doi:10.1111/evo.13074

Sexual selection expedites the evolution of pesticide resistance

Frances Jacomb,[1] Jason Marsh,[1] and Luke Holman[2,3]

[1]*Division of Evolution, Ecology and Genetics, Research School of Biology, Australian National University, Canberra, ACT 0200, Australia*

[2]*School of Biosciences, University of Melbourne, Melbourne, VIC 3052, Australia*

[3]*E-mail: luke.holman@unimelb.edu.au*

Received April 17, 2016
Accepted September 12, 2016

The evolution of insecticide resistance by crop pests and disease vectors causes serious problems for agriculture and health. Sexual selection can accelerate or hinder adaptation to abiotic challenges in a variety of ways, but the effect of sexual selection on resistance evolution is little studied. Here, we examine this question using experimental evolution in the pest insect *Tribolium castaneum*. The experimental removal of sexual selection slowed the evolution of resistance in populations treated with pyrethroid pesticide, and also reduced the rate at which resistance was lost from pesticide-free populations. These results suggest that selection arising from variance in mating and fertilization success can augment natural selection on pesticide resistance, meaning that sexual selection should be considered when designing strategies to limit the evolution of pesticide resistance.

KEY WORDS: **Adaptation, artificial selection, experimental evolution, flour beetle, insecticide.**

Sexual selection results from the variance in fitness caused by competition between members of the same sex over opposite-sex mates and their gametes (Darwin 1871). Sexual selection has been hypothesized to augment natural selection, increasing the rate of adaptation and helping to purge deleterious mutations (e.g., Darwin 1871; Manning 1984; Agrawal 2001; Siller 2001; Whitlock and Agrawal 2009). This hypothesis derives partly from observations that many traits that influence mating success, such as sexually selected "ornaments" and mate-seeking behavior, show condition-dependent expression. Thus, any locus that contributes to variation in condition is subject to sexual selection (Rowe and Houle 1996; Whitlock and Agrawal 2009). Sexual selection might therefore affect the majority of the genome, and favor largely the same set of alleles as natural selection, accelerating adaptation in many traits that are seemingly unconnected to reproduction.

Several studies have found evidence consistent with this hypothesis (reviewed in Whitlock and Agrawal 2009; Holman and Kokko 2013). For example, a mutant allele causing alcohol sensitivity was purged more rapidly in *Drosophila* populations in which sexual selection was allowed rather than prevented (Hollis et al. 2009); *Callosobruchus* beetle populations adapted more rapidly to a novel foodstuff when sexual selection was permitted (Fricke and Arnqvist 2007); and sexual selection appeared to augment natural selection on several *Drosophila* mutations (Whitlock and Bourguet 2000; Sharp and Agrawal 2008). However, the evidence is not unanimous. Sexual selection did not elevate the rate at which deleterious mutations were purged in another study of *Drosophila* (Arbuthnott and Rundle 2012), did not help insect populations respond to selection on thermal tolerance (Holland 2002), diet (Rundle et al. 2006), or age-specific reproduction (Maklakov et al. 2009), and did not confer a mating advantage to locally adapted males (Arbuthnott and Rundle 2014). Sexual selection even appeared to hinder adaptation to abiotic factors in studies of yeast (Reding et al. 2013) and Drosophila (Arbuthnott and Rundle 2012; Chenoweth et al. 2015). Additionally, meta-analyses have yielded mixed conclusions regarding the correlation between paternal success in sexual selection and offspring fitness (Jennions et al. 2012).

The lack of a consistent relationship between sexual selection and the rate of adaptation likely stems from the manifold, conflicting consequences of sexual selection for alleles, individuals, and populations. Sexual and natural selection might often

favor different alleles, such that selection on sexual and nonsexual components of fitness would pull the phenotype in conflicting directions. Additionally, when males tend to preferentially direct harmful courtship behaviors toward well-adapted females (Long et al. 2009; Arbuthnott and Rundle 2012; Chenoweth et al. 2015), male harassment (a putative adaptation to sexual selection) will counteract adaptation by reducing or removing the fitness advantage that would otherwise accrue to these females (see e.g., Hollis and Houle 2011). Such negative consequences of sexual selection cooccur with the putative positive consequences, and their relative strengths are thought to vary between and even within species (e.g., Long et al. 2012; Holman and Kokko 2013; Berger et al. 2014), muddying general predictions about the net effect of sexual selection on adaptation.

Sexual selection could theoretically accelerate or retard the evolution of pesticide resistance, a trait with important consequences for agriculture and health (Laxminarayan 2003). This possibility seems understudied; to our knowledge, all theoretical models of the evolution of pesticide resistance do not incorporate sexual selection on pesticide resistance (e.g., Glass et al. 1986; Lenormand and Raymond 1998; Koella et al. 2009; Read et al. 2009). These models consider the effects of pesticide resistance on "naturally-selected" components of fitness only, such as the elevated survival of resistant individuals encountering pesticide, and the pleiotropic costs of resistance to growth, viability, or fecundity (Kliot and Ghanim 2012). However, there is empirical evidence that pesticide-resistant male mosquitoes might be disadvantaged in sexual selection relative to susceptible males (Berticat et al. 2002), and that (contrary to expectations) resistant male *Tribolium* beetles have higher mating and fertilization success than susceptible males (Arnaud and Haubruge 2002; Arnaud et al. 2005). These findings imply that competition for mates or their gametes could affect the rate at which populations evolve increased or decreased pesticide resistance.

Here, we experimentally tested whether sexual selection affects the rate at which populations evolve increased or decreased pesticide resistance. We used the flour beetle *Tribolium castaneum* as a model. This insect is a pest of stored grains with a polygamous "scramble competition" mating system: males continually court and pursue females, both sexes mate multiple times, facilitating both pre- and postcopulatory sexual selection (e.g., Michalczyk et al. 2011; Demont et al. 2013). In a 2×2 experimental design, we allowed populations of *T. castaneum* to evolve in the presence or absence of pyrethroid pesticide, with sexual selection either allowed or experimentally removed.

Assuming that sexual and natural selections are concordant, for example, because pesticide application confers both a survival and a mating advantage to resistant males relative to susceptible ones, we predict that pesticide-exposed populations will evolve pesticide resistance more quickly when sexual selection is allowed rather than prevented. We also predict that pesticide-free populations will re-evolve susceptibility more quickly when sexual selection is allowed, assuming that resistance carries sexually selected costs. Conversely, we could find that sexual selection retards the evolution of pesticide resistance, which might suggest novel ways of limiting adaptation to pesticides.

Methods

PESTICIDE RESISTANCE ASSAY

We diluted technical-grade Deltamethrin TC insecticide (Bayer CropScience, Hawthorn, Australia) in solvent (3:1:1 turpentine/acetone/mineral oil) at 10 g/l (Collins 1998). We applied 0.5 ml of solution evenly to 60 mm filter paper discs suspended on pins, then allowed the volatile solvent to evaporate for less than one hour, giving a dose of 0.026 mg/cm^2. Beetles were then confined to the pesticide-treated paper using a 50 mm glass cylinder with a metal mesh lid for 24 hours. We then recorded the proportion of beetles that were not "knocked down," that is, immobile or displaying abnormally slow or uncoordinated movement, and used this as a measure of resistance (counts were performed blind to the identity of the beetles). For each experimental unit, we set up three replicate filter papers each with 40 beetles (occasionally <40 if a line was not sufficiently productive). The repeatability of knockdown rate across the three replicate filter papers was high (intraclass correlation coefficient $= 0.99$), so we pooled the data from each replicate in subsequent analyses.

ESTABLISHING AND PROPAGATING THE EXPERIMENTAL EVOLUTION LINES

We obtained two cultures of *T. castaneum* from the Postharvest Grain Protection Unit at Agri-Science Queensland. One culture (QTC4; hereafter termed "S" for susceptible) has been in laboratory culture for many years, and is highly susceptible to pyrethroid pesticides (Collins 1998). The S culture was maintained in pesticide-free medium (i.e., 10 g yeast per 100 g sieved organic wholemeal flour). The resistant "R" culture (QTC279) had been kept for >16 years in medium containing 0.1 g "Prolong" brand cyfluthrin pesticide per 100 g of flour-yeast mix, and has a high level of resistance to pyrethroid pesticides. A mutation at a locus in linkage group 9 is thought to be responsible for almost all of the resistance in the QTC279 stock (Stuart et al. 1998), and the stock's resistance apparently derives from overexpression of a cytochrome P450 enzyme in the brain (Zhu et al. 2010).

Throughout, we ensured beetles were virgin by sieving the cultures to collect pupae, sexing the pupae by their external morphology, and placing them in single-sex Petri dishes. We collected 100 male and 100 female seven- to 10-day-old virgins from the S and R cultures, set them up in Petri dishes in 200 monogamous

pairs (each containing one R and one S individual), and then collected and sexed the F1 pupae. We refer to the F1 as Generation 0 of the selection experiment, and we expect the majority of the F1 to be heterozygous at loci affecting pyrethroid resistance. We assayed the pesticide resistance of a random subset of the F1 as described above.

We established the experimental evolution lines using randomly chosen F1 individuals. Individual lines were subjected to one of four different treatments, which we denote as SS+P+, SS+P−, SS−P+, and SS−P−, in reference to the presence or absence (+/−) of sexual selection (SS) and pesticide (P). There were four replicate lines per treatment, for a total of 16 lines. In the SS− treatments, we placed a single male and a single female together in a 50 mm Petri dish containing about 6 g flour/yeast mixture. The SS+ treatment was identical except that dishes contained one female and five males, providing the opportunity for male–male competition for mating and fertilization, as well as pre- and postcopulatory female choice. Readers should note that our experiment has potential confounding effects (as do all past experimental evolution studies that have removed sexual selection): we have manipulated the number and sex ratio of beetles per dish, in addition to manipulating the presence of sexual selection. The P− treatments contained standard flour-yeast mixture, whereas the P+ treatments contained 0.1 g cyfluthrin per 100 g of flour-yeast mixture. We systematically rotated the position of the trays holding the dishes of beetles around the climate room every two to four days, preventing differences in microclimate from confounding the experiment.

To reduce the difference in effective population size between the SS+ and SS− treatments due to their unequal family sizes, we set up 30 SS− families per line per generation, and 18 SS+ families. With these family numbers, the effective population size (N_e) will be 60 in both the treatments in the unlikely event that all five males in the SS+ shared paternity exactly equally (see Equation 8 in Balloux and Lehmann 2003). Otherwise, the SS+ treatment will have $36 < N_e < 60$, depending on the extent of paternity skew. Our manipulation of sexual selection is identical to that of Demont et al. (2013), except that N_e is 50% larger across the board in our study.

After adding Generation 0 beetles to the Petri dishes, we allowed two weeks for mating and oviposition. We then removed all beetles from the dishes and waited a further two weeks for their offspring to develop into pupae. On days 28–30 after adding the beetles, we sieved all the dishes to collect pupae. We then mixed the pupae from all dishes from the same selection line, giving one pool of pupae for each of the 16 lines. This mixing procedure ensures that the most productive families contributed more offspring to the next generation, allowing natural selection to take place (as well as sexual selection on males, in the SS+ lines). The pupae were then separated into single-sex groups and allowed to enclose, giving virgin males and females. At seven- to 10 days posteclosion, a random subset of these virgins was randomly divided into families (i.e., one male and one female in SS− lines, and five males and one female in SS+ lines) and used to establish the next generation. The remaining progeny were used to assay pesticide resistance, as described above. By repeating this procedure we applied five generations of selection, and assayed pesticide resistance every generation.

COMMON GARDEN EXPERIMENT

After five generations of selection, we collected progeny from all lines and raised them in a "common garden," that is, an identical rearing environment designed to verify that phenotypic differences between selection lines were due to changes in allele frequencies and not to nongenetic differences between lines. For all lines, we placed 30 male–female pairs (randomly collected from the progeny of Generation 5) in individual Petri dishes containing 6 g pesticide-free flour/yeast mixture for two weeks, and then collected their offspring. A random subset of the offspring was used to assay pesticide resistance.

STATISTICAL ANALYSIS

The response variable (resistance to knockdown) was binary, so we analyzed the data using binomial generalized linear mixed models (GLMMs; fit using the *lme4* package for R). We accounted for repeated measurements on beetles from the same selection line by fitting "Line" as a random effect. For the multigenerational data in Table 1, we additionally fit a Generation × Line random slope, allowing the Line effect to vary across generations. The type III analysis of deviance tables in Tables 1 and 2 were calculated using the analysis of variance function in the *car* package for R. R code to reproduce all our analyses is archived at Dryad.

Results

Resistance to pythrethroid pesticide strongly increased in the pesticide-treated "P+" lines, and strongly decreased in the untreated "P−" lines. In a binomial GLMM of the pesticide-resistance data from generations 1–5, there was a significant three-way interaction between sexual selection treatment (SS+ or SS−), pesticide treatment, and generation (Fig. 1; Table 1). This interaction indicated that the effect of sexual selection on the rate of evolutionary change in resistance was more positive in the P+ lines than in the P− lines. Figure S1 depicts the same data as Figure 1, but shows the resistance levels of the 16 individual lines.

For beetles reared in a pesticide-free common garden after five generations of selection, there was again a significant interaction between the sexual selection and pesticide treatments (Fig. 1; Table 2). This result suggests that the trends seen in

Table 1. Analysis of deviance table of type III Wald χ^2-tests for a binomial GLMM testing the effects of sexual selection treatment, pesticide treatment and generation on pesticide resistance in generations 1–5 of the 16 selection lines.

	χ^2	*df*	*P*
Intercept	140.9	1	<0.0001
Pesticide	208.4	1	<0.0001
Sexual selection	0.90	1	0.34
Generation	0.49	1	0.48
Pesticide × sexual selection	0.01	1	0.91
Pesticide × generation	21.2	1	<0.0001
Sexual selection × generation	6.5	1	0.011
Pesticide × sexual selection × generation	4.0	1	0.045

Selection line and the line × generation interaction were included as random factors. The sample size was typically 120 beetles assayed per line per generation, although smaller numbers were used if the selection lines were unproductive; in total, 9281 beetles were assayed. The model presented here is the top model, as ranked by AICc, in a model set containing all possible simpler models (ΔAICc = 1.63).

Table 2. Analysis of deviance table of type III Wald χ^2-tests for a binomial GLMM testing the effects of sexual selection, pesticide treatment, and their interaction on pesticide resistance in the offspring of beetles reared in a common garden (n = 120 beetles assayed per selection line; line was included as a random factor).

	χ^2	*df*	*P*
Intercept	81.3	1	<0.0001
Pesticide	135.6	1	<0.0001
Sexual selection	6.7	1	0.0096
Pesticide × sexual selection	7.0	1	0.0084

This model is the top model, as ranked by AICc, in a model set containing all possible simpler models (ΔAICc = 2.27).

generations 0–5 resulted from changes in allele frequencies rather than nongenetic differences between lines. The significant interaction between the P and SS treatments (Table 2; $P = 0.008$) shows that sexual selection augmented the evolution of resistance, susceptibility, or both. We then ran two posthoc analyses to examine the effect of the SS treatment in the P− and P+ lines separately. In the P− lines, the SS+ treatment was more susceptible than the SS− treatment, but not significantly so (GLMM: $z = 1.81$, $n = 8$ lines, $P = 0.070$). In the P+ lines, the SS+ treatment was more resistant than the SS− treatment, but this difference was not significant (GLMM: $z = 1.65$, $n = 8$ lines, $P = 0.10$). Together with Table 2, these results suggest that sexual selection augmented the evolution of both susceptibility and resistance, depending on which was favored by selection (which in turn depended on whether pesticide was absent or present, respectively).

Discussion

The results were consistent with our prediction that sexual selection can expedite the evolution of resistance when pesticide is present, and accelerate the evolution of susceptibility when

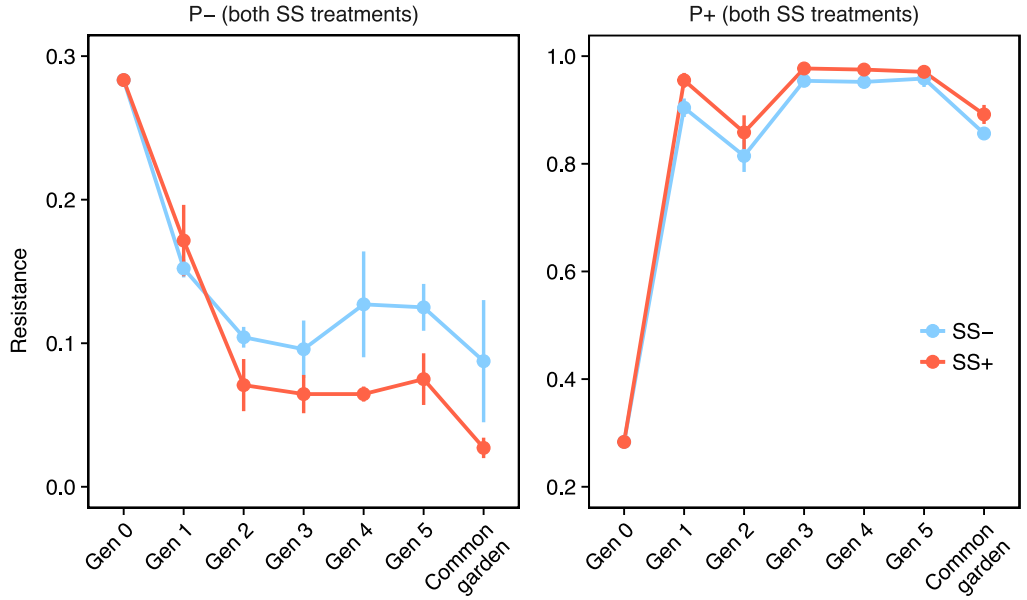

Figure 1. Level of pesticide resistance in generations 0–5 of the selection experiment, and in the common garden. The points show the mean ± one standard error of the four lines in each treatment.

pesticide is absent. Note that our experiment has potentially underestimated the positive effect of sexual selection on the rate of adaptation, because the SS+ lines had a lower effective population size than the SS− lines (see Methods).

The present findings have implications for strategies aimed at limiting the evolution of pesticide resistance. Theoretical work has illustrated that gene flow into pesticide-treated areas can limit the evolution of resistance, because the immigration of susceptible individuals counteracts local adaptation to pesticide (Lenormand and Raymond 1998). These models illustrate that it might be possible to slow the evolution of resistance by ensuring that the pesticide-treated area is as small as possible, since this should increase the number of susceptible immigrants relative to resistant residents, and thus potentially increase introgression of susceptible alleles. Another implication of the models is that one could slow resistance evolution by releasing susceptible individuals into treated areas (particularly males, given that adult males are harmless in many insect pests; Lenormand and Raymond 1998). Our results suggest that such strategies might not work as well as hoped. Residual pesticide in the treated area might reduce the mating success of susceptible immigrants or released males, preventing introgression. There is also evidence that resistance genes pleiotropically increase male reproductive success in at least one pest species (present study; Haubruge and Arnaud 2001; Arnaud et al. 2002), which would similarly hamper the reintroduction of susceptible alleles. Additionally, programs to release susceptible males might fail if these males were laboratory-adapted and had low mating success in the wild. In general, efforts to design or release organisms carrying human-beneficial genes should consider these organisms' success in sexual selection in addition to their survival and fecundity.

Future work could further investigate the effect of sexual selection on the spread of resistance alleles, and in their rate of loss from untreated populations. There is room for more empirical work, as the effects of resistance on sexual components of fitness are understudied relative to nonsexual components (Kliot and Ghanim 2012). One could also seek to unify models of sexual selection and adaptation (e.g., Agrawal 2001; Siller 2001) with resistance evolution models (Lenormand and Raymond 1998; Read et al. 2009; Koella et al. 2009), which could point to new ways to manage the evolution of resistance, or highlight organisms in which sexual selection is likely to have a prominent effect on resistance evolution.

ACKNOWLEDGMENTS

We thank Patrick Collins and colleagues (Postharvest Grain Protection Unit, Queensland) for generously providing beetle stocks and culturing advice, and Bayer CropScience for providing Deltamethrin. This work was supported by a DECRA fellowship from the Australian Research Council to LH (No. DE140101481).

DATA ARCHIVING

The raw data and R code to reproduce our statistical analyses are included as supplementary files.

LITERATURE CITED

Agrawal, A. F. 2001. Sexual selection and the maintenance of sexual reproduction. Nature 411:692–695.

Arbuthnott, D., and H. D. Rundle. 2014. Misalignment of natural and sexual selection among divergently adapted *Drosophila melanogaster* populations. Anim. Behav. 87:45–51.

———. 2012. Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*. Evolution 66:2127–2136.

Arnaud, L., and E. Haubruge. 2002. Insecticide resistance enhances male reproductive success in a beetle. Evolution 56:2435–2444.

Arnaud, L., E. Haubruge, and M. J. G. Gage. 2005. The malathion-specific resistance gene confers a sperm competition advantage in *Tribolium castaneum*. Funct. Ecol. 19:1032–1039.

Arnaud, L., Y. Brostaux, L. K. Assié, C. Gaspar, and E. Haubruge. 2002. Increased fecundity of malathion-specific resistant beetles in absence of insecticide pressure. Heredity 89:425–429.

Balloux, F., and L. Lehmann. 2003. Random mating with a finite number of matings. Genetics 165:2313–2315.

Berger, D., K. Grieshop, M. I. Lind, J. Goenaga, A. A. Maklakov, and G. Arnqvist. 2014. Intralocus sexual conflict and environmental stress. Evolution 68:2184–2196.

Berticat, C., G. Boquien, M. Raymond, and C. Chevillon. 2002. Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes. Genet. Res. 79:41–47.

Chenoweth, S. F., N. C. Appleton, S. L. Allen, and H. D. Rundle. 2015. Genomic evidence that sexual selection impedes adaptation to a novel environment. Curr. Biol. 25:1860–1866.

Collins, P. J. 1998. Inheritance of resistance to pyrethroid insecticides in *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 34:395–401.

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

Demont, M., V. M. Grazer, Ł. Michalczyk, A. L. Millard, S. H. Sbilordo, B. C. Emerson, M. J. G. Gage, and O. Y. Martin. 2013. Experimental removal of sexual selection reveals adaptations to polyandry in both sexes. Evol. Biol. 41:62–70.

Fricke, C., and G. Arnqvist. 2007. Rapid adaptation to a novel host in a seed beetle (*Callosobruchus maculatus*): the role of sexual selection. Evolution 61:440–454.

Glass, E. H., P. L. Adkisson, G. A. Carlson, B. A. Croft, D. E. Davis, J. W. Eckert, G. P. Georghiou, W. B. Jackson, H. M. LeBaron, F. W. Plapp et al. 1986. Pesticide resistance: strategies and tactics for management. National Academy Press, Washington DC.

Haubruge, E., and L. Arnaud. 2001. Fitness consequences of malathion-specific resistance in red flour beetle (Coleoptera: Tenebrionidae) and selection for resistance in the absence of malathion. J. Econ. Entomol. 94:552–557.

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

Hollis, B., and D. Houle. 2011. Populations with elevated mutation load do not benefit from the operation of sexual selection. J. Evol. Biol. 24:1918–1926.

Hollis, B., J. L. Fierst, and D. Houle. 2009. Sexual selection accelerates the elimination of a deleterious mutant in *Drosophila melanogaster*. Evolution 63:324–333.

Holman, L., and H. Kokko. 2013. The consequences of polyandry for population viability, extinction risk and conservation. Phil. Trans. R. Soc. B 368:20120053.

Jennions, M. D., A. T. Kahn, C. D. Kelly, and H. Kokko. 2012. Meta-analysis and sexual selection: past studies and future possibilities. Evol. Ecol. 26:1119–1151.

Kliot, A., and M. Ghanim. 2012. Fitness costs associated with insecticide resistance. Pest Manag. Sci. 68:1431–1437.

Koella, J. C., P. A. Lynch, M. B. Thomas, and A. F. Read. 2009. Towards evolution-proof malaria control with insecticides. Evol. Appl. 2:469–480.

Laxminarayan, R. 2003. Battling resistance to antibiotics and pesticides: an economic approach. Resources for the Future, Washington DC.

Lenormand, T., and M. Raymond. 1998. Resistance management: the stable zone strategy. Proc. R. Soc. B 265:1985–1990.

Long, T. A. F., A. F. Agrawal, and L. Rowe. 2012. The effect of sexual selection on offspring fitness depends on the nature of genetic variation. Curr. Biol. 22:204–208.

Long, T. A. F., A. Pischedda, A. D. Stewart, and W. R. Rice. 2009. A cost of sexual attractiveness to high-fitness females. PloS Biol. 7:e1000254.

Maklakov, A. A., R. Bonduriansky, and R. C. Brooks. 2009. Sex differences, sexual selection, and ageing: an experimental evolution approach. Evolution 63:2491–2503.

Manning, J. T. 1984. Males and the advantage of sex. J. Theor. Biol. 108:215–220.

Michalczyk, L., A. L. Millard, O. Y. Martin, A. J. Lumley, B. C. Emerson, and M. J. G. Gage. 2011. Experimental evolution exposes female and male responses to sexual selection and conflict in *Tribolium castaneum*. Evolution 65:713–724.

Read, A. F., P. A. Lynch, and M. B. Thomas. 2009. How to make evolution-proof insecticides for malaria control. PloS Biol. 7:e1000058.

Reding, L. P., J. P. Swaddle, and H. A. Murphy. 2013. Sexual selection hinders adaptation in experimental populations of yeast. Biol. Lett. 9:20121202.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proc. R. Soc. B 263:1415–1421.

Rundle, H. D., S. F. Chenoweth, and M. W. Blows. 2006. The roles of natural and sexual selection during adaptation to a novel environment. Evolution 60:2218–2225.

Sharp, N. P., and A. F. Agrawal. 2008. Mating density and the strength of sexual selection against deleterious alleles in *Drosophila melanogaster*. Evolution 62:857–867.

Siller, S. 2001. Sexual selection and the maintenance of sex. Nature 411:689–692.

Stuart, J. J., S. Ray, B. J. Harrington, J. J. Neal, and R. W. Beeman. 1998. Genetic mapping of a major locus controlling pyrethroid resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. Econ. Entomol. 91:1232–1238.

Whitlock, M. C., and A. F. Agrawal. 2009. Purging the genome with sexual selection: reducing mutation load through selection on males. Evolution 63:569–582.

Whitlock, M. C., and D. Bourguet. 2000. Factors affecting the genetic load in *Drosophila*: synergistic epistasis and correlations among fitness components. Evolution 54:1654–1660.

Zhu, F., R. Parthasarathy, H. Bai, K. Woithe, M. Kaussmann, R. Nauen, D. A. Harrison, and S. R. Palli. 2010. A brain-specific cytochrome P450 responsible for the majority of deltamethrin resistance in the QTC279 strain of *Tribolium castaneum*. Proc. Natl. Acad. Sci. USA 107:8557–8562.

Associate Editor: T. Kawecki
Handling Editor: M. Servedio

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1: Level of pesticide resistance in generations 0-5 of the selection experiment, and in the common garden.