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MALE-BIASED FITNESS EFFECTS OF SPONTANEOUS MUTATIONS IN *DROSOPHILA MELANOGASTER*

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In populations with males and females, sexual selection may often represent a major component of overall selection. Sexual selection could act to eliminate deleterious alleles in concert with other forms of selection, thereby improving the fitness of sexual populations. Alternatively, the divergent reproductive strategies of the sexes could promote the maintenance of sexually antagonistic variation, causing sexual populations to be less fit. The net impact of sexual selection on fitness is not well understood, due in part to limited data on the sex-specific effects of spontaneous mutations on total fitness. Using a set of mutation accumulation lines of *Drosophila melanogaster*, we found that mutations were deleterious in both sexes and had larger effects on fitness in males than in females. This pattern is expected to reduce the mutation load of sexual females and promote the maintenance of sexual reproduction.

KEY WORDS: **Mutations, population genetics, sexual selection.**

A fundamental principle of evolutionary biology is that selection reduces the frequency of alleles that diminish reproductive success. Any locus is a potential target of selection. In populations with separate sexes, sexual selection may represent a major component of total selection across the entire genome. Males may be subject to a different strength or direction of sexual selection than females due to the divergent reproductive strategies promoted by anisogamy. Will this sex-specific selection impede or facilitate evolution by natural selection?

The answer to this question is not entirely clear. As Darwin (1859, 1871) first proposed, in principle, sexual selection can act against any variation that reduces the overall health and vigor of an individual (Rowe and Houle 1996; Whitlock and Agrawal 2009). If this is true, then sexual selection should have a positive impact on adaptation and population productivity (Whitlock 2000; Lorch et al. 2003). In dioecious populations, sexual selection on males could decrease the genetic load of sexually produced offspring, which could help to offset the cost of sex (Agrawal 2001; Siller 2001; Roze and Otto 2011). On the other hand, sexual selection could promote the maintenance of alleles with sexually antagonistic effects, reducing the fitness of sexual females (Rice 1998; Chippindale et al. 2001; Pischedda and Chippindale 2006; Connallon et al. 2010).

Empirical investigations into the net effect of sexual selection on population fitness have met with mixed results (reviewed in Whitlock and Agrawal 2009). This may be due in part to a difference in the way that sexual selection acts on standing variation as opposed to new mutations. Here, we focus on new mutations. Compared to the case where the strength of selection is equal across the sexes, the genetic load L of females at locus i will be reduced whenever selection on mutations in females is less than the average strength of selection across the sexes, $s_{\mathrm{F},i} < s_{\mathrm{AVG},i}$, assuming that mutations are deleterious to both sexes, $s_{\mathrm{M},i}$, $s_{\mathrm{F},i} > 0$ (Whitlock and Agrawal 2009; Roze and Otto 2011). The ratio $\phi_i = s_{\mathrm{F},i}/s_{\mathrm{AVG},i} = 2s_{\mathrm{F},i}/(s_{\mathrm{M},i} + s_{\mathrm{F},i})$ is then a measure of the potential benefits of sex-specific selection, where load at locus i, $L_i = 2\mu\phi_i$, will be reduced when $\phi_i < 1$, where μ is the mutation rate (Whitlock and Agrawal 2009; Connallon

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et al. 2010). Across loci, the mutation load will be reduced when $E[\phi] < 1$.

The most relevant measure of ϕ will reflect not just sexual selection, but total selection on males and females. Although it is reasonable to speculate that sexual selection will be responsible for many of the differences in selection between the sexes, it is important to remember that total selection is what is most relevant to the theory. The empirical challenge, then, is to measure total selection on new mutations in males and females across many loci. There are a few measures of sex-specific selection against individual "marker" mutations (Whitlock and Bourguet 2000; Pischedda and Chippindale 2005; Sharp and Agrawal 2008). Selection on males was found to be stronger than selection on females for many of these mutations, although some had sexually antagonistic effects (Connallon et al. 2010). This sample of mutations is not only very limited, but also unlikely to reflect patterns of selection on spontaneous mutations, because marker mutations tend to have stronger effects than spontaneous mutations on both fitness and the visible phenotype. On the other hand, the relatively mild effects of spontaneous mutations that make them biologically informative also make them difficult to examine individually. A partial solution is to conduct a mutation accumulation (MA) experiment, allowing the combined effects of a large (but usually unknown) sample of mutations to be studied, as in Mallet et al. (2011), discussed below.

In a MA experiment, populations bearing replicated chromosomes are repeatedly bottlenecked, preventing selection against any new mutations that arise. After many generations, the fitness of these chromosomes is expected to decline relative to that of the ancestral genotype if new mutations are deleterious. If sexual selection has the potential to reduce mutation load, we can make several related predictions. First, MA chromosomes should have a larger effect on relative fitness, w, when expressed in males than when expressed in females, $E[S_M]/E[S_F] > 1$, where $S = 1 - w$ is the decline in relative fitness of MA chromosomes. Second, the ratio of the fitness effect on females to the average fitness effect across sexes for individual MA chromosomes, ϕ, should be less than 1 when averaging across MA chromosomes, $E[\phi] < 1$. Finally, there should be a positive intersexual correlation in the fitness effects of MA chromosomes, $C[S_M, S_F] > 0$, indicating that mutations are generally deleterious to both sexes. These predictions come from considering the effects of individual mutations. However, tests of these predictions involve examination of independent MA lines, each of which may contain multiple mutations, which can complicate interpretation (see Discussion).

Recently, Mallet et al. (2011) used an MA approach to examine the effects of spontaneous X-linked mutations on adult fitness in male and female *Drosophila melanogaster*. In this important study, they found that mutations were harmful in both sexes and had stronger effects on males, providing key evidence that intersexual differences in the strength of selection on spontaneous mutations can reduce mutation load. However, Mallet et al. (2011) measured adult sexual fitness, whereas total fitness is the most relevant measure for the theoretical predictions outlined above. This is an important issue because many mutations will affect juvenile and adult fitness components, with effects likely being similar between the sexes at juvenile stages but more different at adult stages. For example, a mutation that reduces adult male fitness by 10% and adult female fitness by 1% would appear to have a 10-fold stronger effect on males, but if the same mutation also reduces juvenile fitness by 5%, then the effect on total fitness would actually differ by only ~2.4-fold between the sexes. Furthermore, relative to the autosomes, the X chromosome may harbor a relative excess of genes with female-biased expression (Meisel et al. 2012) and genes with sexually antagonistic effects on fitness (Rice 1984; Gibson et al. 2002), which could influence patterns of sex-specific selection.

Here, we report measures of the sex-specific effects of spontaneous mutations on fitness using MA lines of *D. melanogaster*. We performed standardized assays of the effects of homozygous mutant autosomes on adult male and female fitness in a common isogenic background. We also incorporated previous estimates of the effects of the same MA lines on juvenile viability, to better reflect total fitness in each sex. Our results corroborate the above predictions, providing further support for the hypothesis that sexual selection can act against deleterious mutations throughout the genome and reduce mutation load.

Methods

MUTATION ACCUMULATION

To examine the effects of spontaneous mutations on males and females, we used a subset of the MA lines described previously in Sharp and Agrawal (2012). In this experiment, all MA lines shared an initially identical "focal" copy of chromosome 2 (40% of the genome), marked with the recessive mutation *bw*. Each generation, a single male from each MA line bearing this chromosome was crossed out to four virgin females bearing recessive marker mutations, derived from a standard stock. The use of phenotypic markers and the lack of recombination in males ensured that the focal chromosome was transmitted intact to each subsequent generation. The MA lines used in this experiment are among those from Sharp and Agrawal (2012) that accumulated mutations on a wild-type genetic background. We also sampled focal second chromosomes from three control populations, each maintained at a size of 450 adults, where selection will be more effective at eliminating deleterious mutations.

Following 52 generations of MA, we conducted a series of crosses to situate focal chromosomes from MA lines and control

populations on a standard isogenic background with respect to the sex chromosomes and the third chromosome, using balancer chromosomes to suppress recombination. The tiny fourth chromosome was not manipulated. Any focal second chromosomes found to have zero homozygous viability (i.e., those with recessive lethal mutations) were not considered further. Although selection can act against mutations in the control populations and should prevent fixation, we expect some deleterious alleles to be present at low frequency, because of new mutations that arose in these populations during the course of the experiment. To better reflect the fitness of the ancestral genotype, we constructed control lines that were heterozygous for the second chromosome, formed by crossing pairs of chromosomes derived from the same control population in a "round-robin" fashion (see Sharp and Agrawal 2012 for details).

To summarize, the mutant and control flies studied were genetically homogeneous apart from homozygous new mutations on the second chromosome in the MA lines and heterozygous new standing variation on the second chromosome in the control lines. Males and females from a given line were also genetically identical (except that males are XY and females are XX). We can therefore attribute sex differences in relative fitness to the differential effects of accumulated mutations on males and females, minimizing any effects of genetic background.

ADULT FITNESS ASSAYS

All experimental flies were collected as virgins, and maintained in same-sex vials of 25 flies, seeded with live yeast, for two to four days prior to fitness assays. To assess fitness, two focal flies of a given sex from a given line (which are homozygous for the *bw* marker) were placed in a vial with two outbred wild-type competitors of the same sex, and four outbred *bw*/*bw* flies of the opposite sex, and allowed to produce offspring for two days. Flies were then transferred to a new vial, allowed to produce offspring for a further two days, and then discarded. The number of marked (*bw*/*bw*) offspring from each vial was scored after 12 days and after a further three days, and these counts were summed. Counts from the two vials for a given replicate were also summed. All vials were coded with random numbers and scored "blind" with respect to sex and treatment. In principle, these measures of adult reproductive success may reflect multiple fitness components, including mating success, fertility, fecundity, survivorship, and postcopulatory processes such as sperm competition.

We assessed male and female fitness in 30 MA lines and 36 control lines (12 from each control population), with six replicates per line per sex for a total of 792, scoring >64,000 offspring in total. We observed some replicate vials (i.e., not entire lines) with unusual numbers of marked (focal) offspring. This may occur because of food quality variation among vials or stochasticity in the quality of competitor flies. Prior to calculating relative fitness, outliers were identified within each group as those where the number of focal offspring was beyond 1.5 times the interquartile range from the upper or lower quartile. We removed 24 outliers in total (∼3% of replicates), which included replicates from male and female mutants and controls.

Control fitness for a given sex, W_{control}, was determined as the mean number of focal flies in each line, averaged across lines from the three control populations. Relative fitness for a given sex for each MA line was assessed as the mean number of focal flies, W_k, relative to the control mean, that is, $w_{\text{M},k} = W_{\text{M},k}/W_{\text{M,control}}$, and $w_{\text{F},k} = W_{\text{F},k}/W_{\text{F,control}}$. Fitness decline for a given sex was defined as $S = 1 - w$.

JUVENILE VIABILITY DATA

The fitness assays described above are designed to estimate selection via differential adult reproductive success by scoring the number of offspring produced. (These measures of adult fitness could include effects of juvenile mortality in their offspring, but these effects should be small because offspring are heterozygous for the focal chromosome). In some analyses, we estimated the total homozygous effects of mutations by incorporating previous estimates of the effects of these MA chromosomes on juvenile viability. The effects of new mutations on juvenile viability (relative egg-to-adult survivorship in the presence of standard competitors, w_{J}) were assessed at MA generations 16, 30, and 46, as described in Sharp and Agrawal (2012). These measures pool the juvenile survivorship of males and females, which we assume are equivalent; this assumption is supported by the strong positive genetic correlation in juvenile viability between the sexes previously observed in this species (Chippindale et al. 2001). Juvenile viability for a given line at MA generation 52 was estimated as $w_{\text{J},k} = e^{52m}$, where m is the slope of log juvenile relative viability for line k on generations of MA, assuming a relative fitness of 1 at generation 0. We estimated the effect of mutations on total fitness as of generation 52 in each line as $w_{\text{M},k,\text{total}} = w_{\text{M},k} \times w_{\text{J},k}$ and $w_{\text{F},k,\text{total}} = w_{\text{F},k} \times w_{\text{J},k}$. For one line, measures of juvenile viability were not available, and we assumed this line had juvenile viability values corresponding to that expected from the average slope of the remaining 29 lines.

STATISTICAL ANALYSIS

All analyses were conducted in the *R* statistical environment (version 2.14.0, R Development Core Team 2011); *P*-values are from two-sided tests. Our first set of analyses focus on the adult fitness components only (the new data reported here). Our subsequent analyses on total selection integrate both juvenile viability and adult fitness effects. We are particularly interested in the ratio ϕ, which we calculated in two ways: at the group level using mean fitness decline across all 30 MA lines for each sex, that is,

$\Phi = 2E[S_F]/(E[S_F] + E[S_M])$, and at the level of individual lines for those lines where mutations were deleterious in both sexes, that is, $\bar{\phi} = E[2S_F/(S_F + S_M)]$, where $S_{K,j}$ represents fitness decline in sex K of MA line j as of generation 52. Comparing the average effect of mutations in one sex to the average effect of mutations in the other sex (Φ) is not ideal, as this assumes that ϕ is constant across mutations, which is unlikely. The values of Φ and $\bar{\phi}$ can be quite different when ϕ varies among loci. For example, if male and female fitness are reduced by 15% and 1%, respectively, on one MA chromosome, and by 8% and 30% on another MA chromosome, the estimate of Φ would be 1.15, indicating that mutations have stronger effects on females, whereas the estimate of $\bar{\phi}$ would be 0.85, reflecting the fact that, on average, mutations have greater effects on males. The measure Φ is heavily influenced by mutations of large effect (exemplified by the second MA chromosome in this toy example) rather than by the magnitude of ϕ for each MA chromosome. Because $\bar{\phi}$ is a direct function of the ϕ value for each MA chromosome, it is a more theoretically relevant measure than Φ, though estimates of the latter will have less uncertainty.

Confidence intervals were obtained by bootstrapping. For each of 10,000 bootstrap replicates, we sampled with replacement 30 MA lines, taking juvenile viability and adult male and female fitness values. In each bootstrap replicate, we also sampled with replacement from control values for all traits (including controls from each of the three time points at which juvenile viability was measured). All metrics (e.g., fitness declines, Φ, $\bar{\phi}$) were then calculated from the resampled data as described above for the true data. (As in the original dataset, $\bar{\phi}$ was calculated excluding any lines for which the calculated point estimate of either S_F or S_M was negative.) This bootstrapping procedure accounts for the uncertainty in our metrics arising from uncertainty in our estimates of MA values as well as control values for each trait.

Results and Discussion

MA caused substantial declines in adult fitness. As of generation 52, adult male fitness had declined by 46.4% (95% CI = (33.8, 57.9%)) relative to controls ($P < 0.001$), and adult female fitness had declined by 30.9% (95% CI = (16.2, 46.5%)) relative to controls ($P < 0.001$). Fitness decline was 1.5 times greater in males, and this ratio is significantly different from 1 ($P < 0.05$). (An alternative maximum-likelihood analysis [not shown] found that the mean relative fitness decline was 0.459 in males and 0.269 in females. Both sexes showed similar increases in variance in relative fitness: 0.225 and 0.257 for males and females, respectively.) We found that male and female fitness values were positively correlated among MA lines (Pearson $r = 0.42$, $P < 0.05$). Unsurprisingly, there was no correlation among controls ($r = 0.13$, $P = 0.43$).

These measures of adult male and female reproductive success suggest that selection against new mutations is stronger in males than in females. However, with respect to the effects of sex-specific selection on mutation load, our interest is in total selection on each sex, rather than measures of adult reproductive fitness alone. We can obtain a more complete picture of the relative strength of selection on each sex by incorporating estimates of selection on juveniles derived from a previous study that included these MA lines (Sharp and Agrawal 2012). Here, we are assuming that mutations have the same effect on each sex at the juvenile stage, and that mutations have multiplicative effects across the life stages.

Mean decline in juvenile viability as of generation 52 was 17.3%. As reported above, the fitness decline from adult effects alone was 46.4% and 30.9% in males and females, respectively. However, it would be more appropriate to compare across traits after excluding measures of adult fitness that were zero, because we excluded MA chromosomes with zero juvenile viability from this study. Even in this case, we find that adult fitness decline was 40.4% in males ($N = 27$), and 26.0% in females ($N = 28$), indicating that adult fitness is more mutationally sensitive than juvenile viability. Mallet et al. (2012) report a similar result comparing the effects of spontaneous mutations on juvenile viability and adult male fitness *D. melanogaster*.

Figure 1 compares estimates of total fitness, which include selection on both juvenile and adult stages, with estimates of adult fitness alone. As expected, estimates of fitness are lower when the juvenile component of selection is included. However, it is clear that adult fitness explains much of the variation in total fitness, as the correlation between adult and total fitness is very high in both sexes (males: $r = 0.90$; females: $r = 0.91$).

Total fitness in males and females is compared in Figure 2. Incorporating juvenile viability, total male and female fitness decline was 54.7% and 40.3%, respectively. Total fitness decline was 1.36 times greater in males, and this ratio is significantly different from 1 ($P < 0.05$). Equivalently, $\Phi = 0.848$, which is significantly different from 1 ($P < 0.05$). However, we can capture more of the variation in ϕ among loci by considering MA lines individually. For each line, we estimated the ratio of selection in females to the average selection across sexes, and calculated the mean among those lines where mutations were deleterious in both sexes according to the point estimates, $\bar{\phi}$ ($N = 25$). We found $\bar{\phi} = 0.800$, suggesting that, on average, individual MA lines had weaker effects on females than males, though this is marginally nonsignificant (bootstrap 95% CI = (0.689–1.024), $P = 0.09$).

We found evidence that spontaneous mutations have stronger effects on males than on females and that there is a positive

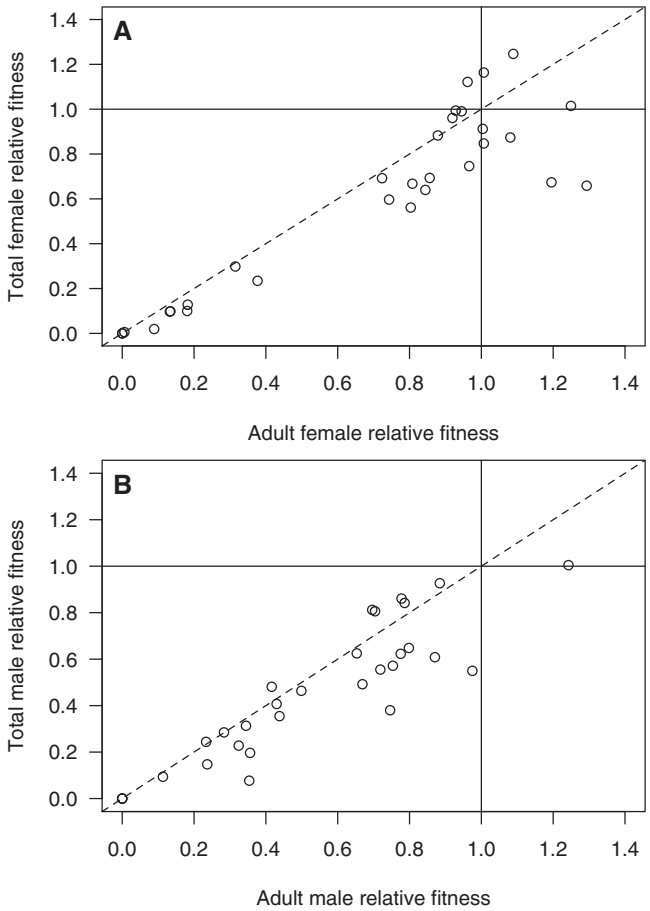

Figure 1. **Comparison of total fitness, incorporating estimates of juvenile viability, to adult fitness alone for females (A) and males (B) from 30 MA lines. Solid lines represent mean control fitness (relative fitness of 1). Dashed lines represent a 1:1 ratio. In (A) two values overlap at (0, 0); in (B) three values overlap at (0, 0).**

correlation in effects between the sexes, suggesting that that sex-specific selection may reduce mutation load. In theory, this pattern can generate stronger selection for sex than that of other forces in models without sex-specific selection (Roze and Otto 2011). In a similar experiment, Mallet et al. (2011) examined the effect of X chromosomes with spontaneously accumulated mutations on adult reproductive fitness, and found that the average effect on male fitness was about 1.5 times larger than the average effect on female fitness (i.e., $\Phi \approx 0.8$). They also identified a positive intersexual correlation for fitness among MA lines. Our results are quite consistent with those of Mallet et al. (2011), but our study differs from theirs in two important respects.

First, we have included the effects of mutations at the juvenile stage, in an attempt to estimate total selection in each sex. It is important to consider the juvenile viability effects of mutations because measuring fitness solely at the adult stage will bias the observed difference in selection between the sexes.

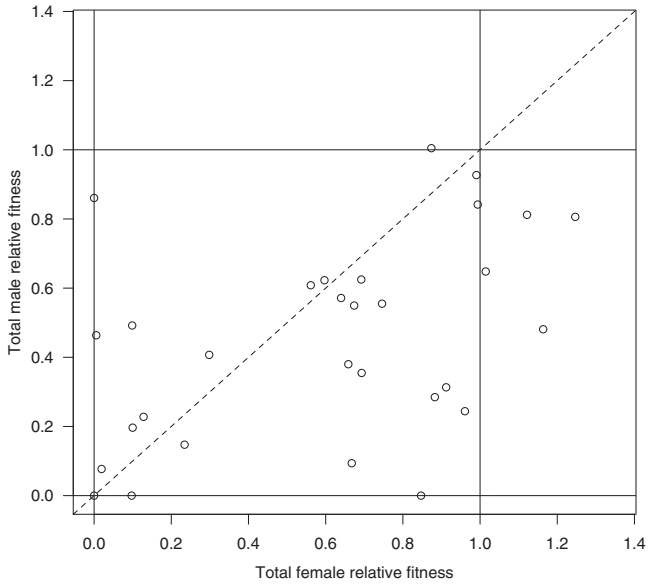

Figure 2. **Total fitness of males and females from 30 MA lines relative to controls, incorporating juvenile viability effects. Solid lines represent mean control fitness for each sex (relative fitness of 1); the dashed line represents equal relative fitness in males and females.**

Second, we have estimated ϕ separately for each MA line, to more appropriately incorporate the effect of variation in ϕ among loci on the mean difference in selection between the sexes. Although $\bar{\phi}$ is more relevant than Φ with respect to theoretical predictions, there are more statistical challenges with measuring the former. Fortunately, our point estimates of these two measures are similar.

We observed a similar pattern of sex-specific selection to that reported by Mallet et al. (2011), but they observed declines in adult fitness in both sexes that were about 1.5 times greater than those observed here (after correcting for differences in chromosome size). It is unclear whether this reflects a pattern of differential selection on the X versus the second chromosome, methodological differences between our studies, or simply estimation error. In any case, all of these data on spontaneous mutations represent a valuable addition to other forms of evidence on this topic, including findings of stronger selection against phenotypic marker mutations in males (Whitlock and Bourguet 2000; Pischedda and Chippindale 2005; Sharp and Agrawal 2008), greater effects of inbreeding depression on males (Mallet and Chippindale 2011), the effect of sexual selection on the rate of MA (Radwan 2004; McGuigan et al. 2011), and others (Whitlock and Agrawal 2009, but see Arbuthnott and Rundle 2012).

To understand the sex-specific effects of mutations, we would ideally estimate ϕ for many individual random spontaneous mutations. Unfortunately, spontaneous mutations are often difficult to study individually. In our experiment, fitness decline in a given

MA line reflects the impact of an unknown number of mutations on fitness. Our metric $\bar{\phi}$ differs from the definition of the true average ϕ among loci because variation in observed ϕ among MA chromosomes will not necessarily capture all of the variation in ϕ among loci. If each MA line carried only one mutation, this would be an exact measure of average ϕ among loci. In each line, we will over- or underestimate the true value of average ϕ when multiple mutations are present and there is variation in ϕ among mutations. If the deleterious mutation rate is 1.2 per genome per generation (Haag-Liautard et al. 2007), we expect there to be approximately $1.2 \times 0.4 \times 0.5 \times 52 = 12.48$ mutations per haploid second chromosome after 52 generations of MA. Based on studies from *Caenorhabditis elegans* (Davies et al. 1999; Bégin and Schoen 2006), it is reasonable to speculate that only a fraction (perhaps 10%) of these will be deleterious in the laboratory. Thus, the "multiple mutation" issue may be relatively minor, though it should be noted that the argument above is based on expected number of mutations and some lines will carry more "laboratory-deleterious" mutations than others. Based on simulations (N. Sharp, unpubl. data) we speculate that estimating average ϕ among chromosomes (i.e., $\bar{\phi}$) will tend to overestimate the true average ϕ across loci. We may therefore be underestimating the true reduction in load due to sex-specific selection.

An additional issue is that when there are multiple mutations in a line, the estimate of ϕ for that line will be driven by the ϕ value of the mutation of largest effect in that line. However, mutations of all effect sizes are of equal importance for determining the relevant average ϕ for mutation load. Thus, our estimate of ϕ in a given line will be biased when there is covariance between effect size and ϕ for individual mutations in that line.

Furthermore, it is difficult to say whether an appreciable fraction of new mutations have sexually antagonistic effects on fitness, both due to the uncertainty associated with fitness estimates of individual lines and because multiple mutations may be present in each line. Although the strength of selection on sexually antagonistic alleles is relevant to the overall impact of sex-specific selection on fitness (Connallon et al. 2010), genome-wide average measures of ϕ should ideally be estimated only for those mutations with sexually concordant effects. Measurement error alone could create the appearance of sexually antagonistic effects in some lines (Fig. 2), even if this is not truly the case. The ϕ estimates from such lines cannot be interpreted biologically in the same way as those from sexually concordant lines and so must be excluded from the calculation of $\bar{\phi}$. If apparent sexual antagonism is simply due to error, then this exclusion of data is unfortunate, and could potentially lead to bias. In contrast, it is possible to include such lines when estimating Φ, which is preferable as long as sexually antagonistic effects are only due to measurement error. Because we cannot be certain whether sexually antagonistic effects are real or not, it is useful to calculate both measures, and reassuring that the two estimates are similar in our dataset. The observed decline in fitness in both sexes and positive intersexual correlation among lines suggests that most new mutations have sexually concordant deleterious effects, although the intersexual correlation among lines may overestimate the true correlation of allelic effects (pleiotropy) between the sexes (Keightley et al. 2000). In addition, if the assay conditions are novel relative to the ancestral laboratory environment, this could inflate estimates of the intersexual correlation by making selection more concordant between the sexes (Long et al. 2012).

Although viable males and females could be obtained from all lines (any line with zero homozygous viability was excluded from this study), we observed that some MA lines had zero adult fitness: one in both sexes, and three in a sex-specific fashion (Fig. 2). This could reflect the presence of recessive mutations that cause sterility, or prevent reproduction through their effects on behavior. The majority of sterility mutations in *D. melanogaster* are thought to be sex-specific, but alleles that cause sterility in both sexes are also known (Ashburner et al. 2005).

In conclusion, data on the sex-specific effects of new mutations from this study and others suggest that sexual selection can have an important impact on the frequency of deleterious alleles throughout the genome, improving population mean fitness. However, evidence from additional species is still needed to assess the generality of this result.

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