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SEXUAL SELECTION IS INEFFECTUAL OR INHIBITS THE PURGING OF DELETERIOUS MUTATIONS IN *DROSOPHILA MELANOGASTER*

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The effects of sexual selection on population mean fitness are unclear and a subject of debate. Recent models propose that, because reproductive success may be condition dependent, much of the genome may be a target of sexual selection. Under this scenario, mutations that reduce health, and thus nonsexual fitness, may also be deleterious with respect to reproductive success, meaning that sexual selection may contribute to the purging of deleterious alleles. We tested this hypothesis directly by subjecting replicate *Drosophila melanogaster* populations to two treatments that altered the opportunity for sexual selection and then tracked changes in the frequency of six separate deleterious alleles with recessive and visible phenotypic effects. While natural selection acted to decrease the frequency of all six mutations, the addition of sexual selection did not aid in the purging of any of them, and for three of them appears to have hampered it. Courtship and mating have harmful effects in this species and mate choice assays showed that males directed more courtship and mating behavior toward wild-type over mutant females, providing a likely explanation for sexual selection's cost. Whether this cost extends to other mutations (e.g., those lacking visible phenotypic effects) is an important topic for future research.

KEY WORDS: **experimental evolution, male harassment, mutation load, natural selection, population mean fitness, sexual conflict.**

Two forms of natural selection are often distinguished: that arising from variation in nonsexual fitness (e.g., viability, fecundity, longevity) and sexual selection, arising from differential fertilization success among living individuals. Although Darwin's (1859) original description of sexual selection suggested that it may often increase nonsexual fitness, the focus of sexual selection research subsequently shifted to elaborate secondary sexual traits that are detrimental to nonsexual fitness and whose evolution can therefore only be explained by sexual selection. However, the past decade has seen renewed interest in the population genetic consequences of sexual selection, in particular its role in facilitating adaptation and purging deleterious mutations, thereby potentially increasing population mean fitness (Whitlock and Agrawal 2009).

The effects of sexual selection on nonsexual fitness are unclear; on the one hand, sexual selection has often been thought to reduce population mean fitness due to the evolution of costly sexual displays and preferences for them (Lande 1980; Kirkpatrick 1982; Gavrilets et al. 2001; Houle and Kondrashov 2002). Sexual selection can also generate sex-specific selection and the resulting intra- and/or interlocus sexual conflict may further reduce population mean fitness (Pischedda and Chippindale 2006; Stewart et al. 2008; Bonduriansky and Chenoweth 2009). For example, sex-specific selection on shared traits may cause males and females to have different evolutionary optima, generating intralocus sexual conflict. Because, at least initially, males and females are assumed to have a common genetic basis for such traits, the evolution of sexual dimorphism is impeded and a gender load

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results (Rice and Chippindale 2001). Sexual selection can also lead to interlocus sexual conflict, a process of antagonistic coevolution between loci that increase male sexual fitness at the expense of female nonsexual fitness, and loci that increase female resistance to these harmful male effects (Trivers 1972; Parker 1979). This process drives coevolutionary arms races that have produced many dramatic examples of traits that are beneficial to males while harmful to females (Arnqvist and Rowe 2005). Finally, in species with such male-induced female harm, male mate choice may cause this harm to be disproportionately directed toward otherwise high-quality (i.e., preferred) females, thereby reducing the variance in realized female fitness and hence the strength of selection (Long et al. 2009).

On the other hand, sexual selection may also facilitate adaptation, and particularly the purging of deleterious mutations, in two main ways. First, positive assortative mating by fitness may arise as a by-product of sexual selection, for instance if high-quality males compete more intensely for access to high-quality females, or if costly mate preferences are expressed to a greater degree in high-quality females (Fawcett and Johnstone 2003; Sharp and Agrawal 2009; Whitlock and Agrawal 2009). A positive correlation in fitness between mates will increase the variance in fitness and hence the efficiency of selection (Rice 1998). Second, sexual selection may directly favor individuals of high nonsexual fitness, aligning with selection arising from variation in nonsexual fitness (hereafter "natural" selection for convenience) to promote the fixation of advantageous alleles and the purging of deleterious ones. This may occur via a classic good genes process in which female mate preferences evolve for males of high breeding value for fitness, driven by the indirect benefits females receive from mating with such males (Iwasa et al. 1991; Kirkpatrick and Ryan 1991; Houle and Kondrashov 2002). It may also occur more generally if fertilization success (i.e., male sexual fitness) is condition dependent. Individuals often invest substantial time and effort in reproducing, often going through several steps to successfully reproduce including finding a potential mate, competing directly or indirectly with members of the same sex for access to that mate, courting or coercing them into mating, and finally achieving fertilization when in competition with the sperm from past and future matings (Andersson 1994). If healthier and more vigorous (i.e., higher condition) males are more likely to succeed at these various stages, then alleles that decrease overall health will also tend to decrease reproductive success (Rowe and Houle 1996; Whitlock and Agrawal 2009). Theoretical models, focusing on good genes benefits, suggest that such an alignment of natural and sexual selection may increase the rate and extent of adaptation (Proulx 1999, 2001, 2002; Lorch et al. 2003), aid in the purging of deleterious alleles (Whitlock 2000), and may even be sufficient to contribute to the maintenance of sexual reproduction (Agrawal 2001; Siller 2001).

There are few direct tests of the effects of sexual selection on nonsexual fitness. Several studies have manipulated the opportunity for sexual selection and then measured the consequences for population fitness or components thereof, either in a constant (Partridge 1980; Promislow et al. 1998; Holland and Rice 1999; Martin and Hosken 2003; Radwan 2004; Crudgington et al. 2005; Tilszer et al. 2006) or a novel environment (Holland 2002; Rundle et al. 2006; Fricke and Arnqvist 2007). These studies have provided mixed results, however, and it is unclear what effect, if any, sexual selection has on nonsexual fitness. Three related studies have manipulated the opportunity for sexual selection within the context of the purging deleterious mutations, either using mutation accumulation or following mutagenesis. Results are again mixed. Consistent with a benefit, sexual selection appeared to reduce the mutation load on productivity in *Drosophila serrata* during mutation accumulation (McGuigan et al. 2011), and embryo viability of populations of bulb mites exposed to ionizing radiation increased significantly after one generation of sexual selection (Radwan 2004). In contrast, following exposure to mutagens, *D. melanogaster* populations with reduced sexual selection showed higher reproductive output after 60 generations as compared to those maintained in the presence of sexual selection (Hollis and Houle 2011). Several of the above studies are multigenerational evolution experiments that measured only population mean fitness (or components thereof). The interpretation of the results from such experiments is hampered because changing the opportunity for sexual selection also alters the opportunity for sexual conflict, confounding their effects. In particular, over the limited time frame of these experiments, a short-term increase in fitness when populations are released from sexual conflict may overwhelm longer term benefits of sexual selection in reducing the mutation load, biasing such studies to finding a cost of sexual selection (Whitlock and Agrawal 2009).

An alternative approach to testing the consequences of sexual selection for nonsexual fitness is to measure the effects on male sexual fitness of individual deleterious mutations. Male reproductive success has been quantified for 17 independent deleterious mutations with visible phenotypic effects in *D. melanogaster*, with natural and sexual selection aligning on 13 of these (Whitlock and Bourguet 2000; Pischedda and Chippindale 2005; Stewart et al. 2005; Sharp and Agrawal 2008; Whitlock and Agrawal 2009; MacLellan et al. 2012). While providing some support for the idea that sexual selection may align with natural selection to reduce mutation load, these studies are not sufficient to show that the combined effects of sexual selection (including sexual conflict) serve to decrease deleterious allele frequencies.

A more powerful and straightforward test of the net effect of sexual selection on nonsexual fitness is to track changes in the frequency of individual deleterious alleles across generations when the opportunity for sexual selection is manipulated. Such an

approach allows the effects of selection on the allele to accumulate across generations, and allows all fitness components to be integrated by the evolutionary process itself. In the only application of this approach of which we are aware, Hollis et al. (2008) found that sexual selection increased the rate at which a single alcohol dehydrogenase null allele was purged from replicate experimental *D. melanogaster* populations. Our understanding of the net effects of sexual selection on nonsexual fitness would therefore benefit from additional data from a wider array of deleterious mutations.

In the current study, we manipulated the opportunity for sexual selection in replicate populations of *D. melanogaster* and directly measured the change, across generations, in the frequency of six independent mutations with recessive, visible phenotypic effects. If sexual and natural selection align, populations with stronger sexual selection should purge these deleterious alleles faster than populations with weak sexual selection. Counter to this prediction, we found that, while natural selection acted against these mutant alleles, sexual selection did not aid in their purging and, for three of the mutations, even appeared to inhibit it. Given known harmful effects of courtship and mating in this species (Fowler and Partridge 1989; Partridge and Fowler 1990; Long et al. 2009), we subsequently explored the possibility that male mate preferences may disproportionately direct this harm to wild-type females, thus hampering the response to selection.

Materials and Methods

STUDY POPULATIONS

The experiment used six different recessive mutations with visible phenotypic effects in adult *D. melanogaster*, all originally obtained from the Bloomington Stock Center. Three of these mutations are autosomal (*brown*, *sepia*, *plexus*) and three are X-linked (*yellow*, *white*, *forked*), and of these, three influence eye color (*brown*, *sepia*, *white*), one affects wing morphology (*plexus*), one affects body color (*yellow*), and one affects bristle morphology (*forked*). Each mutation was separately introgressed, via five rounds of backcrossing, into an outbred and laboratory-adapted stock population, as described in MacLellan et al. (2009). One or more rounds of introgression were initiated by a mating in each direction (i.e., stock female × mutant male and mutant female × stock male) to ensure that the mitochondrial DNA and Y chromosome from each introgressed mutant population were derived from the stock. The resulting populations were each fixed for a different recessive mutation yet shared ∼97% of their outbred genetic background with the original stock.

These mutations have been previously used to investigate the importance of varying male mate search ability in generating sexual selection against them (MacLellan et al. 2009), and in quantifying the effects of dietary stress on the strength of selection against them (MacLellan et al. 2012). The mutations are presumably deleterious relative to the wild-type allele at each locus, although only one (*sepia*) significantly reduced productivity relative to the stock in a previous assay, with a second (*white*) approaching significance ($P = 0.066$; MacLellan et al. 2012). Four of them (*brown*, *sepia*, *white*, and *yellow*) significantly reduced male mating success in multiple-choice trials with virgin females.

EXPERIMENTAL EVOLUTION

For each mutation, six replicate populations at Hardy–Weinberg proportions were created by mixing homozygous mutant, heterozygote, and homozygous wild-type individuals to produce an initial mutant allele frequency of 0.7. Three replicate populations were assigned to each of two experimental treatments that manipulated the opportunity for sexual selection: sexual selection present (+S) or sexual selection greatly reduced (−S). Within each population, a female's expected contribution to the next generation was proportional to the number of adult offspring she produced, allowing natural selection to operate unhindered. This design therefore allows us to compare changes in the frequency of the mutant allele caused by natural selection alone with that caused by the combined presence of natural and sexual selection.

Populations were maintained via nonoverlapping generations by allowing 100 adult females to lay eggs for 24 h in two bottles (50 females/bottle), after which the females were discarded. Offspring developed in these bottles and 120 male and 120 female adult virgins were collected at emergence, using light CO_2 anesthesia and pooling all individuals among the two bottles, and then placed in their respective mating treatments to manipulate the opportunity for sexual selection. In the +S treatment, these virgin adults were transferred to two new food bottles (60 males and 60 virgin females each) and allowed to interact and mate for 3 days, allowing the opportunity for various forms of sexual selection including mate choice, male–male competition, and sperm competition. In the −S treatment, the virgin adults were randomly assigned to 110 separate male–female pairs in individual vials, enforcing monogamy and thereby greatly restricting the opportunity for sexual selection. Following the 3-day mating period, groups of 50 randomly chosen females were transferred to each of two new laying bottles (in the +S treatment, the 50 females in a laying bottle all originated from the same mating bottle) such that, except for the mating environment, conditions were similar across treatments throughout the rest of their life cycle. In both the mating and laying environments, enough live yeast was added to the substrate to allow flies to eat ad libitum throughout the experiment.

Due to a slow and variable response to selection after nine generations of the *brown* experimental trials, we attempted to reduce genetic drift by doubling the population size of all six populations for the remainder of the experimental generations

(i.e., 200 females laid eggs to produce the next generation in each). Population density is a stressor that can influence the strength of selection on new mutations (Agrawal and Whitlock 2010); so as not to alter density this doubling was done by creating twice as many bottles/vials every generation in every *brown* population (both for egg laying and in the mating treatments), keeping the number of flies per bottle/vial constant. More generally, across mutations and treatments we attempted to control population egg/larval density by standardizing the number of females and their egg laying times (i.e., 50 females for 24 h), and by keeping sex ratios the same at 1:1 in both mating treatments (to reduce the possibility of differences in total harassment causing female fecundity to vary between +S and −S populations).

Each mutation is visible only when homozygous and the persistence of the deleterious allele was tracked via the frequency of visible mutants (i.e., homozygotes) every generation. At least 200 individuals per population were phenotyped every generation and these individuals were collected both before and after the individuals used to maintain the populations during the evolution experiment, thereby minimizing any bias in the frequency estimates caused by difference in emergence time of mutant versus wild-type individuals. To determine the true frequency of the mutant allele, a set of test-crosses were performed separately for each of the six populations of a given mutation on three (two for *brown*) separate occasions (i.e., generations). These crosses were initiated when the visible frequency decreased below approximately 0.3, although this varied among mutations in response to the rate and consistency of the decline. These crosses involved mating virgin wild-type individuals from an experimental population with a virgin homozygous mutant of the opposite sex. 50–55 crosses were performed per replicate population in each assay and the resulting offspring of each pair were scored for the presence of the mutant phenotype, indicating that the parent from the experimental population was heterozygous at that locus.

To determine whether natural selection acted against each of these mutations, we tested whether the true allele frequency of a particular mutant allele decreased over time in the three replicate −S populations (i.e., the treatment in which "natural" selection acted alone). The analysis treated populations as replicates and used a repeated measures analysis of variance (ANOVA) with generation as a within-subjects factor. Huynh–Feldt-adjusted *P*-values were employed because the assumption of sphericity could not be confirmed due to limiting degrees of freedom (Huynh and Feldt 1976). To determine whether sexual selection influenced the purging of each mutation, we tested whether there was a significant difference in true allele frequency between the two sexual selection treatments (i.e., −S vs. +S), separately for each mutation and again treating populations as replicates. To do this, for each population we calculated the average allele frequency across the 2–3 replicate measures and then used a two-sample *t*-test to determine whether the sexual selection treatment influenced these frequencies. Results from these analyses were qualitatively the same as those from a repeated measures ANOVA of the true allele frequencies that included treatment as a between-subjects factor (and excluded the first generation in which all populations had an initial frequency of the mutant allele of 0.7). We therefore present the former for simplicity.

MALE PERSISTENCE

Sexual selection appeared to hamper natural selection against several of the mutations (see section Results), either overall or early during the evolution experiment when the mutant frequencies were higher. Courtship and mating in *D. melanogaster* are known to be harmful to females, so to provide insight into the contribution of male mate choice in directing such harm preferentially to wild-type females and thereby hampering natural selection, we carried out two independent male choice persistence assays, using the *plexus* and *sepia* mutations, following Long et al. (2009). In each assay, both homozygous wild-type and homozygous mutant males were separately presented with two females, one being a wild-type homozygote and the other being either a mutant homozygote or a heterozygote. Vials were scored every 40 min for male persistence behavior (defined as active courtship, mating, or males facing female at a distance of 5 mm or less, as in Long et al. 2009) recording which (if any) female the behavior was directed toward. A total of seven observations were made for each of 200 vials per trial, with 50 replicates for each of the four combinations of male and female type.

Flies for use in the assays were collected as offspring of known crosses (involving individuals from the wild-type and introgressed-mutant stock populations), using light CO_2 anesthesia, as nonvirgins on day 12 of their life cycle. Males and females were held separately for 24 h in vials that lacked the usual addition of live yeast, at densities of seven individuals/vial. Females were marked by transferring them for an additional 24 h to new vials that contained abundant yeast paste formed by mixing live yeast with red or blue food coloring (McCormick, Canada). The colored yeast the females eat is visible through their abdomen, temporarily marking them either red or blue. All combinations of female color and genotype were tested in a balanced design. Extensive use of such markings in previous experiments has shown no effect on mating patterns (Rundle et al. 1998, 2007; Mooers et al. 1999; Rundle 2003), and there was no evidence of a male preference for red or blue females in the current experiment when testing either the *plexus* (paired *t*-test; wild-type males: $t_{99} = 0.29$, $P = 0.773$; *plexus* males: $t_{99} = 0.11$, $P = 0.92$) or *sepia* (wild-type males: $t_{99} = 0.78$, $P = 0.44$, *sepia* males: $t_{99} = 0.75$, $P = 0.46$) mutations. Assays were set up on day 14 by aspirating, without anesthesia, a single male and two females into a new vial for observation.

Total male persistence directed toward the two females in a vial was expressed as a difference score (persistence toward wild-type female—persistence toward mutant female); these scores were unimodal and symmetrically distributed among vials. A two-way ANOVA was used to determine whether this difference score depended on the male type (homozygous wild-type vs. homozygous mutant) and the identity of the "mutant" female (mutant homozygote vs. heterozygote), separately for each mutation. Paired *t*-tests were then used to determine whether male persistence was preferentially directed toward wild-type vs. mutant females, treating vials as replicates, conducted separately for those treatments (male type and female identity) that differed significantly from the ANOVA.

Results

EXPERIMENTAL EVOLUTION

For all six mutations, the average visible (i.e., homozygous mutant) and true allele frequencies across the three replicate −S populations decreased during experimental evolution from their initial values of 0.7, implying natural selection acted against these alleles (Figs. 1 and 2). For five of the mutations, this decrease resulted in a significant generation effect in a repeated measures analysis of the true allele frequencies (Table 1). Among-population variation was substantial for the sixth mutation (*yellow*), driven in large part by a decrease in allele frequency in two of the replicate populations that was absent in the third (in which both the visible and true allele frequencies varied across generations around the starting value, showing no directional trend; Fig. S1).

In contrast to the natural selection against these mutations, there was no evidence that sexual selection promoted their purging (Figs. 1 and 2). Average allele frequency did tend to be lower in the +S as compared to the −S treatment for one mutation (*forked*), but this difference was not significant (Table 1). For four of the other mutations, average allele frequencies were very similar in the +S and −S populations (*brown*, *plexus*, *white*, and *yellow*), generating no significant treatment effect (Table 1). This did not change qualitatively for *yellow* if the outlier −S population was excluded ($t_3 = -1.98$, $P = 0.142$). A significant effect of sexual selection was present for the sixth mutation (*sepia*) but the difference was in the opposite direction, with the average allele frequency of the deleterious *sepia* allele being higher in the +S compared to the −S populations after 5–7 generations of experimental evolution (Fig. 1; Table 1).

These allele frequency data were collected via test-crosses after a number of generations of experimental evolution and therefore do not directly address potential treatment effects occurring in earlier generations. Two mutations in particular (*brown* and *plexus*; Fig. 1) showed an initial pattern in the frequency of visible mutants (i.e., mutant homozygotes) in which sexual selection

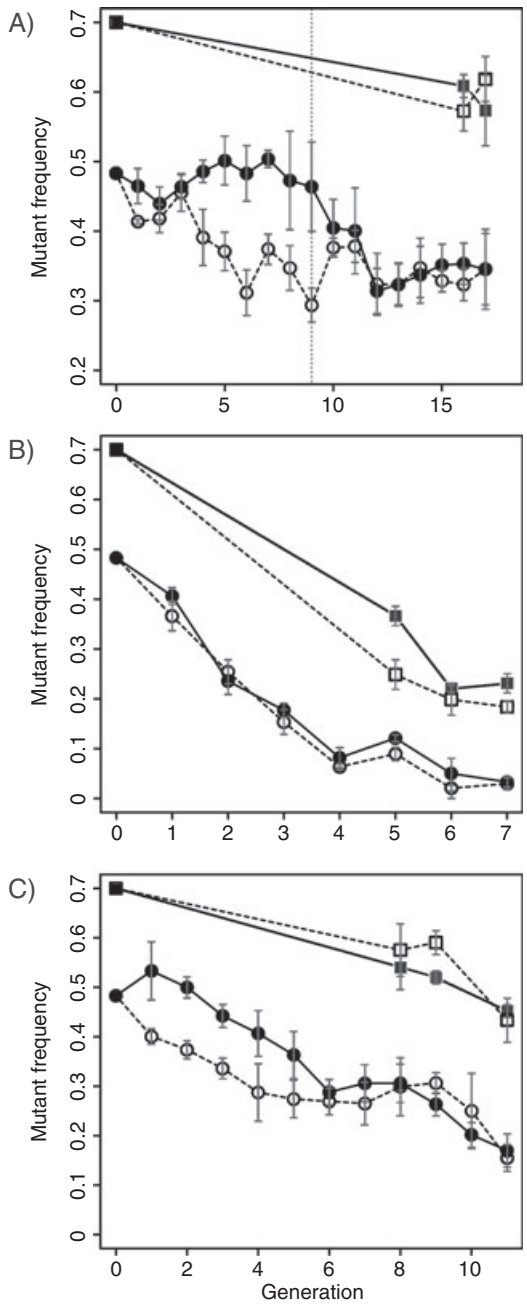

Figure 1. Average (±SE) visible (i.e., homozygous mutant; circles) and true allele (squares) mutant frequencies from the three replicate populations in the +S (solid lines, closed symbols) and −S (dashed lines, open symbols) treatments for the autosomal mutations (A) *brown*, (B) *sepia*, and (C) *plexus*. The vertical dotted line in (A) indicates when population size was doubled (see section Methods).

appeared to hamper purging, but this disappeared in later generations. This varying effect of sexual selection was sufficient to generate a significant treatment × generation interaction in a repeated measures ANOVA of the visible frequency for these mutations (*brown*: $F_{17} = 2.07$, $P = 0.02$; *plexus*: $F_{11} = 2.24$,

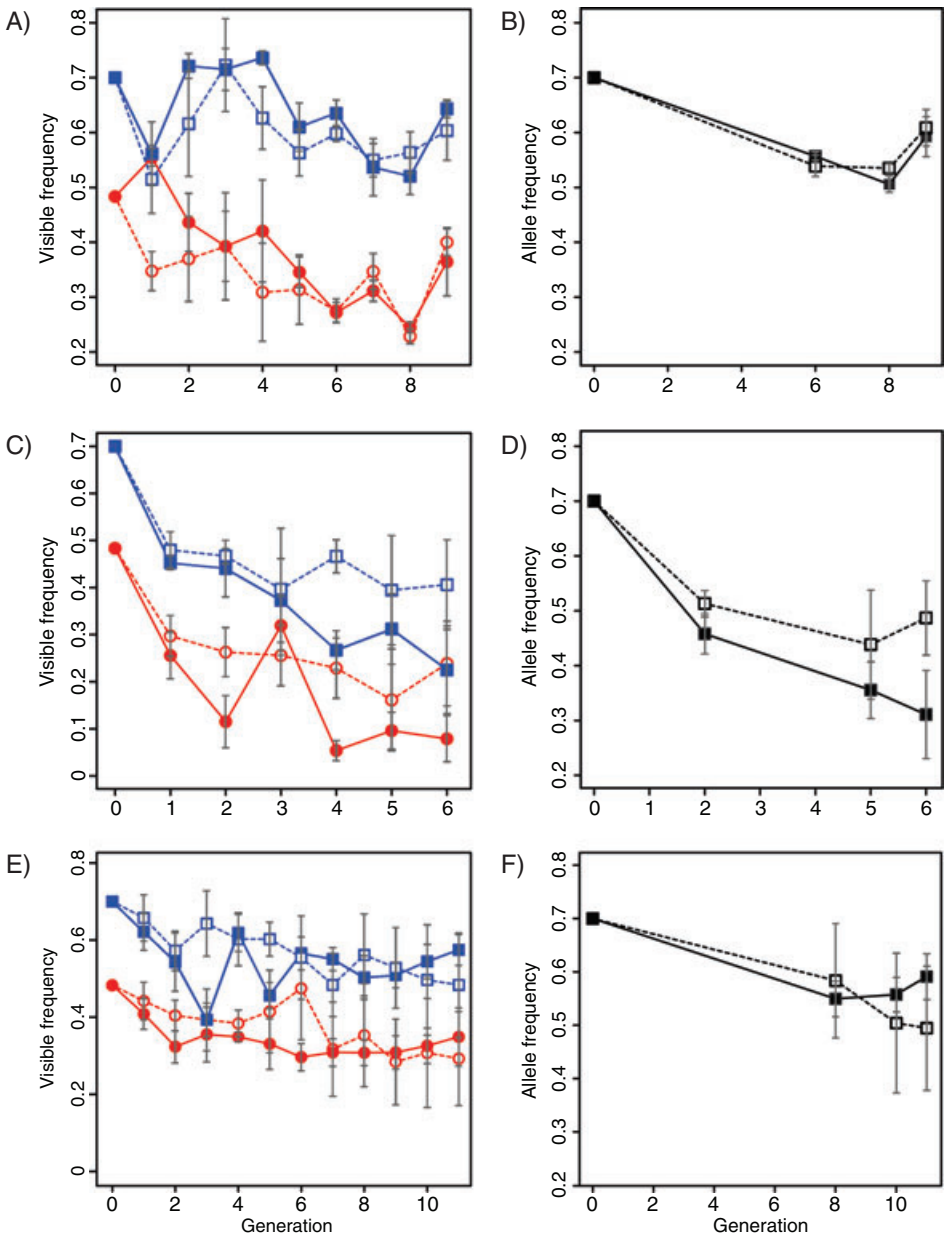

Figure 2. Average (±SE) visible frequency of male (blue) and female (red) mutant individuals (left column, panels A, C, and E), and true mutant allele frequencies (right column, panels B, D, and F), from the three replicate populations in the +S (solid lines, closed symbols) and −S (dashed lines, open symbols) treatments for the X-linked mutations (A and B) *white*, (C and D) *forked*, and (E and F) *yellow*.

$P = 0.03$). For *brown*, the disappearance of this initial hampering effect of sexual selection roughly coincided with the doubling of the population size at generation 9 (Fig. 1A). In particular, prior to this doubling, visible mutant frequency had declined in the −S populations, but not in the +S; after it, visible mutant frequency in the +S populations declined whereas little change occurred in the −S populations.

MALE PERSISTENCE

To gain insight into why sexual selection may have hampered natural selection, male persistence assays were performed for two mutations, one for which sexual selection showed a significantly harmful effect in the analysis of the true allele frequencies (*sepia*), and one for which the effect of sexual selection appeared variable, hampering the decline in visible frequency in the early generations of experimental evolution but not in later generations (*plexus*). For the *sepia* mutation, the difference in male persistence toward wild-type versus mutant females was independent of the identity of the mutant female (heterozygous vs. homozygous mutant female: $F_{1,196} = 0.37$, $P = 0.543$), but showed a borderline significant difference depending on the identity of the males (wild-type vs. mutant homozygote: $F_{1,196} = 3.84$, $P = 0.051$). In particular,

Table 1. Effects of natural and sexual selection on allele frequencies for six replicate mutations.

	Natural selection[1]			Sexual selection[2]	
Mutation	F	df	P	t_4	P
Brown	11.52	2,4	0.022	−0.11	0.917
Sepia	92.55	3,6	0.002	4.35	0.012
Plexus	20.09	3,6	0.022	−0.64	0.556
White	27.27	3,6	0.019	−0.37	0.731
Forked	5.85	3,6	0.033	−1.29	0.277
Yellow	2.47	3,6	0.240	0.32	0.767

[1]Generation effects from repeated measures ANOVA of −S populations. Significance values (*P*) are Huynh–Feldt adjusted.
[2]Two-sample *t*-tests comparing final allele frequencies between sexual selection treatments.

sepia males were significantly more persistent toward wild-type than mutant females (paired t-test, $t_{99} = 2.14$, $P = 0.035$), whereas wild-type males showed no such difference (paired t-test, $t_{99} = -0.52$, $P = 0.301$; Fig. 3A). The interaction of male and female type was nonsignificant ($F_{1,196} = 0.11$, $P = 0.734$). *Sepia* males were also more active than wild-type males overall, displaying significantly more persistence behaviors toward the two females combined (mean number of persistence behaviors ±SE, *sepia* males = 1.38 ± 0.12, wild-type males = 0.77 ± 0.10; two-sample t-test, $t_{198} = -3.86$, $P < 0.001$).

For the *plexus* mutation, the difference in male persistence toward wild-type versus mutant females was independent of the type of male (wild-type vs. mutant homozygote: $F_{1,196} = 1.03$, $P = 0.313$) but varied depending on the identity of the mutant female (heterozygous vs. homozygous mutant female: $F_{1,196} = 4.01$, $P = 0.044$). In particular, males were significantly more persistent toward wild-type females when choosing between these females and *plexus* homozygous females (paired t-test, $t_{99} = 2.17$, $P = 0.032$), but showed no difference when choosing between wild-type and heterozygous females (paired t-test, $t_{99} = -0.78$, $P = 0.440$). Although the interaction between male and female type was nonsignificant ($F_{1,196} = 1.03$, $P = 0.313$), this preferential courtship and mating of wild-type over plexus homozygous female arose largely from wild-type (paired t-test: $t_{49} = 2.56$, $P = 0.014$) as opposed to mutant (paired t-test, $t_{49} = 0.47$, $P = 0.636$) males (Fig. 3B). Wild-type males were also more active than *plexus* males overall, displaying a significantly greater number of persistence behaviors toward the two females combined (mean number of persistence behaviors ±SE, *plexus* males = 0.75 ± 0.09, wild-type males = 1.05 ± 0.11; two-sample t-test: $t_{198} = 2.05$, $P = 0.041$).

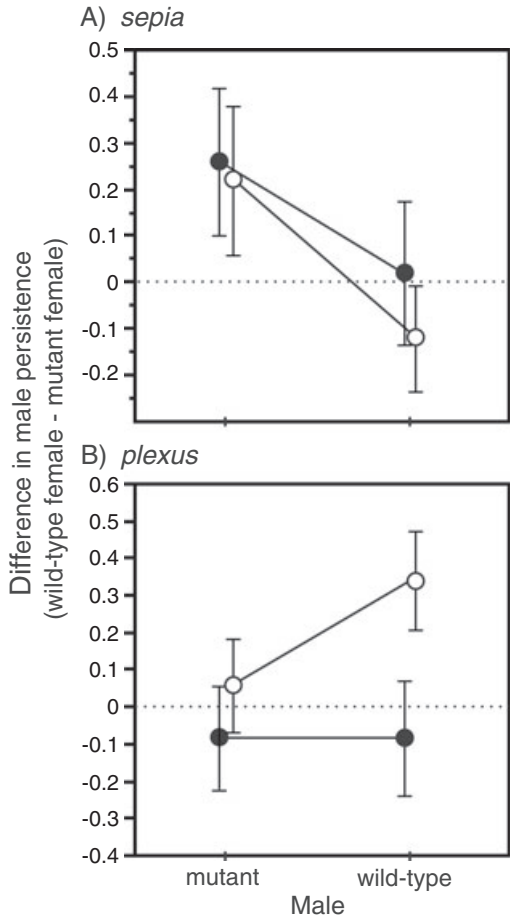

Figure 3. Difference in male persistence behavior toward wild-type and mutant females for the (A) *sepia* and (B) *plexus* mutations. Single males, either homozygous mutant or wild-type, were given a choice between a single wild-type female and either a single homozygous mutant (open circles) or a single heterozygous mutant (closed circles) female. Persistence was quantified as the difference (±SE) in the total number of observed male courtship behaviors and matings involving the two types of females (wild-type—mutant). Positive values indicate that males disproportionately courted and mated wild-type over mutant females.

Discussion

Average allele frequency of all six mutations decreased through time in the absence of sexual selection, confirming that these mutations are deleterious with respect to nonsexual fitness. This decrease was nonsignificant for one mutation (*yellow*), due in large part to a single replicate −S population in which the *yellow* allele showed no directional trend despite decreasing in the other two populations and all three +S populations (Fig. S1). The reason for this discrepancy is unknown, although it is possible that a compensatory mutation that reduced the fitness cost of *yellow* was segregating in the original mutant stock population, or arose de novo early during experimental evolution in this particular replicate. Nevertheless, our conclusions concerning the effects of

sexual selection on *yellow* are robust to the inclusion or exclusion of this single population. Only one of these six mutations (*sepia*) significantly reduced productivity in a previous assay using these same introgressed mutant stocks, with a second approaching significance (*white*; MacLellan et al. 2012). That natural selection against these alleles was detectable in the current experiment is a testament to the power of experimental evolution in integrating net fitness effects across generations.

Contrary to our prediction, sexual selection did not act to promote the purging of any of the six mutations tested. Rather, the addition of sexual selection significantly hampered the purging of the *sepia* mutation and, based on the more extensive visible (i.e., mutant homozygote) frequency data, sexual selection also appeared to slow the initial decline of *brown* and *plexus*, although this effect disappeared in later generations. Four of the tested mutations (*brown*, *sepia*, *white*, and *yellow*) were previously shown to significantly reduce one component of male sexual fitness (mating success with virgin females in multiple choice trials; MacLellan et al. 2012). That the net effects of sexual selection on these mutations, as estimated across generations in the current study, did not promote their purging despite these previous results highlights the danger of drawing general conclusions about net fitness effects from nonevolutionary assays of particular fitness components.

For the *brown* mutation, the disappearance of the hampering effect of sexual selection appears to have coincided with the doubling of the population size (Fig. 1A). This suggests that the initial cost of sexual selection for this mutation may have arisen from a lowering of the effective population size by sexual selection (via increased variance in male reproductive success; Whitlock and Agrawal 2009) to the point at which the *brown* allele was effectively neutral in the +S, but not the −S treatment. The subsequent doubling then reduced drift, allowing selection to act on the mutation in the +S populations. Costs of sexual selection arising from its lowering of effective population size have received little attention, yet will occur even when natural and sexual selection align. Additional, direct tests of this potential cost of sexual selection are an important topic for future work.

Given known or suggested costs to females of courtship and mating in *D. melanogaster*, mediated via male harassment, physical harm during mating, and accessory gland proteins (Fowler and Partridge 1989; Partridge and Fowler 1990; Chapman et al. 1995; Wigby and Chapman 2005; Kamimura 2007; Wolfner 2009), we also sought to determine whether sexual selection's interference in the purging of two mutations (*sepia* and *plexus*) could be explained, at least in part, by male mate choice. In particular, as originally suggested by Long et al. (2009), in species with male-induced harm (like *D. melanogaster*) male mate preferences for high condition females may cause this harm to be disproportionately allocated to high condition (in this case nonmutant) females, decreasing their fecundity and thereby reducing the variance in realized female fitness and inhibiting adaptation. Male mate preferences for larger, and hence more fecund, females have been previously shown in *D. melanogaster*, the consequence of which is a reduction in the variance of female fecundity that should hamper the purging of any deleterious allele that also reduces female body size (Long et al. 2009).

With respect to the *sepia* mutation, mutant males disproportionately courted and mated wild-type over mutant females (both homozygous and heterozygous), whereas wild-type males showed no such preference. *Sepia* males were also significantly more active overall, courting and mating females ∼80% more often during the assay than wild-type males, although this apparently does not translate into increased sexual fitness of these males based on the current results. That mutant males differentiated between wild-type and heterozygous females implies that, despite its recessive phenotypic effects on eye color, this mutation is not fully recessive in its effects on one or more other traits that are targets of male mate choice. Preferential harassment of mutant females by *sepia* but not wild-type males should, all else being equal, lead to a cost of sexual selection that diminishes across generations as the frequency of the *sepia* mutation declines and these males become less common. Although there was no evidence of such a diminishing cost in our results (Fig. 1B), data are limited given the rapid decline and thus short time frame of the experiment with this mutation (seven generations). In addition, mate preferences of heterozygous males were not evaluated; a preference of these males for wild-type over mutant females, including heterozygotes, could maintain a cost of sexual selection at much lower mutant allele frequencies.

For *plexus*, mutant and wild-type males did not differ in their persistence behavior, with both preferentially courting and mating wild-type over homozygous mutant females. When choosing between wild-type and heterozygous females, however, there was no evidence of a significant preference by either type of male, suggesting that the effects of *plexus* are fully recessive in this regard. Such a pattern of male mate choice should produce a diminishing cost of sexual selection because, as the frequency of *plexus* declines, these alleles will to an increasing extent be found primarily in the heterozygous state, therefore shielding wild-type females from preferential male harassment. Changes in the visible frequency of the *plexus* mutation across generations show precisely this pattern (Fig. 1C), with an initial cost of sexual selection that vanishes as allele frequency declines. Whether sexual selection may shift to facilitating the purging of this mutation at low allele frequencies is an interesting possibility for which we lack sufficient data to properly evaluate.

A failure of sexual selection to aid in the purging of these mutations could alternatively be caused by a failure of our treatments to alter sexual selection. As discussed above, the overall cost of

sexual selection in purging *sepia*, and its apparent costs early during the purging of *brown* and *plexus*, are inconsistent with this interpretation. In addition, compared to our monogamous −S treatment, our +S treatment mixed 60 males and females for 3 days, providing extensive opportunities for both male–male interactions, including sperm competition arising from multiple matings, and female mate choice. Indeed, benefits of sexual selection in purging an *Adh* null allele in *D. melanogaster* were previously found in a +S treatment that combined five males and five females for 2 days (Hollis et al. 2008). It is possible that the higher densities in our +S treatment may have increased the opportunity for male harassment and hence sexual conflict, thereby altering the balance between the costs and benefits of sexual selection, and that this might contribute to the differences between our results and those of Hollis et al. (2008). However, this density is reflective of that experienced by our stock populations during their normal life cycle, and the net effects of sexual selection under these conditions are therefore relevant to the evolution of mean fitness in these populations.

In addition to differential male harassment, another cost of sexual selection could arise via density-mediated changes in the strength of natural selection, deriving ultimately from male harassment. Increased population density is one of the few environmental stressors that appear to cause a consistent strengthening of selection against new mutations (Agrawal and Whitlock 2010). If +S females were more harassed than −S females overall, then these females may have had reduced fecundity and subsequently laid fewer eggs than their −S counterparts during the set 24 h laying period every generation. The resulting decreased density in the +S treatment bottles may therefore have weakened natural (i.e., nonsexual) selection against the mutant alleles through decreased larval competition, potentially offsetting other benefits of sexual selection or even generating a net cost. No consistent treatment differences in larval density were observed during our experiments (D. Arbuthnott, pers. obs.) and sex ratios were constant between treatments at 1:1 (suggesting that the average harassment received by females should be the same). Nevertheless, we did not directly control nor measure egg or larval density, so it remains possible that such density-mediated effects contributed to the observed lack of a benefit of sexual selection.

The mutations used in the current study are not representative of a random sample of deleterious mutations. Rather, these mutations have large and visible phenotypic effects when homozygous, and natural selection against them is likely to be stronger than that acting on typical new mutations of small effect. Whether the effects of sexual selection on deleterious mutations depends on the strength of nonsexual selection against them has not been previously considered, although there is no reason to think that these mutations will behave any differently in this respect. However, the visible nature of these mutations may also provide a direct target for mate preferences, potentially causing sexual selection to act differently on them than on other new mutations. In particular, such direct effects may allow individuals to differentiate between mutant and nonmutant mates more easily than would otherwise be possible for deleterious mutations in which the phenotypic effects are visibly manifested only indirectly via changes in condition-dependent traits (e.g., body size). The opportunity for such direct choice could cause sexual selection to enhance or hamper the purging of these mutations. For example, in theory female mate preferences for nonmutant males could reduce the sexual fitness of mutant individuals, causing sexual selection to align with natural selection. Alternatively, the visible effects of these mutations may facilitate increased male persistence of nonmutant females, thereby reducing the variance in female fitness and hampering purifying selection. The net effect of sexual selection on such mutations is therefore an empirical question, and our results suggest that it may often be costly. The effects of sexual selection on the purging of randomly occurring new mutations remains an important, albeit challenging, goal for future studies.

Past studies of sexual selection's effects on nonsexual fitness have used a diversity of approaches and have provided conflicting results. This is true specifically with respect to the purging of deleterious alleles, with several studies suggesting a benefit of sexual selection (Radwan 2004; Hollis et al. 2008; McGuigan et al. 2011) while others, including this study, suggest a cost (Hollis and Houle 2011). These studies differ in the nature of the genetic variance (e.g., individual deleterious alleles vs. those introduced by mutagenesis or mutation accumulation) and the response variable measured, with some addressing the net effect of sexual selection on mean fitness and others addressing the frequency of individual alleles. While it is clear from past results that sexual selection in *D. melanogaster* reduces population mean fitness via sexual conflict (Chippindale et al. 2001; Stewart et al. 2005, 2008; Pischedda and Chippindale 2006; Long et al. 2009; Hollis and Houle 2011), its potential benefits in promoting the purging of deleterious mutations may accrue over much longer timescales, making them hard to detect via short term changes in fitness (Whitlock and Agrawal 2009) or possibly even via effects on the frequency of individual mutations.

In conclusion, theory suggests that sexual selection may increase population mean fitness (e.g., Rowe and Houle 1996; Lorch et al. 2003) and some empirical evidence suggests that sexual selection may increase nonsexual fitness (Promislow et al. 1998; Radwan 2004) and speed adaptation under directional selection (Fricke and Arnqvist 2007). However, our results agree with a number of other studies that failed to find a net benefit of sexual selection (e.g., Holland and Rice 1999; Rundle et al. 2006; Hollis and Houle 2011). Furthermore, direct evidence of preferential male harassment of wild-type over mutant females is consistent with previous results showing a substantial cost of sexual conflict

(e.g., Chippindale et al. 2001; Stewart et al. 2005, 2008; Pischedda and Chippindale 2006; Fricke and Arnqvist 2007; Long et al. 2009). A detailed understanding of the contribution of sexual selection to population mean fitness will require consideration of its short- and long-term effects under constant and novel environmental conditions, and with respect to various types of genetic variance (e.g., segregating vs. new mutations) in a wide variety of organisms, and this remains an important goal in evolutionary genetics.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Average visible (i.e., homozygous mutant) frequency of the *yellow* mutation in each of the three +S (circle, solid lines) and three -S (square, dashed lines) experimental populations.

Supporting Information may be found in the online version of this article.

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