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No evidence that experimental manipulation of sexual conflict drives premating reproductive isolation in *Drosophila melanogaster*

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Abstract

Theoretical models predict that sexual conflict can drive reproductive isolation by decreasing the probability of matings between individuals from allopatric populations. A recent study in dung flies supported this prediction. To test the generality of this finding we used replicate lines of *Drosophila melanogaster* that had been selected under high, medium and low levels of sexual conflict, in which the females had evolved differences in their level of resistance to male-induced harm. We compared the proportion of virgin pairs that mated by set time points, for flies from the same replicate within each sexual conflict level vs. flies from different replicates within each sexual conflict level. The results did not support the prediction that, in *D. melanogaster*, sexual conflict drives population divergence via changes in female willingness to mate. The results were unlikely to be explained by differential inbreeding or by a lack of response to sexual conflict.

Introduction

Sexual conflict and reproductive isolation

Sexual conflict occurs when males and females have divergent evolutionary interests (Parker, 1979). Conflict can occur over male-female interactions such as courtship, mating frequency, time or place of mating, mate choice, fertilization, rate of female reproductive output, total female reproductive output, clutch size or parental care (reviewed in Chapman *et al.*, 2003; Arnqvist & Rowe, 2005). Sexual conflict can result in a reduction in fitness of individuals of one sex as a result of the effects of mating adaptations in individuals of the opposite sex (although at a population-wide level the fitness of males and females is equivalent). Individuals of the sex whose fitness is most reduced by the interaction are predicted to evolve traits to minimise the extent harm sustained (i.e. 'resistance' traits, Holland & Rice, 1998) if such traits result in a net fitness gain. If resistance by the harmed sex reduces the beneficial effects of the harming trait to the harming sex then theory predicts that there will be selection on individuals of the harming sex to overcome this resistance by increasing the level of their harming trait. Thus, sexual conflict may lead to sexually antagonistic coevolution in which members of each sex evolve traits that elevate their own fitness relative to other members of their same sex but at the expense of members of the opposite sex (Rice, 1996).

Theoretical models predict that such sexual conflict can lead to antagonistic coevolution between the sexes (e.g. Parker, 1979; Arak & Enquist, 1993, 1995; Holland & Rice, 1998; Wachtmeister & Enquist, 2000) and that this may facilitate reproductive isolation and speciation (Parker & Partridge, 1998; Rice, 1998; Gavrilets, 2000; Gavrilets *et al.*, 2001; Gavrilets & Waxman, 2002; Martin & Hosken, 2003b). For example, models show that sexual conflict over mating rate could lead to rapid coevolutionary change in reproductive traits in both sexes, promoting the formation of premating or post-mating (e.g. Reyer *et al.*, 1999) reproductive barriers, both in allopatric populations (Gavrilets, 2000) and in sympatry (Gavrilets & Waxman, 2002). Furthermore, Gavrilets' (2000) model predicts that reproductive isolation will evolve faster in large, high-density allopatric populations due to higher levels of sexual conflict in such

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populations. This contrasts with classical-population genetics theory, which predicts faster reproductive isolation by random genetic drift in small allopatric populations (Lande, 1981). Game theory models also suggest that sexual conflict could play a major role in speciation, although when populations meet after a period of allopatry 'females will act as a force favouring premating isolation and males as a force against it' (Parker & Partridge, 1998). Thus, the probability of speciation may depend upon whether the strength of selection is greater in males to force mating or in females to avoid mating and also upon which sex has the greater 'power' to enforce it's evolutionary interests (Parker & Partridge, 1998).

Several empirical studies have provided evidence that sexual conflict can drive the coevolution of antagonistic traits between the sexes (Rice, 1996; Holland & Rice, 1999; Knowles & Markow, 2001; Arnqvist & Rowe, 2002; Rowe & Arnqvist, 2002; Arnqvist & Rowe, 2005). However, fewer studies to date have provided empirical evidence for reproductive isolation through sexual conflict (Arnqvist *et al.*, 2000; Martin & Hosken, 2003b). Martin & Hosken (2003b) used experimental evolution to investigate the role of sexual conflict in the evolution of female resistance to male mating attempts in the dung fly *Sepsis cynipsea*. Three replicates of each of three treatments were set up: monogamous, low density and high density. At higher densities, the flies experienced increased levels of polyandry and therefore elevated sexual conflict (Martin & Hosken, 2003b). Martin & Hosken's (2003b) results showed that females that had evolved in higher density populations resisted male mating attempts significantly more than females that had evolved in lower density populations. This was consistent with the idea that the females had evolved resistance to males by decreasing their receptivity to mating. Females that evolved at high density also resisted mating attempts from males from different populations significantly more than they resisted mating attempts from same-population males. In contrast, the willingness to mate of monogamous females was independent of the origin of their mating partner. This is consistent with the prediction (Gavrilets, 2000) of higher divergence and hence stronger reproductive isolation in the higher density (higher sexual conflict), larger populations (Martin & Hosken, 2003b).

It is not yet clear whether increased divergence under strong sexual conflict is likely to be a general phenomenon (e.g. Parker & Partridge, 1998), or whether similar traits are likely to be involved across different species with different mating systems. To address these issues, we tested the prediction of greater premating reproductive isolation under high levels of sexual conflict, using *Drosophila melanogaster*. Sexual conflict in *D. melanogaster* stems from the higher optimum mating frequency for males as compared to females (Bateman, 1948). In addition, female *D. melanogaster* suffer reduced survival and lifetime reproductive success from mating at high frequencies (Fowler & Partridge, 1989). These mating costs and other male-imposed costs, such as courtship (Partridge & Fowler, 1990; Friberg & Arnqvist, 2003), are likely to be larger than any indirect benefits of multiple mating (Orteiza *et al.*, 2005; Stewart *et al.*, 2005). The cost of mating is mediated by male accessory gland proteins (Acps) transferred to females during mating in the seminal fluid (Chapman *et al.*, 1995). A major part of the female cost of mating is attributable to a single Acp, the sex peptide (Acp70A) (Wigby & Chapman, 2005). Female *D. melanogaster* should evolve to minimise male-induced harm via reduced mating frequencies or via physiological resistance to the harmful effects of the sex peptide or of other harmful Acps. This prediction is supported by results from experimental evolution studies (Holland & Rice, 1999; Wigby & Chapman, 2004), although the exact mechanism by which females respond is not yet known.

Testing sexual conflict predictions using experimental evolution in *Drosophila melanogaster*

Female *D. melanogaster* exhibit a variety of mating avoidance behaviours including taxis away from males, abdomen bending, ovipositor extension and kicking (Connolly & Cook, 1973). Females therefore have the potential to reduce the probability of copulation initiation. If *D. melanogaster* responds similarly to the dung flies studied by Martin & Hosken (2003b), then we predict that females that evolve under experimentally elevated levels of sexual conflict should show reduced willingness to mate. Furthermore, this could promote premating reproductive isolation if the willingness of females to mate with males from allopatric populations was lower than the willingness of females to mate with males from their own population. To test this prediction we used flies from the selection lines described in Wigby & Chapman (2004). In these selection lines the adult sex ratio was manipulated to alter the level of sexual conflict. There were three replicates each of male-biased (MB), female-biased (FB) and equal sex-ratio (ES) lines. These lines have been shown, during selection, to differ in male and female mating rates and hence in the intensity of sexual conflict (Wigby & Chapman, 2004). Female mating frequency and sexual conflict was highest in the MB lines, intermediate in the ES lines and lowest in the FB lines (Wigby & Chapman, 2004). Females responded to the differences in the levels of sexual conflict by evolving differences in the level of resistance to male-induced harm (Wigby & Chapman, 2004).

To test the hypothesis that sexual conflict promotes premating reproductive isolation in *D. melanogaster*, we measured, for pairs of virgin flies, the time from introduction until mating in our MB, FB and ES lines. We assumed that the time until mating would be longer in pairings in which females were less willing to mate.

Pairs of flies consisting of a male and female from the same replicate and same treatment ('within' replicate pairs) and pairs consisting of a male and female from different replicates of the same selection treatment ('between' replicate pairs) were tested. We predicted that females from lines evolving under high levels of sexual conflict should show reduced willingness to mate (presumably to avoid the costs of mating) relative to females from lines evolving under lower levels of sexual conflict. In addition, if sexual conflict leads to increased divergence in premating traits then females from high sexual conflict lines should show a lower willingness to mate with males from different replicates compared to males from their own replicate, than females from low sexual conflict lines. This is based on the idea that there may be fitness benefits (e.g. increased genetic compatibility and/or fertility) for females in mating with males from the same replicate as opposed to mating with males from other replicates (from which they may have genetically diverged, Martin & Hosken, 2003b). Thus, under the hypothesis that increased sexual conflict leads to increased reproductive isolation we predicted that MB females would be the least willing to mate and would show stronger resistance to mating in between-replicate, as compared to within-replicate matings. FB females on the other hand were predicted to be most willing to mate and show the least difference in resistance to mating in between- vs. within-replicate matings.

Methods

Manipulating sexual conflict by experimental evolution

The wild-type stock was originally collected in Dahomey (now Benin) in 1970 and has been maintained since then in the laboratory in four replicate cages held at large population size. To initiate the selection lines, eggs were collected from all four population cages, to provide a genetically varied population. Flies were then randomly drawn from this population for allocation to each of the three selection regimes. The selection protocol is as described in Wigby & Chapman (2004). Briefly, three replicate lines each of male biased (MB, 75 males and 25 females), equal sex (ES, 50 males and 50 females), and female biased (FB, 25 males and 75 females) sex ratio treatments were set up (i.e. nine lines in total). Each line was maintained in a plastic cage with a gauze-covered top. Flies had access to water and were fed every 2 or 3 days with two vials of sugar-yeast (SY) food (e.g. Wigby & Chapman, 2004) with added live yeast in excess. After 9 days of selection, eggs were collected from each cage to propagate the next generation. We estimate that this period represents approximately one-third to one half of the average lifespan of the selected females (e.g. see Wigby & Chapman, 2004). Three hundred first instar larvae of each line were picked and raised at standard density and, to minimize selection on development time, all adults were allowed to eclose over 2 days before being allocated to the same sex-ratio treatment and replicate number as their parents. At the time of the experiments the lines had undergone selection for 41 generations.

Willingness to mate of female virgins from the selection lines

To measure the willingness of females to mate we measured the time taken for pairs of virgin flies to begin mating, and to test for differences between treatments we compared the proportion of pairs that had mated at set time points. Eggs from all selection lines were collected over a 16-hour period. One thousand first instar larvae from each replicate of each selection treatment were placed into SY food vials in batches of 100. Standard density culturing minimized environmentally derived differences in body size arising from competition for food between larvae and from variation in food quality arising from contamination with larval faeces. One hundred virgin females and 100 virgin males from each replicate of each selection treatment were collected using ice anaesthesia and flies were placed into SY vials with added live yeast granules. Females were housed individually and males were housed in batches of five. Four days after eclosion, single males from the same ('within') or different ('between') replicate populations of the same selection treatment were added, without anaesthesia, to the vials containing single females. Fifty within-replicate vials and 50 between-replicate vials were created for each replicate (e.g. for replicate MB1: within replicate mating, 50 MB1 females × 50 MB1 males; between replicate mating, 25 MB1 females × 25 MB2 males + 25 MB1 males × 25 MB3 females). To avoid problems caused by pseudoreplication we limited the number of between replicate comparisons (Martin & Hosken, 2003a). For between-replicate crosses, individuals of each sex were paired with only one of the two possible other replicates (i.e. the between-replicate crosses in each treatment consisted of replicate one females × replicate two males + replicate two females × replicate three males + replicate three females × replicate one males). This made a total of three within- and three between-replicate crosses for each treatment. The time (to the nearest minute) that it took for each pair to begin mating and the number of matings were recorded for 1 h after the flies were placed together.

Statistical analysis

The normality and homogeneity of variances of all raw data and residuals from models were checked by Shapiro-Wilk (Shapiro & Wilk, 1965) and Bartlett's tests (Zar, 1999). To maximise the probability of finding differences between the lines, we analysed the proportion of pairs of flies that had mated or were still mating at the time point

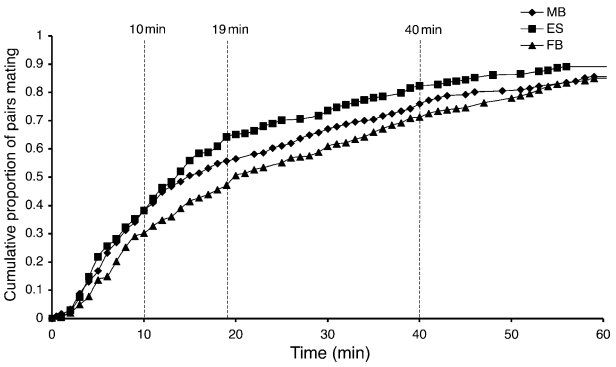

Fig. 1 Proportion of pairs that had mated or were still mating, over time (replicates and mating types (within- and between-replicates) combined). The greatest divergence between any two treatments occurred 19 min after placing flies together. The other time points analysed were 10 and 40 min.

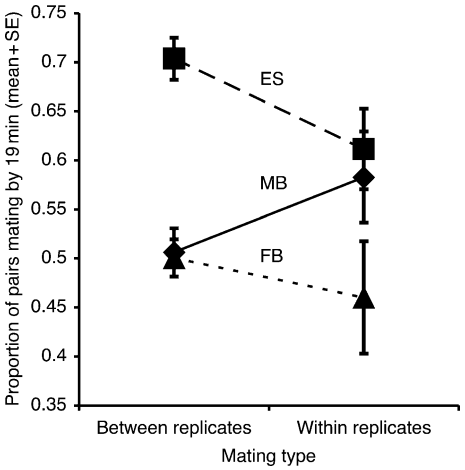

Fig. 2 Mean proportion of pairs that had mated or were still mating after 19 min (±SE) in within- and between-replicate pairings.

at which the greatest deviation between treatments occurred, i.e. at 19 min following introduction (Fig. 1). However, to ensure that the results were not contingent upon this one time point of maximum divergence, we also analysed the proportion of pairs of flies that had mated or were still mating at 10 min and at 40 min. The proportion of pairs mating at 10, 19 and 40 min out of all pairs that were placed together were compared between mating types (within- and between-replicates) and between treatments (MB, ES and FB) using a two-way ANOVA (Zar, 1999). To compare between pairs of treatments, one-way ANOVAS (Zar, 1999) were performed followed by Student-Newman-Keuls (SNK) tests (Zar, 1999). To determine accurate probabilities from Q-values for SNK tests, the software program 'R' (Ihaka & Gentleman, 1996) was used.

Results

At 10 min there were no significant differences in the proportion of matings between selection treatments (MB, ES and FB) or between mating types (within- and between-replicate pairings), and no significant interaction between selection treatment and mating type (selection treatment, $F_{2,12} = 0.92$, $P = 0.43$; mating type, $F_{1,12} = 0.007$, $P = 0.93$; selection treatment × mating type: $F_{2,12} = 1.95$, $P = 0.19$). There were significant differences in the proportion of matings at 19 min between selection treatments but no significant differences between mating types and no significant interaction between selection treatment and mating type (selection treatment, $F_{2,12} = 11.30$, $P = 0.002$; mating type, $F_{1,12} = 0.36$, $P = 0.56$; selection treatment × mating type: $F_{2,12} = 2.62$, $P = 0.11$; Fig. 2). A significantly higher proportion of ES pairs mated at 19 min than FB pairs ($\bar{X}$ ± SE, ES = 0.658 ± 0.029, FB = 0.480 ± 0.028, $Q_{3,15} = 6.13$, $P = 0.0006$) and MB pairs ($\bar{X}$ ± SE, MB = 0.545 ± 0.029, $Q_{2,15} = 3.91$, $P = 0.014$). There was no significant difference in the proportion of pairs mating at 19 min between MB and FB treatments ($Q_{2,15} = 2.22$, $P = 0.14$). The pattern of results for 40 min was the same as at 19 min: there were significant differences in the proportion of matings at 40 min between selection treatments but no significant differences between mating types and no significant interaction between selection treatment and mating type (selection treatment, $F_{2,12} = 4.77$, $P = 0.03$; mating type, $F_{1,12} = 2.20$, $P = 0.16$; selection treatment × mating type: $F_{2,12} = 1.57$, $P = 0.25$). A significantly higher proportion of ES pairs mated at 40 min than FB pairs ($\bar{X}$ ± SE, ES = 0.812 ± 0.022, FB = 0.711 ± 0.022, $Q_{3,15} = 3.74$ $P = 0.046$) and MB pairs ($\bar{X}$ ± SE, MB = 0.724 ± 0.036, $Q_{2,15} = 3.24$, $P = 0.037$). There was no significant difference in the proportion of pairs mating at 40 min between MB and FB treatments ($Q_{2,15} = 0.50$, $P = 0.73$).

The prediction from sexual conflict theory was that the proportion of flies mating would be highest in FB pairs followed by ES pairs and lowest in MB pairs. The results did not conform to this pattern and show that differences in the level of sexual conflict did not predict the observed pattern of differences in mating probability. Furthermore, flies in all treatments did not show a higher proportion of matings in within-replicate pairings as compared to between-replicate pairings, at any time point analysed. Thus, there was no evidence for increased divergence in high conflict lines relative to low conflict lines.

Discussion

The pattern of the proportion of pairs mating at any of the three times tested was not as predicted by sexual conflict. The proportion of mating pairs from lines that

evolved under high levels of sexual conflict was not lower than the proportion of mating pairs from lines that evolved under lower levels of sexual conflict. Also, the proportion of pairs mating was not higher in within-replicate crosses than in between-replicate crosses in any selection treatment and at any time point measured. Hence, there was no indication that high levels of sexual conflict led to increased divergence in premating traits between replicate populations in the trait measured, in contrast to the results obtained in *S. cynipsea* (Martin & Hosken, 2003b). Our data provides no support for the hypothesis that sexual conflict drives reproductive isolation via female willingness to mate in *D. melanogaster*.

One explanation for the difference in the two studies is that increased sexual conflict may not inevitably lead to increased divergence in these particular traits. The nature of pre and post-mating selection arising from sexual conflict in the two species (*S. cynipsea* and *D. melanogaster*) may be very different. The results of this study show that *D. melanogaster* females may not be able to decrease the level of harm inflicted upon them by evolving decreased willingness to mate. Instead, these results support the interpretation that the evolved female resistance to male-induced harm may be primarily via post-mating responses that decrease the harmful effects of Acps (Chapman *et al.*, 1995; Wigby & Chapman, 2005) or responses that decrease the harmful effects of courtship (Partridge & Fowler, 1990; Friberg & Arnqvist, 2003). Although insufficient numbers of generations of selection or insufficient strength of selection cannot be excluded as potential explanations for the lack of divergence in the predicted directions, these explanations seem unlikely given that differences in female resistance to male-induced harm evolved rapidly between treatments and were apparent after 18 and 22 generations of selection (Wigby & Chapman, 2004). The previous findings of divergence in the lines (Wigby & Chapman, 2004) and the fact that the flies were derived from a population that had adapted to the laboratory environment for over 30 years, also render it unlikely that any naturally selected responses to the new selection environments could have masked adaptations to the variation in sexual conflict.

It is possible that examining the proportion of pairs mating by certain time points might not accurately reflect the overall willingness of females to mate. For example, if males differed between treatments in their ability to gain matings (e.g. if they differed in the intensity or frequency of courtship) this could confound this measure of female willingness to mate. However, males from these same selection lines, when housed with wild-type females did not differ between treatments in their courtship frequency (Wigby & Chapman, 2004). This suggests that there were no differences in courtship frequency attributable to the selection line males. Nevertheless, in the same experiments there were differences in mating frequency between treatments (Wigby & Chapman, 2004). This suggests that there is some difference between selection treatments, aside from courtship frequency, that causes differences in the ability of males to gain mates or differences in the ability to reduce female receptivity. Further work is required to examine these possibilities.

Female preferences for within-replicate males could also occur because of genetic drift (Lande, 1981). However, this scenario would not predict that preferences for within-replicate males should be stronger in high conflict lines than in low conflict lines. The outcomes predicted by drift and by sexual conflict are therefore distinct and in fact neither is supported by the pattern of results from this study.

One factor that could potentially affect our measure of female willingness to mate is inbreeding, which has a negative impact on male mating ability (Sharp, 1984). The highest effective population size (Wright, 1938) was in the ES lines ($N_e = 100$). MB and FB lines both had a lower effective population size ($N_e = 75$ for both). However, the variance in mating success (and therefore reproductive success) between males in a population tends to exceed the variance in reproductive success between females (e.g. Joshi *et al.*, 1999). Thus, the MB lines are likely to be more inbred than the FB lines because the MB lines contain the fewest and the FB lines the most females. If males in FB and MB lines were less effective at courtship due to inbreeding then this could explain why more ES pairs had mated than MB or FB pairs at 19 and 40 min, but it cannot explain the lack of differences between the MB and FB treatments. When wild-type females were continuously housed with selection line males in a previous study, no differences in the courtship frequency of males were detected between treatments, and the mating frequency of ES males was found to be significantly lower than that of MB and FB males (Wigby & Chapman, 2004). This suggests that our finding here that there was a higher proportion of ES pairs mating at 19 and 40 min was a result of higher willingness to mate in ES females and not because ES males were more successful at mating or less inbred than MB and FB males. The effective population size, N_e, is for an ideal population in which all individuals contribute offspring to the next generation. The breeding population, which is ultimately important, is not known for our selected flies, as it is difficult to measure owing to unknown variance in reproductive success among individuals. Differential inbreeding cannot therefore be completely excluded as an explanation for our results.

Virgin flies from the selection lines were used in the assays in this study. It may be that the willingness to mate of virgin females does not vary between treatments but that females vary between treatments in their resistance to subsequent matings. However, the fitness of females during selection depended on their on their ability to produce fertile eggs approximately 10 days

after eclosion. Therefore, there will have been little benefit, and potentially large costs, for females in mating too soon after eclosion. This study used 4-day-old virgin males and females and 4 days is almost half the duration of the adult phase of the selection regime. Therefore, one potential avenue for future research would be to assay the willingness to mate of pairs of more recently eclosed flies (e.g. 24 h after eclosion) when there might be more pronounced differences between treatments.

In our previous work, when selection line females were housed continuously with wild-type males, no differences in mating frequency were detected (Wigby & Chapman, 2004). Our finding here that 4-day-old virgin females did not vary between treatments in their willingness to remate is therefore consistent with the mating frequency results from Wigby & Chapman (2004). Together, these results provide no evidence for evolved differences between treatments in the willingness of virgin or nonvirgin females to mate. This supports findings from other *D. melanogaster* selection lines (described in Holland & Rice, 1999) in which females selected under higher levels of sexual conflict did not evolve reduced mating frequencies (Pitnick *et al.*, 2001).

The body sizes of flies from the selection lines were previously measured after 32 generations of selection (Wigby & Chapman, 2004) and no differences were detected between treatments in either sex. Given this result and the fact that selected and experimental flies were raised at standard densities, the results of this study are unlikely to be confounded by size-related variation among males (such as differences in courtship intensity, or female preference for large males, e.g. Partridge & Farquhar, 1983; Partridge *et al.*, 1987a,b). However, the parents of the experimental flies experienced different environmental conditions during selection, which could have resulted in variation between treatments arising from differential maternal effects. Furthermore, the possibility that there were unidentified differences in male behaviour or morphology between treatments that could have affected the results, or that body size differences had evolved between generations 32 and 41, cannot be excluded.

Conclusions

Our results provide no evidence that sexual conflict had a predictable effect on the evolution of female willingness to mate or that sexual conflict drove population divergence via changes in female willingness to mate in our *D. melanogaster* selection lines. The proportion of pairs of flies that had mated or were still mating at 10, 19 and 40 min after introduction did not differ in within- or between-replicate matings. These results suggest that premating female mate discrimination is unlikely to be a universal mechanism by which sexual conflict may drive reproductive isolation. Alternative mechanisms by which sexual conflict could drive reproductive isolation are through variation in female post-mating responses such as egg production, egg fertility, refractory period and response to male-induced harm. These traits have the potential to affect the proportion of offspring produced from within-population relative to between-population pairings and thus could be mechanisms by which reproductive isolation evolves.

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References

Arak, A. & Enquist, M. 1993. Hidden preferences and the evolution of signals. *Philos. Trans. R. Soc. London B* **340**: 207–213.

Arak, A. & Enquist, M. 1995. Conflict, receiver bias and the evolution of signal form. *Philos. Trans. R. Soc. London B* **349**: 337–344.

Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. 2000. Sexual conflict promotes speciation in insects. *Proc. Natl. Acad. Sci. USA* **97**: 10460–10464.

Arnqvist, G. & Rowe, L. 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature* **415**: 787–789.

Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton University Press, Princeton, New Jersey.

Bateman, A.J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349–368.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003. Sexual conflict. *Trends Ecol. Evol.* **18**: 41–47.

Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F. & Partridge, L. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**: 241–244.

Connolly, K. & Cook, R. 1973. Rejection responses by female *Drosophila melanogaster*: their ontogeny, causality and effects upon the behaviour of the courting male. *Behaviour* **44**: 142–167.

Fowler, K. & Partridge, L. 1989. A cost of mating in female fruitflies. *Nature* **338**: 760–761.

Friberg, U. & Arnqvist, G. 2003. Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. *J. Evol. Biol.* **16**: 797–811.

Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**: 886–889.

Gavrilets, S., Arnqvist, G. & Friberg, U. 2001. The evolution of female mate choice by sexual conflict. *Proc. R. Soc. London B* **268**: 531–539.

Gavrilets, S. & Waxman, D. 2002. Sympatric speciation by sexual conflict. *Proc. Natl. Acad. Sci. USA* **99**: 10533–10538.

Holland, B. & Rice, W.R. 1998. Chase-away sexual selection: antagonistic seduction *versus* resistance. *Evolution* **52**: 1–7.

Holland, B. & Rice, W.R. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl. Acad. Sci. USA* **96**: 5083–5088.

Ihaka, R. & Gentleman, R. 1996. R: A language for data analysis and graphics. *J. Comp. Graph. Stat.* **5**: 299–314.

Joshi, A., Do, M.H. & Mueller, L.D. 1999. Poisson distribution of male mating success in laboratory populations of *Drosophila melanogaster*. *Genet. Res.* **73**: 239–249.

Knowles, L.L. & Markow, T.A. 2001. Sexually antagonistic coevolution of a postmating-prezygotic reproductive character in desert *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98**: 8692–8696.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA* **78**: 3721–3725.

Martin, O.Y. & Hosken, D.J. 2003a. Costs and benefits of evolving under experimentally enforced polyandry or monogamy. *Evolution* **57**: 2765–2772.

Martin, O.Y. & Hosken, D.J. 2003b. The evolution of reproductive isolation through sexual conflict. *Nature* **424**: 979–982.

Orteiza, N., Linder, J.E. & Rice, W.R. 2005. Sexy sons from remating do not recoup the direct costs of harmful male interactions in the *Drosophila melanogaster* laboratory model system. *J. Evol. Biol.* **18**: 1315–1323.

Parker, G.A. 1979. Sexual selection and sexual conflict. In: *Sexual Selection and Reproductive Competition in Insects* (M. S. Blum & N. A. Blum, eds), pp. 123–166. Academic Press, New York.

Parker, G.A. & Partridge, L. 1998. Sexual conflict and speciation. *Philos. Trans. R. Soc. London B* **353**: 261–274.

Partridge, L., Ewing, A. & Chandler, A. 1987a. Male size and mating success in *Drosophila melanogaster* – the roles of male and female behavior. *Anim. Behav.* **35**: 555–562.

Partridge, L. & Farquhar, M. 1983. Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. *Anim. Behav.* **31**: 871–877.

Partridge, L. & Fowler, K. 1990. Nonmating costs of exposure to males in female *Drosophila melanogaster*. *J. Insect Physiol.* **36**: 419–425.

Partridge, L., Hoffmann, A. & Jones, J.S. 1987b. Male size and mating success in *Drosophila melanogaster* and *Drosophila pseudoobscura* under field conditions. *Anim. Behav.* **35**: 468–476.

Pitnick, S., Brown, W.D. & Miller, G.T. 2001. Evolution of female remating behaviour following experimental removal of sexual selection. *Proc. R. Soc. Lond. B* **268**: 557–563.

Reyer, H.U., Frei, G. & Som, C. 1999. Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. *Proc. R. Soc. London B* **266**: 2101–2107.

Rice, W.R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**: 232–234.

Rice, W.R. 1998. Intergenomic conflict, interlocus antagonistic coevolution and the evolution of reproductive isolation. In: *Endless Forms Species and Speciation* (D. J. Howard & S. H. Berlocher, eds), pp. 261–270. Oxford University Press, UK.

Rowe, L. & Arnqvist, G. 2002. Sexually antagonistic coevolution in a mating system: combining experimental and comparative approaches to address evolutionary processes. *Evolution* **56**: 754–767.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* **52**: 591–611.

Sharp, P.M. 1984. The effect of inbreeding on competitive male mating ability in *Drosophila melanogaster*. *Genetics* **106**: 601–612.

Stewart, A.D., Morrow, E.H. & Rice, W.R. 2005. Assessing putative interlocus sexual conflict in *Drosophila melanogaster* using experimental evolution. *Proc. R. Soc. London B* **272**: 2029–12035.

Wachtmeister, C.A. & Enquist, M. 2000. The evolution of courtship rituals in monogamous species. *Behav. Ecol.* **11**: 405–410.

Wigby, S. & Chapman, T. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution* **58**: 1028–1037.

Wigby, S. & Chapman, T. 2005. Sex peptide causes mating costs in female *Drosophila melanogaster*. *Curr. Biol.* **15**: 316–321.

Wright, S. 1938. Size of population and breeding structure in relation to evolution. *Science* **87**: 430–431.

Zar, J.H. 1999. *Biostatistical Analysis*. Prentice-Hall, NJ, USA.

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