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# Harm to females increases with male body size in *Drosophila melanogaster*

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Previous studies indicate that female *Drosophila melanogaster* are harmed by their mates through copulation. Here, we demonstrate that the harm that males inflict upon females increases with male size. Specifically, both the lifespan and egg-production rate of females decreased significantly as an increasing function of the body size of their mates. Consequently, females mating with larger males had lower lifetime fitness. The detrimental effect of male size on female longevity was not mediated by male effects on female fecundity, egg-production rate or female-remating behaviour. Similarly, the influence of male size on female lifetime fecundity was independent of the male-size effect on female longevity. There was no relationship between female size and female resistance to male harm. Thus, although increasing male body size is known to enhance male mating success, it has a detrimental effect on the direct fitness of their mates. Our results indicate that this harm is a pleiotropic effect of some other selected function and not an adaptation. To the extent that females prefer to mate with larger males, this choice is harmful, a pattern that is consistent with the theory of sexually antagonistic coevolution.

**Keywords:** sexual selection; sexual conflict; remating; longevity; body size; fecundity

## 1. INTRODUCTION

Bigger is generally better when it comes to reproduction. Larger females are almost universally more fecund (Stearns 1992; Roff 2002) and are often preferred by males (Andersson 1994). For males, larger size typically confers an advantage in intra-sexual competition for mates and, to the extent that females exert a choice of mates, 'bigger is better' is again considered to be the general outcome (Darwin 1871; Andersson 1994).

Recently, the evolution of traits that contribute to differential male reproductive success in species where males provide no direct benefits to females or their young is being re-considered with respect to sexually antagonistic coevolution. For example, the function of male ornaments may not be to honestly signal male quality to females, but rather to coerce or manipulate females into acting in ways that benefit the males' reproductive interests to the detriment of the females' fitness (Parker 1979; Rice 1996, 1998, 2000; Gowaty 1997; Gowaty & Buschhaus 1998; Holland & Rice 1998; Gavrilets *et al.* 2001). Owing to the fact that male and female reproductive interests are only confluent under strict genetic monogamy, sexual conflict is expected to be widespread (Rice 2000).

As with any 'arms race', sexually antagonistic coevolution is expected to cycle. Any evolutionary advantage gained by one sex will intensify selection on the other sex to evolve analogous counteradaptations (Chapman & Partridge 1996*a*; Rice 1996). At any point in time, therefore, one sex may be 'winning' or the balance of power may be at equilibrium. The degree of male armament, for example, across species will thus not be expected to correlate with the extent of harm to females (Arnqvist & Rowe 2002*a*,*b*; Rowe & Arnqvist 2002). Within a population at any time, however, as male size and ornamentation increases, male manipulation of female reproductive biology, and consequently male-induced harm to females, should increase. This relationship may exist irrespective of where the balance of power resides in the coevolutionary struggle. The influence of body size or extent of ornamentation of males with which females mate on the direct lifetime fitness of females, in species where males provide no direct benefits to their mates, has received little attention (Andersson 1994).

Here, we examine the effects of male size on direct female lifetime fitness in *Drosophila melanogaster*. Empirical studies of the reproductive biology of this species have played a central part in the recent development of sexual conflict theory (Fowler & Partridge 1989; Chapman *et al.* 1995; Rice 1996; Holland & Rice 1998; Pitnick *et al.* 2001*a*,*b*). Male *D. melanogaster* modify female behaviour and physiology in various ways that have been interpreted as possible examples of sexually antagonistic coevolution. Copulation has been demonstrated to

(i) reduce female receptivity to courting males;
(ii) increase the egg-production rate; and
(iii) decrease female lifespan.

Owing to the fact that both prior and potential future mates of females attempt to manipulate them, these interactions and their consequences for female fitness are complex.

Prior mates, via seminal fluid proteins, reduce female receptivity to courting males (Chen 1984; Chen *et al.* 1988; Aigaki *et al.* 1991; Kalb *et al.* 1993) and increase their egg-production rate (Kalb *et al.* 1993; Herndon & Wolfner 1995; Heifetz *et al.* 2000). At the same time, 'future mates' coerce females into mating more frequently

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than is optimal from the females' perspective (Fowler & Partridge 1989). The net consequences of all these interactions are a decrease in female lifespan (Partridge *et al.* 1987*a*; Fowler & Partridge 1989; Chapman *et al.* 1993, 1995; Rice 1996; Civetta & Clark 2000) and lifetime progeny production (Chapman *et al.* 1993, but see Chapman & Partridge 1996*b*). It has been convincingly demonstrated that the reduction in female lifespan resulting from exposure to males is a consequence of harmful effects of both male courtship and seminal fluid (Partridge *et al.* 1987*b*; Partridge & Fowler 1990; Chapman 1992; Chapman *et al.* 1995; Lung *et al.* 2002). It is not a consequence of receiving and storing sperm (Chapman *et al.* 1993).

The aim of the present study was to examine the roles of male and female size in mediating sexual conflict. Relationships between males' size and their influence upon female egg production, remating frequency and longevity were examined. In addition, the relationship between females' size and their ability to resist male manipulation and any male-size by female-size interaction effects were examined.

## 2. MATERIAL AND METHODS

Using the laboratory strain Oregon R of *D. melanogaster*, phenotypic variation in male and female body size was generated by varying larval density, as body size is known to decrease with increasing density (Atkinson 1979; Wilkinson 1987). To set up the rearing vials, *ca*. 100 adult flies were placed on oviposition plates containing a cornmeal–molasses–agar medium and a paste of live yeast. Soon after hatching of the resulting eggs began, the first-instar larvae from these plates were transferred using a pin to eight dram glass shell vials each containing 50 ml of media and live yeast. Numerous vials for each of three larval densities: 25, 75 and 150 were set up. Virgin adult males and females from these vials were collected a few hours after eclosion. As an index of total body mass (Robertson & Reeve 1952; Pitnick & Markow 1994), the thorax length of each fly was measured after anaesthetization with $CO_2$. Flies were then placed in food vials specific to each sex and size measurement, with sizes separated by discrete units of 0.0125 mm. The variation in body size generated was presumed to be primarily environmental rather than genetic in nature.

For this experiment, 100 females were chosen that represented the full range of body sizes (thorax lengths, 0.825–1.062 mm). Females were then assigned, in order of increasing body size, an identification number. These females were then designated to receive as their mates either a 'small', 'medium' or 'large' male (although the exact size of males was known; range, 0.724–0.981 mm) as follows: female no. 1: small male; female no. 2: medium male; female no. 3: large male; female no. 4: small male; etc. Throughout their lives, each female was exposed to males of nearly identical body size (the mean size of all males copulated with was determined for each female). Thus, the full range of interaction between varying female and male sizes was achieved.

The experiment began when the virgin females were 4 days of age and continued until all females were dead (95 days). All females were exposed to a similar 4-day cycle. On day 0 of the cycle, each female was placed in a fresh food vial with two males (both males of identical size class) and left for 5 h. Males remained in vials with females for the full 5 h, irrespective of whether copulation occurred. On rare occasion, females were observed to copulate twice in a single morning ($N = 21$, compared with $N = 689$ single matings); all copulations were counted equally in tallying female-remating frequency and mean mate size. After removing males, females remained in these vials overnight and then were switched to fresh vials on both days 1 and 2. Females remained in this latter vial for 2 days, after which they were transferred to a fresh vial containing two males to begin the next cycle. All eggs laid in the day 0 and 1 vials were counted. Thus, all egg numbers reported probably represent more than 50% of each female's productivity (oviposition rate declines with time following remating, see e.g. Kalb *et al.* (1993)). Regression analysis of data from a preliminary experiment with an extensive range of male sizes (thorax lengths, 0.787 – 0.950 mm) indicated that female productivity during the first 2 days was a robust indicator of productivity over 4 days ($F_{1,152} = 618.00$, $r^2 = 0.804$, $p < 0.0001$) and that adding male body size to the analysis did not significantly improve the regression model (new $r^2 = 0.805$, male thorax $t = -0.9$, $p = 0.34$). Thus, there was no relationship between male size and the pattern of egg allocation by females within the 4-day cycle.

Males were used for three successive cycles and then discarded. For each new cycle, male pairs were rotated among the females, such that potential mates for each female were replaced by different males of approximately equal size. Thus, the females had the opportunity to mate with new males in every cycle. The exact size of males supplied to each female each cycle was recorded. New rearing vials of the three standard larval densities were set up every 12 days to ensure a continuous supply of males throughout the experiment.

For statistical analyses, male size was determined for each female as the mean size of all of the males that she copulated with. Both lifetime egg production and lifetime number of mates were calculated. In addition, number of eggs laid and number of matings in the first 20 days of the experiment were analysed. These latter variables were used to determine male influence on egg production and remating behaviour early in the females' lives and without the confounding influence that male effects on female lifespan have on total egg production and mating opportunity. A period of twenty days was chosen, prior to data analysis, as this was the latest interval for which no females had yet died. Relationships between the independent variables (male and female size) and the dependent variables (egg production, remating frequency and female lifespan) were investigated with a combination of univariate and multivariate statistical analyses using SAS (SAS Institute, Inc. 1989). The sample size for all analyses was $N = 94$ females; one female was lost during the experiment, one was injured and four females were excluded from the analyses because they produced no eggs (inclusion of these females did not qualitatively influence the results).

Owing to the breeding design, there was no statistical relationship between female size and the size of males with which they copulated ($r = 0.06$, $p = 0.54$; table 1). Moreover, initial multivariate regression analyses of all dependent variables revealed that in no case were male size by female size interaction effects statistically significant and that addition of this interaction term never improved model fit. This interaction effect was subsequently excluded from all analyses presented here. The statistical analyses first examined the univariate relationships between continuous variation in male size or female size and female lifespan, fecundity and remating frequency. Pearson's correlation coefficients among all the dependent and independent variables are presented in table 1. Multivariate analyses

Table 1. A matrix of the Pearson's correlation and Pearson's partial correlation coefficients.
(The table shows the simple correlation coefficients (Pearson's correlation coefficients; upper values), Pearson's partial correlation coefficients (upper values) and *p* values (lower values) for the dependent variables: female lifespan, female lifetime fecundity and female-remating frequency (lifetime number of mates) and the independent variables: male body size and female body size.)

| | simple correlations | | | | partial correlations | |
|---|---|---|---|---|---|---|
| | male size | female size | remating | fecundity (lifetime) | male size | female size |
| female lifespan | −0.3184<br>0.0017 | 0.1096<br>0.2902 | 0.5482<br>0.0001 | 0.6345<br>0.0001 | −0.0771<br>0.4626 | −0.1254<br>0.2312 |
| female fecundity (lifetime) | −0.4016<br>0.0001 | 0.3552<br>0.0004 | 0.6446<br>0.0001 | | −0.0297<br>0.0297 | 0.3898<br>0.0001 |
| female fecundity (20 days) | −0.1984<br>0.0539 | 0.4556<br>0.0001 | 0.5070<br>0.0001 | | −0.2051<br>0.0474 | 0.4682<br>0.0001 |
| female remating frequency (lifetime) | −0.3035<br>0.0028 | 0.1072<br>0.3011 | | | −0.0441<br>0.6748 | −0.1382<br>0.1864 |
| female remating frequency (20 days) | −0.0215<br>0.8364 | 0.0579<br>0.5771 | | | 0.0572<br>0.5841 | −0.1341<br>0.1975 |
| female size | 0.0636<br>0.5402 | | | | | |

were then used to examine the simultaneous effects of male and female size on female reproductive biology in an attempt to discern underlying causation for certain patterns observed in this correlational study. Partial correlation coefficients were calculated to examine the relationships between male and female size and each of the three dependent variables: female lifespan, female lifetime fecundity and female-remating frequency, while controlling for the effects of the remaining two dependent variables (table 1). Partial correlations of productivity in the first 20 days of the experiment held remating over these 20 days constant, and vice versa. Due to multicollinearity among all of the dependent variables (table 1), however, there were limitations on the extent to which variation in female traits could be successfully partitioned among the multiple variables.

## 3. RESULTS

There was a highly significant negative relationship between female lifespan and the mean body size of their mates (figure 1*a*; table 1). The partial correlation between female lifespan and male size was not significant (table 1). However, this statistical outcome is difficult to interpret and is presumed to be a statistical artefact of multicollinearity between all of the dependent variables (Steel & Torrie 1990). It makes no biological sense to conclude that reduced longevity of females is mediated through male effects on remating or egg production. First, females paired with larger males did not remate at a higher rate. Also, females paired with larger males produced fewer eggs (figure 1*b*). It is conceivable, however, that females mating with larger males increased their egg-production rate for a short time and that this 'ramping up' of productivity decreases both lifetime egg productivity and lifespan. To test this hypothesis, we examined the relationships between the mean number of eggs produced per day and male size over three time-scales: the first two days of the experiment, the first 20 days and the lifetime of females. Significant relationships were found for the latter two time periods (table 2) that were consistent with the analysis of male effects on productivity. More importantly, these relationships were consistently negative, indicating that females mated to larger males at no time increased their egg-production rate, thereby reinforcing the interpretation that larger males differentially harmed females by reducing their lifespan, independent of the harm to fecundity. The relationship between female size and female lifespan was positive but not significant (table 1).

Both male and female sizes were highly correlated with the total number of eggs produced by females. The relationship between male body size and female productivity, however, was positive for females and negative for males (figure 1*b*; table 1). Thus, the direct lifetime fitness of females declined as the size of their mates increased. Evidence that the effect of male size on female lifetime productivity was not simply mediated through the negative effect of male size on female lifespan is twofold. First, there was a significant negative partial correlation between male size and female productivity, while holding constant the effects of the variables lifespan and remating (table 1). Second, there was a negative relationship between the number of eggs laid by females during the first 20 days of the experiment, before any female died, and male size. The correlation for this relationship was marginally nonsignificant and the partial correlation was marginally significant (table 1).

There was also a highly significant negative relationship between male size and the number of times that females mated over their lifetime (figure 1*c*, table 1). This relationship, however, was not due to any influence of male size on female-remating rate. Rather, it resulted from females mated to larger males dying sooner and consequently having fewer opportunities to remate. Again, evidence for this conclusion is twofold. First, the partial correlation between female remating and male size was not significant (table 1), although this interpretation could be erroneous

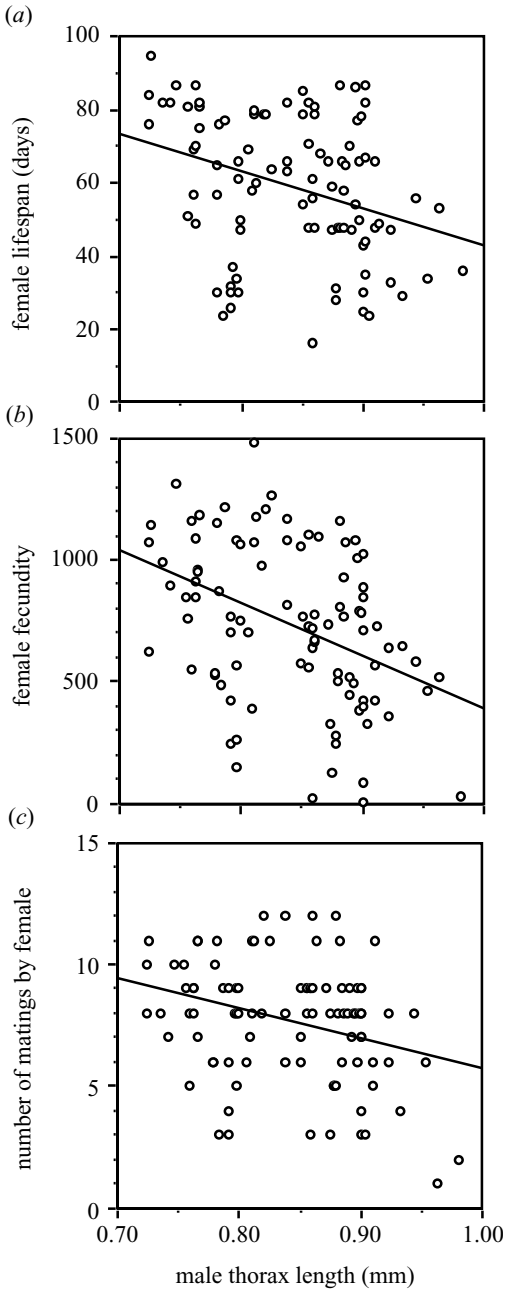


Figure 1. Relationships between (*a*) female lifespan, (*b*) lifetime fecundity (eggs counted on two of every four days; see § 2) and (*c*) the number of times females mated, and the mean body size of the males with which the females mated. Lines show the fit by least-squares linear regression.

due to the effects of multicollinearity. Second, there was no significant relationship between male size and the number of times that females mated in the first 20 days of the experiment (table 1). There was no significant relationship between the number of times that the females mated and female size (table 1).

## 4. DISCUSSION

In *D. melanogaster*, whose reproductive biology has been extensively studied in both the laboratory and the wild, body size is a key predictor of male reproductive success (reviewed in Partridge 1988). Of particular importance is the positive relationship between male size and mating success (Ewing 1961, 1964; Partridge & Farquhar 1983; Partridge *et al.* 1987*a*,*c*; Markow 1987*a*, 1988). This relationship is at least partially attributable to the advantages that larger males have in male–male competition (Dow & von Schilcher 1975; Hoffmann 1987, 1991; Partridge *et al.* 1987*a*,*c*). A contribution of female discrimination among potential mates to patterns of nonrandom mating in general (Iliadi *et al.* 2001), and discrimination based on male size in particular, is far more conjectural for *D. melanogaster*. Some authors have interpreted their data as showing a mating advantage of larger males 'as a purely male effect, with no involvement of female choice' (Partridge *et al.* 1987*c*; see also Partridge *et al.* 1987*a*; Wilkinson 1987), whereas other authors have indicated a more active role for females (Markow 1987*a*,*b*; Pitnick 1991). To the extent that female choice contributes to the enhanced mating success of larger males in *D. melanogaster*, the negative relationship between male size and female fitness identified in the current study indicates that females prefer males that are harmful to them. Such a pattern would be consistent with the theory of sexually antagonistic coevolution for male courtship traits and female resistance to such traits (Holland & Rice 1998). If female choice for male size is not important in this species, then our results simply indicate that a trait favoured by sexual selection on males is detrimental to some components of female fitness. The lack of female size by male size interaction effect in all analyses indicates that the mechanisms underlying male harm to females (e.g. the candidate toxic seminal fluid protein, Acp62F, with protease inhibitor activity (Lung *et al.* 2002)) does not differentially impact females according to their size. Similarly, with regard to male influence on female lifespan, any putative mechanisms of female resistance to male harm were not size-dependent.

It is also important to note for the current study that, although sexual conflict was clearly identified, only the consequences for direct female fitness were examined. It is possible that females mating with relatively large males, despite producing relatively few progeny, realize relatively high net fitness through the production of more fecund daughters and 'sexy sons' (Parker 1979; Weatherhead & Robertson 1979) and thus the production of relatively many grandchildren. However, given the magnitude of the direct costs to females of mating with larger males identified here, it seems unlikely that these costs could be outweighed by indirect, genetic benefits (Møller & Alatalo 1999). In this context, we obviously assume that genetic variation in male size would exhibit similar relationships with female fecundity and longevity. This important assumption remains to be tested. Nevertheless, body-size variation in nature will largely be environmentally determined in *Drosophila* (see Coyne & Beecham 1987; Weigensberg & Roff 1996 and references therein) and so direct effects on female fitness of phenotypic variation in male size identified here will be relevant to selection in natural populations. One final caveat pertains to the history of the population used in this study. Oregon R is a laboratory strain of *D. melanogaster* that has certainly been subject to different selective pressures from flies in nature: sperm competition is probably more intense and there is weaker selection on late-life traits (see Sgrò & Partridge 2000). However, U. Friberg and G. Arnqvist (unpublished

Table 2. The results of multiple-regression analyses.
(The dependent variables were the egg-production rate (examined over 2 days, 20 days and the female lifetimes) and the independent variables were the female and male body sizes.)

| | overall model | | | female size | | male size | |
|---|---|---|---|---|---|---|---|
| egg-production rate | $r^2$ | $F_{2,94}$ | $p$ | $t$ | $p$ | $t$ | $p$ |
| 2 days | 0.234 | 14.06 | $< 0.0001$ | 5.30 | $< 0.0001$ | $-0.19$ | 0.85 |
| 20 days | 0.260 | 16.12 | $< 0.0001$ | 5.23 | $< 0.0001$ | $-2.54$ | $< 0.02$ |
| lifetime | 0.186 | 10.50 | $< 0.0001$ | 3.82 | $< 0.001$ | $-2.77$ | $< 0.01$ |

data) have simultaneously and independently conducted similar experiments using a more 'natural' laboratory strain of *D. melanogaster* (Dahomey stock; maintained in the laboratory for over 30 years, but in mass culture in population cages with overlapping generations); they obtained qualitatively similar results with regard to the male-size effect on female lifespan, indicating that at least some of the patterns reported here are robust to replication with other material.

The observed negative relationship between male size and female lifetime fecundity confirms and extends an earlier report of this relationship for *D. melanogaster* based on single-mate productivity (Pitnick 1991). This pattern has also been noted for the yellow dung fly *Scathophaga stercoraria* (Martin & Hosken 2002) and the water strider *Gerris incognitus* (Arnqvist *et al.* 1997). Irrespective of the net effects on female fitness, size-dependent harm to females will contribute to antagonistic selection on male size (Parker 1979). It has been presumed up to this point that sexual selection favours larger size in *D. melanogaster* and that viability selection provides the stabilizing selection on body size (Wilkinson 1987). Only recently has it been recognized that post-copulatory sexual selection can antagonistically select on male size (Gage 1995; Danielsson 2001). Our results demonstrate that at least one component of sexual selection contributes to antagonistic selection on size in *D. melanogaster*.

The decline in female lifespan associated with the increasing size of their mates was presumably mediated through male ejaculate toxicity or courtship (Partridge *et al.* 1987*b*; Partridge & Fowler 1990; Chapman 1992; Lung *et al.* 2002); no discrimination between these two modes of action is possible with the experimental design here employed. The effect on lifespan obviously was not a result of male influence on egg production, as females mated to larger males both produced fewer eggs and died sooner. The decline in female longevity was also clearly independent of the female-remating rate, as there was no relationship between male size and female-remating frequency in the first 20 days of the experiment and, across their lifetimes, females paired with larger males remated fewer times (a consequence of dying sooner). These patterns are consistent with genetic analysis of variation in the detrimental effects of males upon female longevity in *D. melanogaster* (Sawby & Hughes 2001) and with patterns observed in other taxa (Chapman *et al.* 1998).

The lack of influence of male size on the female-remating rate fails to support an earlier claim that females are more likely to remate when courted by larger males (Pitnick 1991). In the previous study (Pitnick 1991), however, females were tested daily for their first remating rather than every fourth day for life, and this may account for the disparity. An independent study of fitness consequences to female *D. melanogaster* of mate size by U. Friberg and G. Arnqvist (unpublished data) did find a significant positive relationship between male size and female mating rate. This difference may be attributable to differing experimental designs, as in the U. Friberg and G. Arnqvist (unpublished data) study females were continuously exposed to males. We presume that the lack of a relationship between male size and female-mating frequency in our study renders the other results more conservative, as a positive relationship between male size and mating frequency would have magnified any additive harmful effects of male size on female lifespan and productivity.

It has been presumed by most investigators that the male-induced decline in female longevity in *D. melanogaster* is a by-product of the male seminal product's selected function, that of mediating sperm competition (Chapman *et al.* 1995; Rice 1996; Holland & Rice 1999; Civetta & Clark 2000; Lung *et al.* 2002). A positive association between male success in sperm competition and the level of harm to females has been demonstrated both experimentally (Rice 1996) and through a comparative study of the correlation between male sperm competitive ability and female death rate among chromosome-extracted lines (Civetta & Clark 2000). A theoretical model, however, has indicated otherwise (Johnstone & Keller 2000). If the cumulative damage of multiple mating has an accelerating impact on female fitness, it was argued, then seminal toxins may be an adaptation, rather than a pleiotropic effect, by which males can induce females to delay or avoid remating (Johnstone & Keller 2000). Our results do not support this contention. The Johnstone & Keller (2000) model would predict that if males harm functions to delay female remating, then there should be a positive relationship between the extent of harm and the female-remating interval. Although we found a strong positive relationship between male size and harm, there was no relationship between male size and female remating during the first 20 days or over the lifetime, after controlling for longevity. Females exposed to greater harm did not delay remating longer than females exposed to less harm. As mentioned above, U. Friberg and G. Arnqvist (unpublished data) observed a positive relationship between male size and female remating, thus reinforcing our interpretation of the Johnstone & Keller (2000) model.

Ironically, while this study provides strong confirmation of the extent to which interactions between the sexes involves conflict in *D. melanogaster*, it also raises concerns

about the interpretation of one of the most important experimental demonstrations of sexually antagonistic coevolution in this species. Holland & Rice (1999) removed sexual selection through enforced monogamy with random mate assignment in two replicate lines of *D. melanogaster* and then, after 47 generations, compared the reproductive biology of the monogamy-line flies with those from paired, promiscuous control lines. Three principal results were attributed to the removal of sexual antagonistic coevolution:

(i) monogamy-line males evolved to be more benign to females;
(ii) monogamy-line females evolved lowered resistance to male-induced harm; and
(iii) sexual selection places a load on populations.

Results of the current and other published studies on the effects of body size raise doubts about all three of the conclusions of Holland & Rice (1999).

Selection in the experiment of Holland & Rice (1999) was generated by collecting all flies for the subsequent generation on day 1 of their culturing protocol (see Holland & Rice (1999) for a detailed description); all flies eclosing on subsequent days were discarded. Any male-induced harm that diminished early female productivity would thus be selected against. This protocol inadvertently subjected all lines to strong selection for rapid development time, for which only the monogamy lines were free to respond due to the removal of sexual selection (male–male competition favours larger males in *D. melanogaster*). Monogamy-line flies consequently evolved to be significantly smaller (genetically large flies take longer to develop; Robertson 1960) than control-line flies (Pitnick *et al.* 2001*b*). With this in mind, and given that Holland & Rice (1999) did not statistically control for body-size differences between lines in any analyses, re-evaluation of the results of the study of Holland & Rice (1999) is warranted.

First, Holland & Rice (1999) found that monogamy-line males evolved to be more benign to females. Specifically, both the survival and the net reproductive rate of 'test' females was greater when paired with monogamy males compared with control males. The current study demonstrates that these results could be due to the smaller size of monogamy males.

Second, Holland & Rice (1999) found that monogamy-line females evolved lowered resistance to male-induced harm. Specifically, monogamy-line females died faster than control-line females when housed continuously with control-line males. Although the current study found no significant relationship between female size and longevity (the relationship was positive) or resistance to male harm, studies have overwhelmingly found significant positive relationships between size and longevity for both females and males of *D. melanogaster* and related species (Robertson 1957; Tantawy & Vetukhiv 1960; Tantawy & Rakha 1964; Tantawy & El-Helw 1966; Partridge & Farquhar 1983; Partridge *et al.* 1986). Thus, monogamy-line females may have died faster because they were smaller, not because they were less resistant to male-induced harm.

Third, Holland & Rice (1999) found that sexual selection places a load on populations. Specifically, the net reproductive rate of the monogamy populations was greater than that of the controls. This experiment was conducted under the same conditions for which selection had been imposed—all progeny not eclosing by the end of day 1 of the experiment were not included in this assay. However, Holland & Rice (1999) also determined for this experiment the fraction of the total adult progeny that was available for collection on day 1. They report that the total number of surviving progeny did not differ significantly between lines and that the greater reproductive rate of the monogamy lines was due to a significantly faster development rate (again, smaller flies result from faster development). It may be argued that increased male size represents the proximate basis by which sexual selection places a load on populations. The relevance of this interpretation to sexually antagonistic coevolution requires careful consideration, however, because evidence indicates that the evolutionary divergence between the Holland & Rice (1999) lines in body size resulted from the removal of male–male competition rather than from post-copulatory interaction between the sexes (Pitnick *et al.* 2001*b*).

In conclusion, female fitness is significantly influenced by variation in the size of their mates. This result cautions that models of sexual selection might benefit from inclusion of costs to females of mate choice that escalate with increased male displays (Houle & Kondrashov 2002). Although a positive relationship between male size and mating success is widespread throughout the animal kingdom, the prevalence of male harm to females is not known. Sexually antagonistic coevolution may frequently generate a trade-off between the detrimental effects of 'preferred' mates upon the females' direct fitness and the beneficial effects of mates upon their indirect fitness. To the extent that females are able to choose their mates, the balance between direct and indirect fitness effects (Kirkpatrick 1985, 1996; Curtsinger & Heisler 1988, 1989; Heisler & Curtsinger 1990; Kirkpatrick & Ryan 1991; Pomiankowski *et al.* 1991; Kirkpatrick & Barton 1997; Møller & Alatalo 1999) will determine where females draw the evolutionary line and stabilize selection on male size or ornamentation.

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## REFERENCES

Aigaki, T., Fleischmann, I., Chen, P.-S. & Kubli, E. 1991 Ectopic expression of sex peptide alters reproductive behavior of female *Drosophila melanogaster*. *Neuron* **7**, 557–563.

Andersson, M. 1994 *Sexual selection*. Princeton University Press.

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

Arnqvist, G. & Rowe, L. 2002*b* Correlated evolution of male and female morphologies in water striders. *Evolution* **56**, 936–947.

Arnqvist, G., Thornhill, R. & Rowe, L. 1997 Evolution of animal genitalia: morphological correlates o fitness components in a water strider. *J. Evol. Biol.* **10**, 613–640.

Atkinson, W. D. 1979 A field investigation of larval competition in domestic *Drosophila*. *J. Anim. Ecol.* **48**, 91–102.

Chapman, T. 1992 A cost of mating with males that do not transfer sperm in female *Drosophila melanogaster*. *J. Insect Physiol.* **38**, 223–227.

Chapman, T. & Partridge, L. 1996*a* Sexual conflict as fuel for evolution. *Nature* **381**, 189–190.

Chapman, T. & Partridge, L. 1996*b* Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. *Proc. R. Soc. Lond.* B **263**, 755–759.

Chapman, T., Hutchings, J. & Partridge, L. 1993 No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males. *Proc. R. Soc. Lond.* B **253**, 211–217.

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.

Chapman, T., Miyatake, T., Smith, H. K. & Partridge, L. 1998 Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. *Proc. R. Soc. Lond.* B **265**, 1879–1894. (DOI 10.1098/rspb.1998.0516.)

Chen, P. S. 1984 The functional morphology and biochemistry of insect male accessory glands and their secretions. *A. Rev. Entomol.* **29**, 233–255.

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M. & Bohlen, P. 1988 A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell* **54**, 291–298.

Civetta, A. & Clark, A. G. 2000 Correlated effects of sperm competition and postmating female mortality. *Proc. Natl Acad. Sci. USA* **97**, 13 162–13 165.

Coyne, J. A. & Beecham, E. 1987 Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics* **117**, 727–737.

Curtsinger, J. W. & Heisler, I. L. 1988 A diploid 'sexy son' model. *Am. Nat.* **132**, 439–453.

Curtsinger, J. W. & Heisler, I. L. 1989 On the consistency of sexy-son models: a reply to Kirkpatrick. *Am. Nat.* **134**, 978–981.

Darwin, C. 1871 *The descent of man, and selection in relation to sex*. London: Murray.

Danielsson, I. 2001 Antagonistic pre- and post-copulatory sexual selection on male body size in a water strider (*Gerris lacustris*). *Proc. R. Soc. Lond.* B **268**, 77–81. (DOI 10.1098/rspb.2000.1332.)

Dow, M. A. & von Schilcher, F. 1975 Aggression and mating success in *Drosophila melanogaster*. *Nature* **254**, 511–512.

Ewing, A. W. 1961 Body size and courtship behaviour in *Drosophila melanogaster*. *Anim. Behav.* **9**, 93–99.

Ewing, A. W. 1964 The influence of wing area on the courship behaviour of *Drosophila melanogaster*. *Anim. Behav.* **12**, 316–320.

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

Gage, M. J. G. 1995 Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. Lond.* B **261**, 25–30.

Gavrilets, S., Arnqvist, G. & Friberg, U. 2001 The evolution of female mate choice by sexual conflict. *Proc. R. Soc. Lond.* B **268**, 531–539. (DOI 10.1098/rspb.2000.1382.)

Gowaty, P. A. 1997 Sexual dialectics, sexual selection, and variation in reproductive behavior. In *Feminism and evolutionary biology* (ed. P. A. Gowaty), pp. 351–384. New York: Chapman & Hall.

Gowaty, P. A. & Buschhaus, N. 1998 Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis, and social monogamy. *Am. Zool.* **38**, 207–225.

Heifetz, Y., Lung, O., Frongillo, E. A. & Wolfner, M. F. 2000 The *Drosophila* seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. *Curr. Biol.* **10**, 99–102.

Heisler, I. L. & Curtsinger, J. W. 1990 Dynamics of sexual selection in diploid populations. *Evolution* **44**, 1164–1176.

Herndon, L. A. & Wolfner, M. F. 1995 A *Drosophila* seminal fluid protein, Acp26Aa, stimulates egg laying in females for 1 day after mating. *Proc. Natl Acad. Sci. USA* **92**, 10 114–10 118.

Hoffmann, A. A. 1987 A laboratory study of male territoriality in the sibling species *Drosophila melanogaster* and *D. simulans*. *Anim. Behav.* **35**, 807–818.

Hoffmann, A. A. 1991 Heritable variation for territorial success in field-collected *Drosophila melanogaster*. *Am. Nat.* **138**, 668–679.

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.

Houle, D. & Kondrashov, A. S. 2002 Coevolution of costly mate choice and condition-dependent display of good genes. *Proc. R. Soc. Lond.* B **269**, 97–104. (DOI 10.1098/rspb.2001.1823.)

Iliadi, K., Iliadi, N., Rashkovetsky, E., Minkov, I., Nevo, E. & Korol, A. 2001 Sexual and reproductive behaviour of *Drosophila melanogaster* from a microclimatically interslope differentiated population of 'Evolution Canyon' (Mount Carmel, Israel). *Proc. R. Soc. Lond.* B **268**, 2365–2374. (DOI 10.1098/rspb.2001.1822.)

Johnstone, R. A. & Keller, L. 2000 How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am. Nat.* **156**, 368–377.

Kalb, J. M., DiBenedetto, A. J. & Wolfner, M. F. 1993 Probing the function of *Drosophila melanogaster* accessory glands by directed cell ablation. *Proc. Natl Acad. Sci. USA* **90**, 8093–8097.

Kirkpatrick, M. 1985 Evolution of female choice and male parental investment in polygynous species: the demise of the 'sexy son'. *Am. Nat.* **125**, 788–810.

Kirkpatrick, M. 1996 Good genes and direct selection in the evolution of mating preferences. *Evolution* **50**, 2125–2140.

Kirkpatrick, M. & Barton, N. H. 1997 The strength of indirect selection on female mating preferences. *Evolution* **94**, 1282–1286.

Kirkpatrick, M. & Ryan, M. J. 1991 The evolution of mating preferences and the paradox of the lek. *Nature* **350**, 33–38.

Lung, O., Tram, U., Finnerty, C. M., Eipper-Mains, M. A., Kalb, J. M. & Wolfner, M. F. 2002 The *Drosophila melanogaster* seminal fluid protein Acp62F is a protease inhibitor that is toxic upon ectopic expression. *Genetics* **160**, 211–224.

Markow, T. A. 1987*a* Genetic and sensory basis of sexual selection in *Drosophila*. In *Evolutionary genetics of invertebrate behavior* (ed. M. D. Huettel), pp. 89–95. New York: Plenum.

Markow, T. A. 1987*b* Behavioral and sensory basis of courtship success in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **84**, 6200–6204.

Markow, T. A. 1988 Reproductive behavior of *Drosophila*

*melanogaster* and *D. nigrospiracula* in the field and in the laboratory. *J. Comp. Psychol.* **102**, 169–173.
Martin, O. Y. & Hosken, D. J. 2002 Asymmetry and fitness in female yellow dung flies. *Biol. J. Linn. Soc.* **76** (In the press).
Møller, A. P. & Alatalo, R. V. 1999 Good-genes effect in sexual selection. *Proc. R. Soc. Lond.* B **266**, 85–91. (DOI 10.1098/rspb.1999.0607.)
Parker, G. A. 1979 Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (ed. M. S. Blum & N. A. Blum), pp. 123–166. New York: Academic.
Partridge, L. 1988 Lifetime reproductive success in *Drosophila*. In *Reproductive success: studies of individual variation in contrasting breeding systems* (ed. T. H. Clutton-Brock), pp. 11–23. University of Chicago Press.
Partridge, L. & Farquhar, M. 1983 Lifetime mating success of male fruit flies (*Drosophila melanogaster*) is related to their size. *Anim. Behav.* **31**, 871–877.
Partridge, L. & Fowler, K. 1990 Non-mating costs of exposure to males in female *Drosophila melanogaster*. *J. Insect Physiol.* **36**, 419–425.
Partridge, L., Fowler, K., Trevitt, S. & Sharp, S. 1986 An examination of the effects of males on the survival and egg-production rates of female *Drosophila melanogaster*. *J. Insect Physiol.* **32**, 925–929.
Partridge, L., Ewing, A. & Chandler, A. 1987*a* Male size and mating success in *Drosophila melanogaster*: the roles of male and female behaviour. *Anim. Behav.* **35**, 555–562.
Partridge, L., Green, A. & Fowler, K. 1987*b* Effects of egg-production and of exposure to males on female survival in *Drosophila melanogaster*. *J. Insect Physiol.* **33**, 745–749.
Partridge, L., Hoffmann, A. & Jones, J. S. 1987*c* Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. *Anim. Behav.* **35**, 468–476.
Pitnick, S. 1991 Male size influences mate fecundity and remating interval in *Drosophila melanogaster*. *Anim. Behav.* **41**, 735–745.
Pitnick, S. & Markow, T. A. 1994 Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. *Proc. Natl Acad. Sci. USA* **91**, 9277–9281.
Pitnick, S., Brown, W. D. & Miller, G. T. 2001*a* Evolution of female remating behaviour following experimental removal of sexual selection. *Proc. R. Soc. Lond.* B **268**, 557–563. (DOI 10.1098/rspb.2000.1400.)
Pitnick, S., Miller, G. T., Reagan, J. & Holland, B. 2001*b* Males' evolutionary responses to experimental removal of sexual selection. *Proc. R. Soc. Lond.* B **268**, 1071–1080. (DOI 10.1098/rspb.2001.1621.)
Pomiankowski, A., Iwasa, Y. & Nee, S. 1991 The evolution of costly mate preferences. I. Fisher and biased mutation. *Evolution* **45**, 1422–1430.
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 evolution, and the evolution of reproductive isolation. In *Endless forms: species and speciation* (ed. D. J. Howard & S. H. Berlocher), pp. 261–270. Oxford University Press.
Rice, W. R. 2000 Dangerous liaisons. *Proc. Natl Acad. Sci. USA* **97**, 12 953–12 955.
Robertson, F. W. 1957 Studies in quantitative inheritance. XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. *J. Genet.* **55**, 428–443.
Robertson, F. W. 1960 The ecological genetics of growth in *Drosophila*. I. Body size and development time on different diets. *Genet. Res.* **1**, 288–304.
Robertson, F. W. & Reeve, E. C. R. 1952 Studies in quantitative inheritance. I. The effects of selection for wing and thorax length in *Drosophila melanogaster*. *J. Genet.* **50**, 416–448.
Roff, D. A. 2002 *Life history evolution*. Sunderland, MA: Sinauer.
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.
SAS Institute, Inc. 1989 *SAS user's guide: statistics*, v. 5. Cary, NC: SAS Institute, Inc.
Sawby, R. & Hughes, K. A. 2001 Male genotype affects female longevity in *Drosophila melanogaster*. *Evolution* **55**, 834–839.
Sgrò, C. M. & Partridge, L. 2000 Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *Am. Nat.* **156**, 341–353.
Stearns, S. C. 1992 *The evolution of life histories*. Oxford University Press.
Steel, R. G. D. & Torrie, J. H. 1990 *Principles and procedures of statistics: a biometrical approach*, 2nd edn. New York: McGraw-Hill.
Tantawy, A. O. & El-Helw, M. R. 1966 Studies on natural populations of *Drosophila*. V. Correlated response to selection in *Drosophila melanogaster*. *Genetics* **53**, 97–110.
Tantawy, A. O. & Rakha, F. A. 1964 Genetic variances of and correlations between four characters in *D. melanogaster* and *D. simulans*. *Genetics* **50**, 1349–1355.
Tantawy, A. O. & Vetukhiv, M. O. 1960 Effects of size on fecundity, longevity and fertility in populations of *Drosophila pseudoobscura*. *Am. Nat.* **94**, 395–403.
Weatherhead, P. J. & Robertson, R. J. 1979 Offspring quality and the polygyny threshold: the 'sexy son' hypothesis. *Am. Nat.* **113**, 201–208.
Weigensberg, I. & Roff, D. A. 1996 Natural heritabilities: can they be reliably estimated in the laboratory? *Evolution* **50**, 2149–2157.
Wilkinson, G. 1987 Equilibrium analysis of sexual selection in *Drosophila melanogaster*. *Evolution* **41**, 11–21.

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