Although XO males do not transfer sperm, they do transfer normal amounts of seminal proteins (Ingman-baker & Candido, 1980; Chapman, 1992). In addition, it has been shown that XO males are less vigorous at courtship than XY males (Partridge & Fowler, 1990; Chapman, 1992). However, this difference was unimportant in our experiment because females in all experimental treatments received identical exposure times to XO (6 h) and XY (4 h) males (see Fig. 1). The XO and XY males used in our study were obtained from duplicate populations that were temporally advanced by 3 days, compared with the LH_M base population used to obtain females, so that on day 10 of the lifecycle, when virgin males and females were first combined (see Experimental design section below), the males were fully mature.

Experimental design

The objective of the assay (Fig. 1) was to experimentally vary the number of times that a female mated, while still closely matching the conditions to which the LH_M-population had previously adapted. On day 9 of the generation cycle (the third to last day of the juvenile competition phase of the life cycle and the point where most females first start to eclose, but few have mated), females were collected as virgins by brief CO_2 gas anaesthesia (<2 min) and housed separately in 16 vials with 16 females per vial. Three-day old (post-eclosion) XY and XO males were collected on the same day from a separate set of vials that were 3 days advanced in their life cycle. On day 10 (the second last day of the juvenile competition phase in the 14-day life cycle, and the point when females commonly start mating), the experiment was initiated by adding either 24 XY or XO males to these vials for a 2-h duration. Within the experiment, vials of females were randomly assigned to one of four treatments (1–4; Fig. 1) that varied only in the timing of daily exposures of females to XO and XY males, but not in total exposure to either type of male (i.e. all treatments received three 2-h daily exposures to XO males and two 2-h daily exposures to XY males, but the ordering of these two types of exposures was experimentally varied among treatments, as illustrated in Fig. 1). Because long-term (>1 day) suppression of female mating requires the presence of sperm (Xue & Noll, 2000; Liu & Kubli, 2003), and because females that have successfully stored sperm



Treatment	day 10	day 11	day 12	day 13	day 14
1	**XY ♂**	**XY ♂**	XO♂	XO♂	XO♂
2	XO♂	**XY♂**	**XY♂**	XO♂	XO♂
3	XO♂	XO♂	**XY ♂**	**XY ♂**	XO♂
4	XO♂	XO♂	XO♂	**XY ♂**	**XY ♂**

Fig. 1 Experimental setup. Sixteen females were repeatedly exposed for 2 h day^{-1} to groups of 24 males that were either all intact (XY males that transferred sperm during mating) or all spermless (XO males that transfer no sperm, but normal amounts of seminal fluid during mating) for five subsequent days, after which the fecundity of individual females was measured by placing them in individual vials for 6 h. During each 2-h exposure the number of matings was tallied at 5-min intervals by direct observation. Females mated first to spermless males (XO) were expected to be highly receptive to remating on subsequent days, because they did not successfully store sperm from the mating. Once mated to intact XY males, females were expected to be recalcitrant to remating on subsequent days, because they had successfully stored sperm. The earlier females were exposed to the 2 days of intact XY males, the lower the expected remating number.

are highly recalcitrant to remating during 2-h exposures to males (Newport & Gromko, 1984), our use of 2-h exposures of females to XY males at progressively later days (days 10 and 11 in treatment 1 to days 13 and 14 in treatment 4), enabled us to control the timing and duration of female receptivity and, therefore, the average number of times that females mated. Our design caused the number of matings to increase from an average of about one in treatment 1 to successively higher values with increasing treatment numbers. A 50% excess of males was used to ensure that all females could be mated during the 2-h period. Data on matings were collected by counting mating pairs (via visual observations) every 5 min during these 2 h. After 2 h, males and females were separated with brief CO_2 gas anaesthesia (<2 min). The 2-h exposure to males was repeated daily until the end of the 14-day life cycle, for a total of five exposures. On the 12th day females were transferred 1 h before the onset of the mating trial to a fresh vial containing a small amount of live yeast (10 mg) applied to the surface of the killed yeast medium (cornmeal, molasses, agar, killed yeast, propionic acid, tegosept and H_2O), as occurs during the normal propagation of the LH_M population. The live yeast was provided on day 12 to match the timing of this event during the routine culture of the LH_M base population (see LH_M culturing protocol above). After the last mating trial on day 14, females were immediately separated from males under brief CO_2 anaesthesia (<2 min) and put into individual egg-laying vials where eggs were deposited for a period of 6 h. After this time the females were removed and the eggs chilled to arrest development, and then counted. The normal egg-laying period (18 h) was abbreviated to 6 h in order to reduce the risk of females becoming sperm depleted if they had recently remated with an XO male on day 14. A comparison between 6- and 18-h fecundities of females from the LH_M base population demonstrated that a 6-h egg-laying period comprises 69.3 ± 4.6% (mean ± SE) of total egg deposition, so we believe that our measures are an accurate index of lifetime fecundity. To assure that all females were actually fertilized, they were transferred from the oviposition vials to an additional set of individual egg-laying vials, which were checked for the presence of larvae after 3 days. Ten of 1248 assayed females (0.8%) died or were lost in the course of the experiment and 39 females (3.1%) were infertile. Both groups of females were subsequently removed from further analysis. The remating assay was replicated five times. The experiment was carried out over five replicates with four vials of 16 females per treatment per replicate. Two vials were lost, leading to a total sample size of $n = 78$ vials.

Although we strove to make the experimental conditions match as much as possible the normal culturing protocol to which the LH_M base population was adapted, some departures were necessary during the experiments: (1) females were exposed to mature males (XY and XO at a sex ratio of 1.5 males per female) for only 2 h day^{-1} on days 10–14 (instead of continuous exposure at this time to XY males at a one-to-one sex ratio – this was necessary to enable us to reliably count all matings), (2) female oviposition occurred individually, at one female per test tube for 6 h, as opposed to groups of 16 females in a in vial for 18 h – this was necessary to track the individual fecundity of each female. Despite these differences we think that the environment was sufficiently similar to that of the base population to enable us to explore the costs to females of multiple mating in this microcosm.

The experimental treatments changed the timing of receipt of sperm by females and this might influence female fecundity. A control was carried out to test this possibility. The control assay had an identical experimental design, except that females were not exposed to any males on days that females in the main experiment were exposed to XO males, and males in the control were all 12 days (post-egg deposition) old when used on the 2 days of exposure. Sample sizes for the control were eight vials of 16 females for each of the four experimental treatments (32 vials total), with one vial being lost during the experiment in treatment 1.

Analysis

The total number of matings per vial was calculated by using a 15-min 'sliding-window' across each 2-h mating period. Mating in *D. melanogaster* averages approximately 18 min and rarely exceeds 20 min (Grant, 1983; Singh & Singh, 2004). We scored total mating pairs in each vial every 5 min. By sliding a 15-min window across the counts of matings – starting at the first 5-min count and continuing across all 24 successive counts – and only adding new matings to the tally if they were separated by more than 15 min, we were able to discern new vs. old matings and sum the total number of matings. Scoring matings when there are many matings pairs in a vial was difficult because the pairs were motile. To accommodate this difficulty, we conservatively did not count a mating pair if there was any ambiguity as to whether it had been previously counted.

All statistical analysis was done using JMP version 5 (SAS Institute, Cary, NC, USA). When we carried out a Pearson's product–moment correlation between number of matings and lifetime fecundity of females we observed that the variance of the number of matings increased with its magnitude. To insure that our *P*-value was robust to this departure from the underlying assumptions of the statistical correlation model, we also carried out a non-parametric bootstrap analysis. The 31 bivariate data points were randomly sampled with replacement 10 000 times, and for each resampled data set the product–moment correlation coefficient was calculated. The bootstrap *P*-value was determined by the value of α at which a symmetrical $100(1 - \alpha)\%$ confidence interval [estimated by the interior $100(1 - \alpha)\%$ of the bootstrapped correlation values] overlapped zero (Manly, 1991).

To measure the effect of successive matings on female lifetime fecundity, we carried out a polynomial regression of ln(female lifetime fecundity) vs. number of matings. Curvilinearity of the regression was tested by the statistical significance of a quadratic term over and beyond the linear regression. Remating number was controlled experimentally by varying the order in which females were exposed to XO males (spermless and infertile) and XY males (with sperm and fertile), as described above. Nearly all the variation in female mating number was determined by this experimental treatment, but there was random variation in mating number around the experimentally controlled values. Least-squares regression remains valid when the average value of an independent variable is experimentally controlled and there is random variation about these values (Neter *et al.*, 1985, pp. 166–167).

Results

Although females in all our experimental treatments received the same number of hours of exposure to sperm-less (XO; 6 h) and intact (XY; 4 h) males over the course of the experiment, females in lower treatment numbers received sperm earlier and mated less often (Fig. 2; average number of matings were 0.96, 2.03, 2.44 and 3.15 for treatments 1–4 respectively; two-way ANOVA, treatment, $F_{3,12} = 277.06$, $P_{\text{treatment}} < 0.0001$; replicate, $F_{4,12} = 4.43$, $P_{\text{replicate}} = 0.01$; interaction, $F_{12,58} = 0.85$ $P_{\text{treatment} \times \text{replicate}} = 0.6$). The lack of a significant interaction between treatment and replicate indicates that there was no measurable inconsistency among replicates of the experimental treatments on remating number. Although it was possible for an XO male to remate a female previously mated by an XY male, and displace its sperm, we observed nearly no remating during daily 2-h exposure to males once a female had mated with an XY male, in agreement with previous studies (Newport & Gromko, 1984).

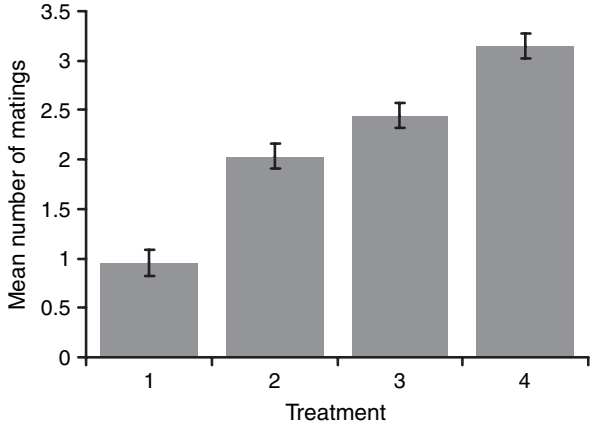


Fig. 2 Female mating numbers per treatment. The average number of matings that were observed in vials of 16 females including no pre-fertilization exposure to XO ales (treatment 1) to increasing amounts of prefertilization exposure to XO males (treatments 2–4). Data points are vial averages.

To evaluate the association between the number of matings and fecundity of females, we tested for a correlation between these variables. Our measure of fecundity (egg production during the first 6 h of the 18-h oviposition phase of the lifecycle) is an useful index of lifetime fecundity because it includes 69.3% of total reproductive output (see *Methods*). Because females experiencing the same environment (vial) are not stochastically independent data points, and because it was not possible to measure the number of times that each individual female mated (while we could measure the total number of female matings in each vial), we correlated vial averages of fecundity and mating number. There was a strong negative association between fecundity of females and the number of times that females mated ($r = 0.538$, $t_{76} = -5.56$, $P < 0.0001$; Fig. 3). Because the variance of the number of matings increased with its average value, we also carried out a nonparametric bootstrap analysis, which corroborated the significance of this correlation ($P < 0.0001$). To investigate the degree to which sequential rematings harmed females, we used polynomial regression of the logarithm of female fecundity against the number of matings per female (Fig. 4). This regression will be a straight line if the proportional cost to females is constant across sequential matings, but will be curvilinear (i.e. quadratic) when this cost significantly changes with additional matings. We found a significant negative quadratic term from this regression ($F_{1,75} = 4.88$, $P = 0.03$), and its significance markedly increased ($F_{1,74} = 7.90$, $P = 0.006$) after a single outlier point was removed from the analysis (this point fell far outside the 95% confidence bounds for the regression function; see Fig. 4). The steeper than log-linear regression between fecundity and remating level indicates that each subsequent remating has proportionately larger harm to females.

One possible explanation for the negative association between remating level and fecundity is that the timing of fertilization influences female fecundity. Although we observed nearly no matings after females received sperm from intact (XY) males, the time since mating might influence fertilization efficiency, e.g. female fecundity might differ if females were fertilized on day 10 of their 14-day life cycle compared with day 14, simply because of factors such as depletion of stored sperm. We tested for this possibility in a separate control experiment, which was identical to the main experiment, except that 'no males present' replaced the sperm-less males (XO) treatments, as described in the *Methods* section. In this case, all females received identical exposure to male behaviour and seminal fluid from intact males, and only the timing of fertilization was experimentally varied. Unlike the main experiment, no differences between

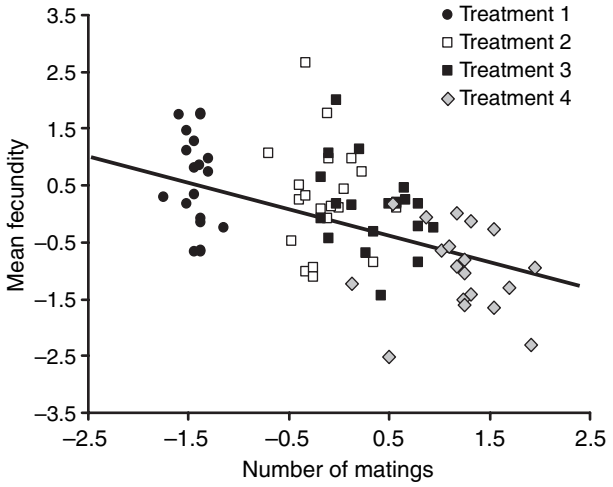


Fig. 3 Correlation between mean number of matings and female fecundity. There was a negative correlation ($P < 0.0001$) between the number of matings and female fecundity. Mean fecundity and number of matings are expressed as standardized values [(observed − sample mean)/sample standard deviation]. Data points are vial averages.

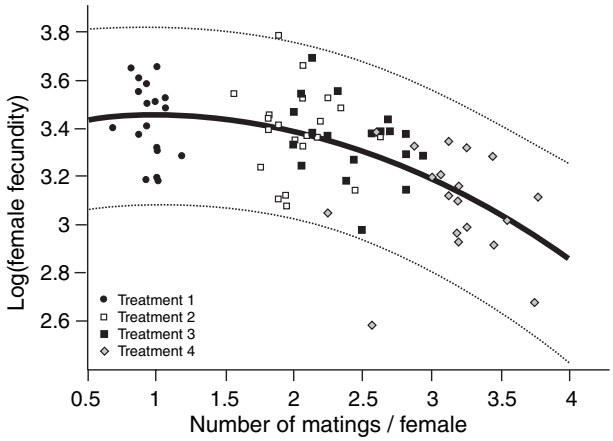


Fig. 4 Semi-log plot of mean fecundity vs. mean number of matings. There was a steeper than log-linear decay in log(female fecundity) as the number of matings increased. Data points are vial averages. The quadratic term was significantly negative ($P = 0.03$) and this significance increased ($P = 0.006$) when the extreme outlier was removed from the analysis.

treatments were observed (ANOVA, $F_{3,27} = 1.0146$, $P = 0.4016$); mean fecundities (±SE) were: 30.5 (±0.89), 26.8 (±1.25), 28.9 (±2.45) and 27.9 (±0.76) for treatments 1–4 respectively, indicating that the timing of exposure to intact (XY) males and thus the different timing of sperm reception by females had little or no influence on our results.

Discussion

Our results corroborate and extend the findings of previous research by showing that remating is costly to females when measured under environmental conditions that closely match those to which the population had adapted. Moreover, our results show that harm to females can be manifest in a manner that is neither restricted to declining survivorship nor to enriched food conditions, and which can be manifest in short-lived populations with discrete generations. Overall, our results in combination with previous studies (see *Introduction*), indicate that male-induced harm is manifest over widely different environmental and demographic conditions.

Previous studies of our locally adapted LH_M population found a 21.6 ± 0.25% (mean ± SE) decrease in the lifetime fecundity of females that was due to male–female interactions in the adult competition phase of their lifecycle (Rice *et al.*, 2006). The decrease in female fecundity is potentially due to (i) costly behavioural interactions with males (e.g. evading persistent male courtship by running, flying or kicking; Partridge & Fowler, 1990) and/or (ii) costs that are associated with the act of mating, which are due, in part, to the transfer of harmful seminal fluid (Chapman *et al.*, 1995, 2003b; Wigby & Chapman, 2005). The study described here quantifies the second component in our LH_M population by showing that there is a cost per mating after controlling for any nonmating effects. The estimated steeper-than-log-linear decline in fecundity indicates that, while a single remating has only a modest cost to females (−6.2%), the cost rapidly accelerates with additional rematings (−22.4% with two rematings and −48.5% with three rematings). Because there are no measurable countervailing direct (Chapman *et al.*, 1994; Pitnick *et al.*, 1997) or indirect (Orteiza *et al.*, 2005; Stewart *et al.*, 2005) benefits to offset these costs, these data indicate a substantial selection gradient on females to resist remating, a finding that was confirmed in another independent study (Linder & Rice, 2005).

Previous studies that assayed female mating numbers in the LH_M population during the adult competition stage showed that most females remate (Friberg *et al.*, 2005; Linder & Rice, 2005; Morrow *et al.*, 2005; Lew *et al.*, 2006), but that few females remate more than once (4.28–8.73%; Morrow *et al.*, 2005). Because females that remated once incur only an estimated 6.2% cost to fecundity, remating is estimated to account for far less than half of the net 21.6% reduction (Rice *et al.*, 2006) associated with all forms of male–female interactions. The low, but substantial, cost associated with a single remating indicates that most male-induced harm in our laboratory population is neither due to the act of mating, nor the receipt of seminal fluid proteins or pathogens during mating, but rather is due to persistent male courtship.

One of Cordero and Eberhard's major criticisms of past work demonstrating male-induced harm in laboratory populations of *D. melanogaster*, was that these laboratory conditions were too artificial to be extrapolated directly to wild populations. We agree. However, when conducting 'laboratory island analysis' the aim is not to directly extrapolate specific results to wild populations. Instead, the goal is to use these microcosms as locally adapted populations that are of scientific interest in-and-of-themselves, and which can be used to derive general evolutionary principles (Rice *et al.*, 2005, 2006). It is these empirically derived principles that can be applied back to wild populations of *Drosophila*, as well as other species.

The observation that, after hundreds of generations of coevolution, males continue to substantially harm females in a competitive laboratory environment indicates that sexual conflict is a feasible outcome of coevolution in wild populations. Data from microcosms can never prove that sexual conflict is occurring in any specific natural population, but such data can prove that the process is a tenable evolutionary outcome. More importantly, studies of microcosms can empirically test for general conditions under which sexually antagonistic – as opposed to mutualistic – coevolution will ensue. For example, studies of experimentally enforced life-long monogamy (Holland & Rice, 1999; Hosken *et al.*, 2001; Wigby & Chapman, 2004; Rice & Holland, 2005) provide support for the theoretical prediction that sexually antagonistic coevolution will be reversed with monogamy, and move towards mutualistic coevolution between the sexes.

Overall, this study indicates that the cost of remating increases with the number of rematings, and that remating can be costly to females in the currency of net fitness, and in the context of a short-lifespan demography. We have also shown that the cost of remating can be manifest in the currency of reduced daily fecundity, rather than the reduced survival that has been found in previous studies (Partridge *et al.*, 1986, 1987; Fowler & Partridge, 1989; Partridge & Fowler, 1990; Trevitt & Partridge, 1991; Chapman, 1992; Chapman *et al.*, 1993, 1994, 1995), and we have quantified the accelerating cost of each additional remating. Lastly, we agree with Cordero & Eberhard (2003) that it is impossible to directly extrapolate findings from laboratory populations to wild populations, because of the large differences in environmental conditions. However, when locally adapted, outbred laboratory populations are studied (laboratory island analysis), we think that meaningful evolutionary principles can be deduced from these microcosms, and that these principles can be applied to wild populations.

Acknowledgments

We thank J. McLean for technical help and W.G. Eberhard, U. Friberg and E.H. Morrow for helpful comments on earlier versions of this manuscript. We also thank Adam Chippindale for supplying the C(1;y) males and C(1)RM females. This work was supported by grants U.S. National Science Foundation (DEB-0128780 and DEB-0410112) to W.R.R. B.K. was supported by a topmaster grant and a Marco Polo travel grant from the University of Groningen.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

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Received 20 May 2006; accepted 29 May 2006