Evolutionary Ecology Research, 2005, **7**: 633–641

Natural selection favours harmful male *Drosophila melanogaster* that reduce the survival of females

Timothy A. Lew and William R. Rice*

Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106-9610, USA

ABSTRACT

Question: Is there a net selective advantage for alleles that increase male reproductive performance while harming females through pleiotropy.

Organisms: Outbred *Drosophila melanogaster*.

Methods: Both short-term and continuous exposure of females to males.

Conclusions: As male net fitness increased, so too did male harm to female survival. Male-induced harm to females is genetically variable and under directional selection for increased harm.

Keywords: inter-locus contest evolution, sexual conflict, sexual selection, sexually antagonistic co-evolution.

INTRODUCTION

The evidence that males of some species harm their mates comes from many taxa, including birds [Aves (McKinney *et al.*, 1983)], crickets [Orthoptera (Dean, 1981; Burpee and Sakaluk, 1993)], cockroaches [Blattodea (Moore *et al.*, 2001)], cotton strainers and water striders [Hemiptera (Kasule, 1986; Arnqvist, 1989; Rowe, 1994)], beetles [Callosobruchus (Crudgington and Siva-Jothy, 2000)] and flies [Diptera (Cohet and David, 1976; Fowler and Partridge, 1989; Chapman *et al.*, 1998)]. The most extensive body of work documenting this harm focuses on the *Drosophila melanogaster* laboratory model system. Experiments have demonstrated that *D. melanogaster* males harm their mates through both their behaviour and seminal fluid (Fowler and Partridge, 1989; Chapman *et al.*, 1995; Rice, 1996; Holland and Rice, 1999; Civetta and Clark, 2000; Sawby and Hughes, 2000), and that harm can be manifest through reduced survival, fertility and/or fecundity of females (Pyle and Gromko, 1978; Trevitt and Partridge, 1991; Prout and Clark, 2000, Pitnick and García-González, 2002). However, the relationship between a male's harm to females and his lifetime fitness has never been directly measured. Theoretical analysis of antagonistic co-evolution between the sexes requires that at least some male adaptations reduce female fitness – that is, that there be sexually antagonistic

* Author to whom all correspondence should be addressed. e-mail: rice@lifesci.ucsb.edu

pleiotropy for the fitness of a male versus that of his mate (Parker, 1979, Rowe *et al.*, 1994; Rice and Holland, 1997; Rice, 1998; Gavrilets *et al.*, 2000; Chapman *et al.*, 2003). Here we test this theoretical prediction.

Our experiments build on earlier studies of genetic variation for male-induced harm to their mates. Civetta and Clark (2000) and Sawby and Hughes (2000) correlated genetic variation for male reproductive performance in sperm competition with the survival of their mates. Both studies found a negative association between some, but not all, aspects of the efficacy of sperm competition and female survival. Because these studies only measured a subset of the components of male fitness, rather than total lifetime fitness, it could not be determined whether there was net selection favouring male genotypes that were more harmful to their mates. In addition, these studies measured harm from males that were made to be homozygous for 40% of their genome, so some of the observed variation attributed to genotype may have been due to differing amounts of inbreeding depression. Here we extend these previous studies by assaying genetic variation in outbred flies for both total lifetime fitness of males and their harm to female survival. We then determine the regression between these two components of genetic variation and thereby determine the net direction of selection on male-induced harm to female survival.

METHODS

The experiments were conducted with a sample of flies from a large outbred population that had adapted to the laboratory environment for over 300 generations [for a description of the base population, see Chippindale *et al.* (2001)]. A total of 102 haploid genomes (including 99% of all gene loci, consisting of the X and the two major autosomes, but excluding the dot fourth chromosome) were randomly sampled using cytogenetic cloning and screened for total lifetime fitness in males, as described previously (Rice and Chippindale, 2001a). An additional sample of 17 haplotypes was also screened using a similar protocol (Rice and Chippindale, 2001b). A measure of the total fitness of each haploid genome was obtained by following its change in frequency across multiple generations, in a genetic context that caused the entire genomic haplotype to co-segregate as a single, male-limited genomic unit. This multi-generation measure of lifetime fitness included all fitness components, each weighted by its relative contribution to net fitness.

From this combined screen of 119 genome-wide haplotypes, 18 hyperdispersed genomes were selected (Fig. 1): 7 from the top 10% (high fitness), 4 from the middle 10% (average fitness) and 7 from the bottom 10% (low fitness). The high dispersion of male fitness within the selected sample was designed to increase statistical power when testing for a regression with male-induced harm to their mates. Once isolated, the 18 haploid genomes were clonally maintained by repeated cycles of cytogenetic cloning as described in Chippindale *et al.* (2001).

To begin an assay, each genomic haplotype was amplified and placed in multiple males, each with a unique random genetic background, to produce a hemiclone, as described previously (Chippindale *et al.*, 2001). A hemiclone is a group of individuals that share one identical set of genes at each diploid locus in the genome, but the other allele at each locus is a random pick from the base population. Another way to describe a hemiclone is that it is a group of individuals that are produced by randomly drawing a sample of eggs from a population, and then fertilizing the eggs with cloned copies of the same sperm genome.

We tested for male harm to female survival using tester females. The tester females came from a stock with multiple visible mutations [C(1)DX, *y, f*; T(2;3) *rdg*C *st in ri* p^{P} bw^{D}] that

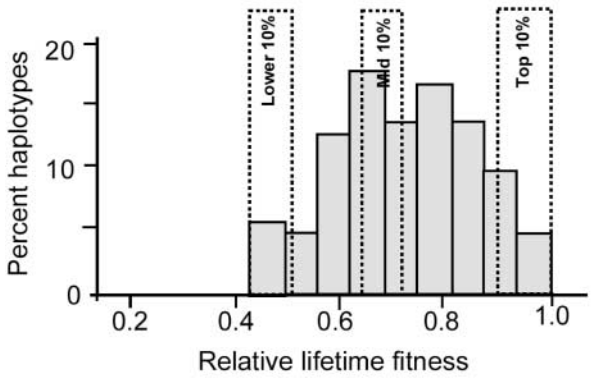

Fig. 1. The dispersion of the three categories of fitness among the male hemiclones selected for analysis. The histogram is based on the 101 hemiclones previously assayed for lifetime fitness (Rice and Chippindale, 2001a). The dashed boxes denote the range of hemiclones selected for the three fitness categories: high, medium and low.

had been backcrossed multiple times through our base population (Rice, 1996; Holland and Rice, 1999; Rice and Chippindale, 2001a). Because the tester females expressed multiple visible mutations, they were less vigorous than wild-type females and their survival, when continuously housed with wild-type males, was lower than that of wild-type females. The increased susceptibility to male harm made the tester females sensitive indicators of subtle differences among males in their degree of harm to female survival (Holland and Rice, 1999).

Male-induced harm to survival of their mates was measured in two ways: (1) short-term exposure of females to males (90 min), and (2) continuous exposure of females to males. The short-term exposure treatment was designed to measure harm to females from seminal fluid proteins and other constituents of the ejaculate, but it also included any harm from the physical act of copulation and other male behaviour during the 90 min that females were exposed to males while they were being inseminated. The long-term exposure treatment was designed to be influenced more strongly by male courtship and copulation behaviour, but it also included harm from the ejaculate.

In the short-term male exposure treatment, tester females were mated a single time by combining 25 three-day-old virgin females with a twofold excess of males (3–5 days post eclosion, all from the same hemiclone) for 90 min. Next, the sexes were separated under light CO_2 anaesthesia (<30 s), the males discarded, and the 25 females were placed into fresh food vials. The females were subsequently transferred to new food vials every two days and dead females were counted at each transfer. Transfers continued until all the females in any one of the individual vials had died. This truncation procedure was used because once all females had died in a vial, any measure of male harm could not increase further in that vial. The protocol produces a sensitive index of male harm to female survival that can be used to compare different male hemiclones. However, because the truncation point of the assay is variable, our mortality index (percent females dead when the experiment is terminated) is correlated with, but not a direct estimate of, the mortality metric typically used in demographic analysis. The continuous male exposure treatment was carried out with the same protocol except that both sexes were housed together throughout the duration of the experiment with a 15 males : 25 females sex ratio after the initial mating.

The short-term and continuous male exposure assays were repeated once to produce two replicated blocks for both experimental treatments. The number of vials per hemiclone per block averaged 3.6 and 6.1 for blocks 1 and 2 of the assay of total harm from males, respectively; and 3.6 and 8.0 for blocks 1 and 2 of the assay of harm from short-term exposure, respectively.

Genetic variation among the 18 hemiclones was measured with random-effects analysis of variance. The model was $Y_{ijk} = \mu + \text{hemiclone}_i + \text{replicate}_j + \text{error}_{ijk}$, where Y is the arcsine-transformed square root of the proportion of females alive in vial_{ijk}, μ is the grand mean of Y_{ijk}, hemiclone_i denotes the i^{th} hemiclone of males, replicate_j is the j^{th} replicate, and error_{ijk} are the error terms assumed to be normally distributed with equal variance. The interaction between hemiclone and replicate was not included in the model because in all cases it was found to be statistically insignificant. Both hemiclone and replicate were modelled as random effects. Statistical analysis was performed using the JMP© statistical package.

RESULTS

We found significant genetic variation among hemiclones for male harm to female survival in both the short-term male exposure treatment ($P = 0.0066$) and the continuous male exposure treatment ($P < 0.0001$; Table 1, Fig. 2). In addition, we found a positive regression between male lifetime fitness and male-induced harm to females for both the continuous exposure to males treatment and the short-term exposure to males treatment ($P = 0.0004$ for continuous male exposure and $P = 0.0094$ for short-term male exposure) (Fig. 3A,B). Based on the positive correlations between lifetime fitness and male-induced harm traits, males that harm their mates more have higher total lifetime fitness, hence there is a positive selection gradient on male-induced harm to females.

Both of our measures of male harm to females included male behaviour, but to different extents. Because tester females mate infrequently when continuously exposed to males (Rice, 1996), the continuous male exposure treatment was more strongly influenced by male behaviour (i.e. courtship intensity and the number of times males re-mated females) than the short-term exposure treatment [in which virtually all tester females mate once during the 90 min exposure to male behaviour (Rice, 1996)]. To obtain an index of male harm that was

Table 1. Analysis of variance for male harm to females

Source	d.f.	SS	MS	*F*-ratio	Prob > *F*
Mean total harm					
Hemiclone	17	13.67	0.803	9.44	< 0.0001
Replicate	1	0.15	0.150	1.76	0.1866
Error	155	13.20	0.085		
Mean short-term exposure harm					
Hemiclone	17	2.05	0.121	2.15	0.0066
Replicate	1	0.05	0.046	0.81	0.3694
Error	203	11.41	0.056		

Note: The dependent variable is the arcsine-transformed square root of the proportion of females that died.

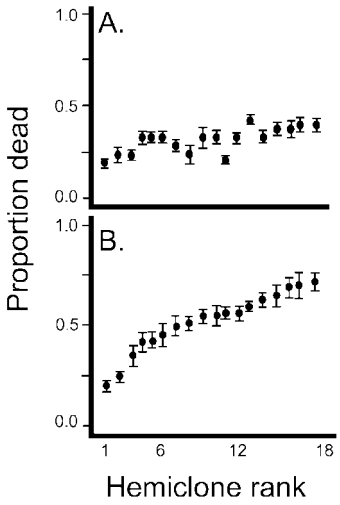

Fig. 2. The mean proportion of females dying after exposure to 18 different hemiclones of males. (A) Continuous exposure to males, (B) short-term exposure to males. Rank order of hemiclones based on the continuous male exposure treatment. Error bars are standard errors.

enriched with the influence of male behaviour, we obtained residuals from the regression of our average index of male harm to female survival from the continuous male exposure treatment on the average measure of male harm from the short-term exposure treatment. The residuals from this regression were significantly positively correlated with male lifetime fitness ($P = 0.0387$) (Fig. 3C).

As a further check on the presence of additive genetic variation among hemiclones for male-induced harm to female survival, we correlated the average mortality values for each hemiclone from the continuous male exposure assay with the corresponding values from the short-term male exposure assay. We found a positive correlation ($r = 0.57$, $P = 0.013$; Fig. 4), as would be expected when genetic variation among hemiclones contributed to variation in the degree of harm to female survival. Because these independent measures of male-induced harm shared no environmental covariance, the observed positive correlation corroborates the conclusion from the analysis of variance that there is additive genetic variation among male hemiclones for harm to female survival.

DISCUSSION

The results of this study demonstrate additive genetic variance among male hemiclones for their harm to females, both in the context of short-term and continuous exposure to males. Because a short exposure to male behaviour was required to inseminate females, our experimental design could not fully resolve harm from the ejaculate from harm from male behaviour. However, the fact that the contact with male behaviour was so brief (90 min) while females were being inseminated in the short-term exposure treatment, suggests to us that seminal fluid or other constituents of the ejaculate probably were the primary factor contributing to harm to female survival in this treatment. Our results also provide limited, indirect evidence for additive genetic variation among male hemiclones for harm to females

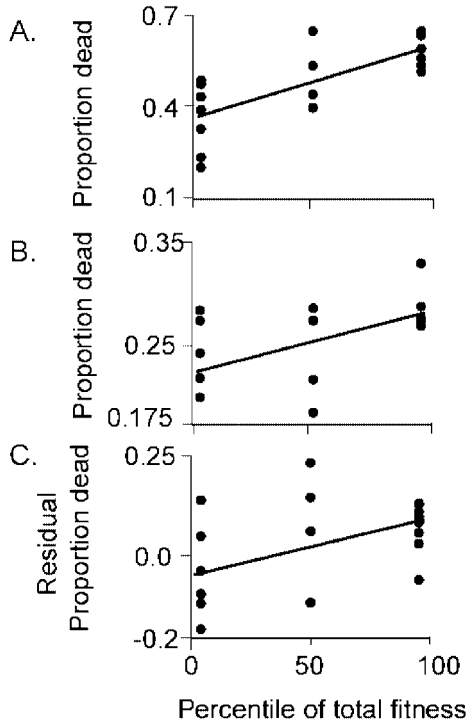

Fig. 3. The regression between harm to females due to their interactions with different hemiclones of males, and the lifetime fitness of the hemiclones of males (measured as the percentile category [bottom 10%, middle 10%, or top 10%] of the total fitness distribution in Fig. 1). (A) Continuous exposure to males; (B) short-term exposure to males; (C) residuals from long-term exposure after adjusting for the harm from short-term exposure.

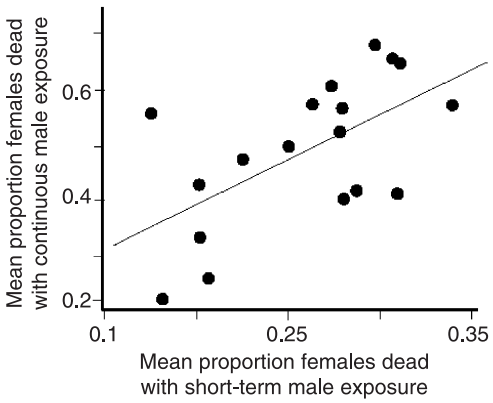

Fig. 4. The correlation between average survival of females when they had short-term or continuous exposure to males.

through behaviour, based on the analysis of the residuals of harm to female survival after adjustment for harm from the short-term exposure treatment. Because the flies used in these assays were outbred, inbreeding depression did not contribute to the observed variation among hemiclones.

The genetic units that we measured were hemiclones. Analysis of hemiclones extends the traditional analysis of genetic variation among individual chromosomes to genome-wide haplotypes. Because members of a hemiclone share only half of their genes in common, the additive genetic variation among hemiclones that we measured represents approximately half of the genetic variation that would occur among diploid individuals in our base population. For this reason, hemiclonal analysis provides a conservative estimate of standing genetic variation among individuals. However, the genetic variation detected by hemiclonal analysis (and the analysis of individual chromosomes) potentially contains non-heritable epistatic genetic variation. Additive genetic variation among hemiclones contains no non-heritable dominance variation, but like the analysis of individual chromosomes, it potentially contains non-heritable epistatic variation among alleles that co-segregate within the same genomic haplotype. The potential to include epistatic variation in the estimate of heritable variation is not unique to hemiclonal analysis (or the analysis of individual chromosomes). The lack of recombination in male *Drosophila* causes epistatic variation to potentially contribute to estimated heritable genetic variation in all forms of paternal half-sib analysis. Theoretical and empirical work, however, suggests that epistatic genetic variation is generally a small subset of the total detected additive genetic variation in *Drosophila* for traits that are closely associated with fitness (for a review, see Tachida and Cockerham, 1988).

Our analysis of the regression between male harm to females and the lifetime fitness of males indicates that the selection gradient on male harm to females is positive. The combination of additive genetic variance among hemiclones and the positive selection gradient on this variation indicates that males are presently evolving in our base population to increase their harm to female survival. Previous studies of lines isogenic for one of the two major autosomes have shown that at least some fitness components concerning sperm competition are positively correlated with the level of male harm to females (Civetta and Clark, 2000; Sawby and Hughes, 2000). These previous studies, however, did not measure total fitness and they may have been influenced by strong inbreeding depression because the flies assayed were completely inbred for 40% of their genome. Our study extends the previous work, and confirms the earlier conclusions, by showing that the same positive selection gradient occurs among outbred flies when the level of male harm to females is regressed on total lifetime fitness.

The observed reduction in the survival of females due to their interactions with males could potentially be offset by increased fecundity due to intersexual interactions, but recent work in our laboratory has shown that this is not the case (Linder and Rice, 2005), as has work in other laboratories (Chapman *et al.*, 1998; Fowler and Partridge, 1989; Trevitt and Partridge, 1991). The reduced survival of females also could be offset by direct and/or indirect benefits (Parker, 1979; Rice and Holland, 1997). Previous studies have shown that *D. melanogaster* males do not provide direct benefits to their mates (Chapman *et al.*, 1994; Pitnick *et al.*, 1997). However, previous experiments with our base population have demonstrated a substantial potential for indirect benefits due to high variation among males and females for net fitness (Chippindale *et al.*, 2001; Rice and Chippindale, 2001a; Gibson *et al.*, 2002). Despite this potential, a previous experiment from our laboratory did not support indirect benefits through the 'good genes' model of sexual selection (Holland, 2002),

and another recent study in our laboratory did not support the 'sexy sons' route to indirect benefits (N. Ortieza, J.E. Linder and W.R. Rice, unpublished). Nonetheless, even if it were shown that females recouped all male-induced harm via indirect benefits, the observed positive selection gradient on male genotypes that are more harmful to female survival could fuel an arms race between the sexes. In this case, females would receive both costs and benefits from males, but because there would be selection on females to reduce the cost component, females would be expected to counter-evolve in response to male-induced harm whenever at least part of this harm can be genetically disassociated from any compensating benefits.

Overall, the observed standing genetic variation for male-induced harm to female survival, in combination with the observed positive selection gradient on this variation, provides direct evidence for the sexually antagonistic pleiotropy that is required to fuel antagonistic co-evolution between the sexes. Co-evolution between loci mediating interactions between the sexes is likely to be a mosaic, with some interacting loci evolving mutualistically and others antagonistically. This study provides evidence that at least some of the co-evolution is antagonistic.

ACKNOWLEDGEMENTS

We thank P. Bryne and A.K. Chippindale for help in the laboratory and A.K. Chippindale, A. Civetta and U. Friberg for comments on an earlier draft of the manuscript. This work was supported by two grants from the National Science Foundation to W.R.R. (DEB-0128780 and DEB-0410112).

REFERENCES

Arnqvist, G. 1989. Multiple mating in a water strider: mutual benefits or intersexual conflict? *Anim. Behav.*, **38**: 749–756.

Burpee, D.M. and Sakaluk, S.K. 1993. Repeated matings offset costs of reproduction in female crickets. *Evol. Ecol.*, **7**: 240–250.

Chapman, T., Trevitt, S. and Partridge, L. 1994. Remating and male-derived nutrients in *Drosophila melanogaster*. *J. Evol. Biol.*, **7**: 51–69.

Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F. and 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. and 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.

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

Chippindale, A.K. and Rice, W.R. 2001. Y chromosome polymorphism is a strong determinant of male fitness in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA*, **98**: 5677–5682.

Chippindale, A.K., Gibson, J. and Rice, W.R. 2001. Negative genetic correlation for adult fitness between the sexes reveals ontogenetic conflict in *Drosophila*. *Evolution*, **98**: 1671–1675.

Civetta, A. and Clark, A.G. 2000. Chromosomal effects on male and female components of sperm precedence in *Drosophila*. *Genet. Res., Camb.*, **75**: 143–151.

Cohet, Y.A. and David, J.R. 1976. Deleterious effects of copulation in *Drosophila* females as a function of growth temperature of both sexes. *Experientia*, **32**: 696–697.

Crudgington, H.S. and Siva-Jothy, M.T. 2000. Gential damage, kicking and early death: the battle of the sexes takes a sinister turn in the bean weevil. *Nature*, **407**: 855–856.

Dean, J.M. 1981. The relationship between lifespan and reproduction in the grasshopper *Melanoplus*. *Oecologia*, **48**: 385–388.

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

Gavrilets, S., Arnqvist, G. and Friberg, U. 2000. The evolution of female mate choice by sexual conflict. *Proc. R. Soc. Lond. B*, **268**: 531–539.

Gibson, J., Chippindale, A.K. and Rice, W.R. 2002. The X chromosome is a hot spot for sexually antagonistic fitness variation. *Proc. R. Soc. Lond. B*, **269**: 499–505.

Holland, B. 2002. Sexual selection fails to promote adaptation to a new environment. *Evolution*, **56**: 721–730.

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

Kasule, F.K. 1986. Repetitive mating and female fitness in *Dysdercus cardinalis* (Hemiptera, Pyrrhocoridae). *Zool. J. Linn. Soc.*, **88**: 191–199.

Linder, J.E. and Rice, W.R. 2005. Natural selection and genetic variation for female resistance to harm from males. *J. Evol. Biol*., **18**: 568–575.

McKinney, F., Derrickson, S.R. and Mineau, P. 1983. Forced copulation in waterfowl. *Behavior*, **86**: 250–294.

Moore, A.J., Gowaty, P.A., Wallin, W.G. and Moore, P.J. 2001. Sexual conflict and the evolution of female mate choice and male social dominance. *Proc. R. Soc. Lond. B*, **268**: 517–523.

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

Pitnick, S. and García-González, J. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B*, **269**: 1821–1828.

Pitnick, S., Spicer, G.S. and Markow, T.A. 1997. Phylogenetic examination of female incorporation of ejaculates in *Drosophila*. *Evolution*, **51**: 833–845.

Prout, T. and Clark, A.G. 2000. Seminal fluid causes temporary reduced egg hatch in previously mated females. *Proc. R. Soc. Lond. B*, **267**: 201–203.

Pyle, D.W. and Gromko, M.H. 1978. Repeated mating by female *Drosophila melanogaster*. *Experientia*, **34**: 449–450.

Rice, W.R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Science*, **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 and S.H. Berlocher, eds.), pp. 261–270. New York: Oxford University Press.

Rice, W.R. and Chippindale, A.K. 2001a. Sexual recombination and the power of natural selection. *Science*, **294**: 555–559.

Rice, W.R. and Chippindale, A.K. 2001b. Intersexual ontogentic conflict. *J. Evol. Biol.*, **14**: 685–693.

Rice, W.R. and Holland, B. 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav. Ecol. Sociobiol.*, **41**: 1–10.

Rowe, L. 1994. The costs of mating and mate choice in water striders. *Anim. Behav.*, **48**: 1049–1056.

Rowe, L., Arnqvist, G., Krupa, J. and Sih, A. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends Ecol. Evol.*, **9**: 289–293.

Sawby, R. and Hughes, K.A. 2000. Male genotype affects female longevity in *Drosophila melanogaster*. *Evolution*, **55**: 834–839.

Tachida, H. and Cockerham, C.C. 1988. Variance components of fitness under stabilizing selection. *Genet. Res.*, **51**: 47–53.

Trevitt, S. and Partridge, L. 1991. A cost of receiving sperm in the female fruitfly *Drosophila melanogaster*. *J. Insect. Physiol.*, **37**: 471–475.