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MATING DENSITY AND THE STRENGTH OF SEXUAL SELECTION AGAINST DELETERIOUS ALLELES IN *DROSOPHILA MELANOGASTER*

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Deleterious alleles constantly enter populations via mutation. Their presence reduces mean fitness and may threaten population persistence. It has been suggested that sexual selection may be an efficient way by which deleterious alleles are removed from populations but there is little direct experimental evidence. Because of its potential role in mutational meltdowns, there is particular interest in whether the strength of sexual selection changes with density. For each of eight visible markers in *Drosophila melanogaster* we have compared the strength of sexual selection at two densities. We find evidence of strong sexual selection against most but not all of these alleles. There is no evidence that sexual selection tends to be stronger (or weaker) at high density relative to low density. In addition, we also measure the effects of these mutations on two key parameters relevant to population productivity—juvenile viability and female fecundity. In most cases, sexual selection is as strong or stronger than these other forms of selection.

KEY WORDS: Deleterious alleles, density, *Drosophila melanogaster*, fecundity selection, mutation load, sexual selection, viability selection.

All populations carry deleterious mutations because of recurrent mutation. As a consequence, not all individuals are as fit as they could be. This reduction in the mean fitness of individuals is known as the mutation load. Under some simplifying assumptions, Haldane (1937) determined the mean fitness at mutation–selection balance to be $\bar{w} = e^{-U}$, where U is the genome-wide rate of deleterious mutation. This result implies that mutation load can greatly reduce the mean fitness of populations if mutation rates are not too low. For example, if there is, on average, a single deleterious allele per genome per generation ($U = 1$), then $\bar{w} = 0.37$, indicating that deleterious mutations reduce mean fitness by over 60%.

Although one can quibble with the assumptions underlying Haldane's equation, his result illustrates the potential for populations to be burdened by large mutation loads provided the mutation rate is sufficiently high (i.e., on the order of 1). Prior to the turn of the millennium, estimates of the mutation rate varied over three orders of magnitude and it was unclear whether mutation rate was high enough to inflict a serious load (Keightley and Eyre-Walker 1999; Lynch et al. 1999). However recent evidence (Denver et al. 2004; Haag-Liautard et al. 2007) suggests that mutation rates are likely to be high. In part, this is because comparative genomics suggests that large amounts of noncoding DNA are under selection, thus vastly increasing the target size for deleterious mutation (Andolfatto 2005; Keightley et al. 2005; Halligan and Keightley 2006).

Given the potential for large reductions in mean fitness due to deleterious alleles, evolutionary biologists have wondered whether mutation load may be important in understanding other evolutionary phenomena such as population persistence (Lande

1994; Lynch et al. 1995), the evolution of sex (Kondrashov 1982; Agrawal 2001; Agrawal and Chasnov 2001; Siller 2001; Keightley and Otto 2006), the evolution of selfing versus outcrossing (Lloyd 1979; Charlesworth 1980; Lande and Schemske 1985), the evolution of specialists versus generalists (Whitlock 1996; Kawecki et al. 1997), and the evolution of genome size and complexity (Lynch and Conery 2003). With respect to population persistence as well as competition between obligately sexual and obligately asexual groups, one can focus on the mean fitness of females because they are the primary determinants of a population's reproductive output. Females will have higher fitness then expected under Haldane's equation if sexual selection on males serves as a strong selective sieve against deleterious mutations (Whitlock 2000; Agrawal 2001; Siller 2001). In essence, females will carry fewer deleterious alleles if these mutations are removed from the population via strong selection on males.

Sexual selection will only reduce the mutation load experienced by females if most new mutations that are deleterious with respect to viability and/or fecundity are also deleterious with respect to male mating success. There are both conceptual reasons to believe that sexual selection acts against most deleterious alleles as well as some indirect evidence (e.g., Mulcahy 1979; Rowe and Houle 1996; Welch et al. 1998; Drickamer et al. 2000; Evans and Magurran 2000; Radwan 2004; Dolgin et al. 2006, reviewed in M. C. Whitlock and A. F. Agrawal, unpubl. ms.). However, there is very little direct evidence showing that sexual selection acts against deleterious alleles (see Discussion).

In addition to simply knowing whether sexual selection acts against deleterious alleles, it is important to understand how this selection changes with varying ecological conditions. The effect of density is particularly important in the context of population persistence. This becomes clear when considering conventional thought on how deleterious mutations might contribute to extinction.

Under normal conditions, most populations are capable of sufficient reproductive excess such that they are able to thrive even in the face of substantial mutation load. However, if a population is reduced in size by extrinsic factors (e.g., climate change, habitat loss, invasive species, elevated predation), drift becomes a more powerful evolutionary force. Some deleterious alleles that were previously held at low frequencies by selection fall into the zone of "effective neutrality" at this reduced population size ($s \ll 1/4N_e$) and are able to drift to high frequency or even fixation. As more deleterious alleles reach high frequency, mean fitness declines and the population becomes intrinsically less healthy. If the accumulation of deleterious alleles reduces mean fitness sufficiently, then the population will no longer have the reproductive capacity to sustain itself and population size will decline further even in the absence of extrinsic stresses. As the population size continues to decline, drift becomes progressively stronger, allowing even more deleterious alleles to reach high frequencies, further reducing mean fitness and dooming the population to extinction. This is the so-called "mutational meltdown" process (Gabriel et al. 1993).

Although existing mutational meltdown models (e.g., Lynch et al. 1995; Schultz and Lynch 1997) have not considered the role of sexual selection, it seems logical that its inclusion would reduce the likelihood of meltdown. This is because females would enjoy higher mean fitness if deleterious mutations were being eliminated from the population via sexual selection on males. However, the importance of sexual selection is likely to depend on how its strength changes with density (assuming density scales with population size). First, consider a scenario in which sexual selection becomes weaker at lower densities. As population size declines, deleterious alleles that were previously held at low frequencies by sexual selection will be able to increase because of the reduced strength of sexual selection in addition to the increased power of drift, thus accelerating the decline of female fitness. On the other hand, if sexual selection becomes stronger at lower densities, then the elevated strength of sexual selection at lower population sizes will prevent the increase of deleterious alleles that may otherwise have occurred with stronger drift. In this case, sexual selection may retard or prevent mutational meltdowns. In addition to mutational meltdowns, there are other contexts (e.g., sex vs. asex) in which density-dependent changes in the strength of sexual selection may lead to alternative evolutionary outcomes. In general, the mutation load of females will change with density if sexual selection against males is density dependent.

There is no general theoretical prediction for how density will affect the strength of sexual selection. Conceptually, it is easy to imagine scenarios in which the strength of sexual selection may increase or decrease as density declines. For example, consider the strength of sexual selection from the perspective of male–male competition. At low density, females may be dispersed over larger areas making it difficult for any male (regardless of genotype) to monopolize more than a single female (i.e., sexual selection is weak at low density). Alternatively, sexual selection may be stronger at low density because only the best males have the energy to find and court multiple females. Empirical study is the only way to determine how, and if, sexual selection changes with density.

We have studied selection on eight visible markers in *Drosophila melanogaster*, measuring their effects on both egg-to-adult viability and female fecundity. Most importantly, we have estimated the strength of sexual selection against each of these markers under two different densities. This is the largest dataset for which selection against specific mutations has been measured separately for juvenile, male, and female fitness components. To our knowledge, this is the first dataset to compare the strength of sexual selection against specific mutations at different densities. These density data are relevant not only to the conceptual issues

discussed above with respect to mutation loads and mutational meltdowns but also to the more practical issue of experimentally measuring sexual selection in *D. melanogaster*. As reported below, our data indicate that there is no general tendency for sexual selection assays employing high mating density to be more sensitive than assays at low mating density.

Methods

STOCK POPULATIONS

Flies were obtained from a large outbred laboratory population (Dah) of *D. melanogaster* originally collected in 1970 in Dahomey (now Benin) West Africa, and maintained in the current laboratory for over three years. Eight deleterious phenotypically dominant mutations obtained from the Bloomington *Drosophila* Stock Center were introgressed into the Dah background for at least six to ten backcross generations. Each mutation has visible effects on eyes (*Dr*, *Gla*, *L*), wings (*Ly*, *U*), bristles (*Pin*, *Sb*), or body color (*Frd*). Although these primary visible phenotypes allow for easy scoring, it is reasonable to assume that all of these mutations have a variety of other pleiotropic effects. This is illustrated by the fact that all of these markers affect juvenile survivorship (as shown below) even though the phenotypes for which these genes were named apply to adult characters. All stocks were cultured at 25°C on a 12L:12D cycle, with 70% RH.

SEXUAL SELECTION AND VIABILITY EFFECTS

Experimental flies were raised on a standard yeast-sugar medium in 10-dram vials at moderate density and collected as virgins. Heterozygous mutant males (*M*/+) and wild-type (+/+) males were collected from the same stock vials; wild-type (+/+) females were collected from a separate set of vials. Flies were housed in same-sex, same-genotype vials for 2–4 days prior to the mating trials on media seeded with live yeast, at a density of 15–20 flies per vial. Mutant and wild-type males were always the same age in a replicate. On average, flies did not differ in age across treatments.

Mating trials were conducted in 13.5-dram vials on media seeded with live yeast. Low mating density trials consisted of 12 wild-type (+/+) females, eight heterozygous mutant (*M*/+) males, and four wild-type (+/+) males. High mating density trials consisted of 36 wild-type females, 24 mutant males, and 12 wild-type males, that is, the two density treatments differed in density by a factor of three. In both treatments, one-third of the offspring are expected to carry the mutant allele if there is no natural or sexual selection against the mutation. Mating trials lasted approximately 48 h, providing some opportunity for both pre- and postcopulatory effects to contribute to sexual selection against mutant males. Only wild-type females were used in these mating trials because deleterious alleles are expected to be sufficiently rare in natural populations that a male carrying a mutation is unlikely to encounter females carrying the same mutant allele. Consequently, sexual selection against a mutation will be imposed primarily by females who do not carry that allele.

At the end of the 48-h mating period groups of 10 randomly chosen females were removed to egg-laying vials. There was one group of 10 females for every low-density replicate, and three such groups for every high-density replicate. Females were allowed to lay eggs in standard, yeasted vials for approximately 24 h, and then for a further 24 h in a second egg-laying vial. This procedure ensures that larval density is reasonably constant between density treatments because there were always 10 females per egg-laying vial, regardless of mating density. These egg-laying vials were scored after 15 days for the number of *M*/+ and +/+ offspring. For a given replicate, offspring counts were summed across first and second egg-laying vials to determine the proportion of *M*/+ offspring. (For high-density treatment replicates, offspring were summed across all three first egg-laying vials and all three second egg-laying vials.)

For a particular density treatment, the extent to which the proportion of offspring carrying the mutation is reduced below one-third is indicative of a reduction in the reproductive success of mutant males, as well as reduced viability of mutant offspring. To quantify the viability effects of these mutations, viability tests were preformed using a method identical to that of the mating trials, as described above, but using 12 wild-type females and 6 heterozygous mutant males per mating group. In these trials, it is not possible for sexual selection to act against the mutation because all males are identical with respect to the focal gene, so a reduction below the expected proportion (in this case one-half) of offspring carrying the mutation is indicative of viability effects only. As described below, we use the data from these "viability" trials to estimate viability selection and then estimate sexual selection from the mating trials after removing the effect of viability selection.

Table 1 shows the number of replicates performed for each mutation in each type of trial. In total 364,941 offspring were scored from the mating trials, and 56,537 offspring were scored in the viability trials.

We compared the strength of sexual selection at high and low densities by examining the frequency of marked offspring produced by females from the mating trials. Although viability effects contribute to the observed frequencies in the sexual selection test vials, these effects should be approximately equal in both mating density treatments. There should be no systematic difference in the average larval density between density treatments as all vials from which offspring were scored had the same number of females (10) laying eggs in them for the same period of time. The proportion of marked offspring from each replicate was arcsine square root transformed for analysis. These data were analyzed with a random coefficients model in *SAS* using Proc Mixed with

Table 1. Number of replicates (average number of offspring scored per replicate). Data for fecundity assays are number of marked and wild-type females assayed (average number of eggs per female).

Gene	Viability	Sexual selection		Fecundity	
		High density	Low density	Wild-type	Mutant
Dr	30 (128)	57 (598)	59 (202)	45 (26)	42 (30)
Frd	32 (321)	51 (882)	52 (302)	48 (45)	41 (37)
Gla	30 (141)	52 (524)	52 (176)	40 (34)	33 (30)
L	38 (149)	51 (505)	53 (184)	47 (31)	43 (29)
Ly	30 (174)	49 (639)	50 (242)	44 (50)	48 (40)
Pin	31 (158)	51 (504)	53 (184)	48 (33)	46 (32)
Sb	30 (310)	51 (674)	49 (227)	46 (34)	42 (32)
U	33 (316)	52 (883)	52 (309)	44 (41)	48 (43)

gene and mating density as fixed effects. Because there was considerable variation within treatments in the number of offspring per vial, the total number of offspring per vial was included as a covariate to help reduce variation arising from density-dependent larval viability effects. The model allows the relationship with total number of offspring per vial to differ among genes and mating density treatments (i.e., random coefficients).

FEMALE FECUNDITY

To produce the flies for fecundity measurements, wild-type (+/+) females were mated to (*M*/+) heterozygous males to produce +/+ and *M*/+ offspring. These experimental flies were raised on a standard yeast-sugar medium in 10-dram vials at moderate density. Heterozygous mutant females and corresponding wild-type females were collected as virgins from the same stock vials; wild-type males were collected from a separate set of vials. Mutant and wild-type females were held together in vials seeded with live yeast for 2–4 days, and were then each mated individually to two wild-type males. Matings took place in grape-agar vials, seeded with live yeast. After 24 h, females were transferred to new grape vials without live yeast (males were discarded). Grape media allows eggs to be easily visualized, but likely limited females nutritionally. Females were flipped into new grape vials every 24 h for four days, and daily egg production was recorded. Vials from the first day were retained to confirm that each female was producing viable eggs. Females that did not produce viable eggs or that did not live to the end of the experiment were excluded from the analysis.

ESTIMATING SELECTION

Let the relative egg-to-adult viability of wild-type (+/+) and mutant (*M*/+) flies be $w_{v[+/+]} = 1$ and $w_{v[M/+]} = 1 - s_v$. The expected frequency of marked individuals among the surviving adults is

$$E[f_{a[M/+]}] = f_{z[M/+]}(1 - s_v)/(1 - f_{z[M/+]}s_v), \quad (1a)$$

where $f_{z[M/+]}$ is the frequency of the mutants among zygotes. In our viability vials, we expect $f_{z[M/+]} = 1/2$ so that equation (1a) reduces to

$$E[f_{a[M/+]}] = (1 - s_v)/(2 - s_v) \quad (1b)$$

Rearranging, we can estimate the strength of viability selection as

$$\hat{s}_v = \left(1 - 2\hat{\bar{p}}_{v[M/+]}\right)/\left(1 - \hat{\bar{p}}_{v[M/+]}\right), \quad (2)$$

where $\hat{\bar{p}}_{v[M/+]}$ is the observed mean frequency of mutant adults surviving in viability test vials. This calculation assumes that 50% of the offspring sired by *M*/+ males will come from *M*-bearing sperm. If there is sperm selection, then our estimates of viability selection will be overestimated. However, this seems unlikely given that only a tiny fraction of genes are expressed in sperm (Dorus et al. 2006) and none of the genes we studied are among those known to be expressed in sperm (according to supplementary table 1 of Dorus et al. 2006).

Let the relative mating success or, more accurately, the relative siring success of wild-type (+/+) and mutant (*M*/+) males be $w_{s[+/+]} = 1$ and $w_{s[M/+]} = 1 - s_s$. The expected frequency of mutant gametes among the pool of successful gametes is

$$E[f_{g[M]}] = \tfrac{1}{2}\, f_{m[M/+]}(1 - s_s)/(1 - f_{m[M/+]}s_s), \quad (3)$$

where $f_{m[M/+]}$ is the frequency of mutants among breeding males; in our sexual selection vials, $f_{m[M/+]} = 2/3$. The $\frac{1}{2}$ in the numerator of equation (3) comes from the fact that only half of the gametes of *M*/+ males will carry the marker mutation. Of course, we did not directly observe successful gametes; rather we scored the offspring that survived to adulthood. Consequently, viability selection will also affect the frequency of marked offspring in our sexual selection vials. Considering both sexual selection and viability selection, the expected frequency of mutants among those offspring surviving to adulthood in our sexual selection vials is

$$E[f_{a[M/+]}] = \frac{(1 - s_s)(1 - s_v)}{3 - s_s(2 - s_v) - s_v} \quad (4)$$

Rearranging equation (4) and combining with equation (2), we can estimate the strength of sexual selection as

$$\hat{s}_s = \frac{2\hat{\bar{p}}_{s[M/+]} - \hat{\bar{p}}_{v[M/+]}(1 + \hat{\bar{p}}_{s[M/+]})}{\hat{\bar{p}}_{s[M/+]} - \hat{\bar{p}}_{v[M/+]}}, \quad (5)$$

where $\hat{\bar{p}}_{s[M/+]}$ is the observed mean frequency of marked individuals among the surviving adults in the sexual selection test vials.

Let the fecundity of wild-type (+/+) and mutant (*M*/+) females be $w_{f[+/+]} = k$ and $w_{f[M/+]} = k(1 - s_f)$. Fecundity selection is estimated as

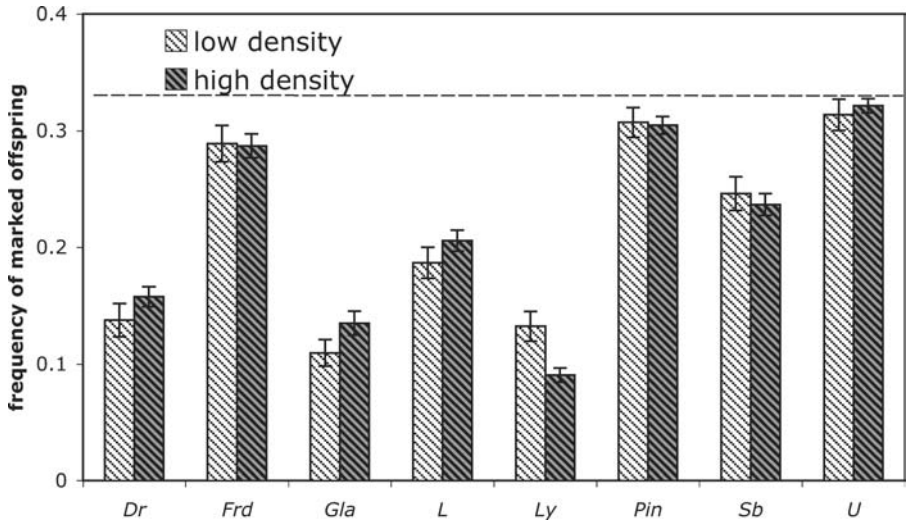

Figure 1. The effect of density on the mean frequency of marked offspring (±1 SE). The dashed line shows the expected frequency under no natural or sexual selection (1/3). Untransformed data are shown here but frequency data were arcsine square root transformed for analysis.

$$\hat{s}_f = \frac{\hat{\bar{n}}_{e[+/+]} - \hat{\bar{n}}_{e[M/+]}}{\hat{\bar{n}}_{e[+/+]}}, \qquad (6)$$

where $\hat{\bar{n}}_{e[x]}$ is the observed mean number of eggs produced by females of genotype x.

The estimators for s_v, s_s, and s_f presented above are known to be negatively biased. Using simulations we determined that the degree of bias was small; in all cases the bias was smaller than −0.02. Ninety-five percent confidence intervals were determined for all selection coefficients by bootstrapping. Simulations indicated that the bootstrap confidence intervals behave as expected, that is, the confidence intervals on simulated datasets included the true parameter value in approximately 95% of simulations.

Results

EFFECTS OF MATING DENSITY

We compared the frequency of mutant adult offspring from the high and low mating density treatments of the sexual selection test vials for each of the eight genes (Fig. 1). As expected, there was a highly significant effect of gene ($F_{7,809} = 16.48$, $P < 0.001$) indicating that the average amount of selection differed across genes. There was no main effect of mating density ($F_{1,809} = 0.00$, $P = 0.998$). However, there was a strongly significant interaction effect between gene and mating density ($F_{7,809} = 2.75$, $P = 0.008$) indicating that mating density had different effects on the strength of sexual selection against mutants for different genes.

We attempted to decompose this interaction with least square mean contrasts for each gene between the two densities (Table 2). Visual inspection of raw means (shown in Fig. 1) and least square means (Table 2) suggests that selection for some genes is stronger at high mating density but stronger at low mating density for other genes. However, there is no statistical support for any effect of mating density on the strength of sexual selection for five of the genes (*Frd, L, Pin, Sb, U*). There was a lower proportion of mutant *Ly* flies in the high-density treatment than in the low-density treatment ($P = 0.017$), implying that sexual selection against *Ly* was stronger at high density. There was weak evidence in the opposite direction for *Dr* and *Gla*. For both of these loci the proportion of mutant flies in the low-density treatment was lower than in the high-density treatment (*Dr*: $P = 0.071$; *Gla*: $P = 0.092$), implying stronger sexual selection at low density. These *P*-values are marginal but the contrasts should be considered in light of the overall model. Our main result is that there is no main effect of mating density but there is a highly significant interaction, implying that

Table 2. Least square mean estimates and contrasts from random coefficients model of the frequency of marked offspring (arcsine square root transformed) from sexual selection trials.

Gene	Least squares mean estimate (SE)		Contrast	
	Low density	High Density	$F_{1,809}$	P
Dr	0.371 (0.035)	0.417 (0.031)	3.26	0.071
Frd	0.560 (0.052)	0.547 (0.048)	0.19	0.667
Gla	0.267 (0.033)	0.311 (0.028)	2.84	0.0923
L	0.456 (0.034)	0.4803 (0.03)	0.087	0.352
Ly	0.380 (0.040)	0.312 (0.034)	5.70	0.017
Pin	0.559 (0.034)	0.552 (0.030)	0.08	0.777
Sb	0.538 (0.036)	0.519 (0.032)	0.047	0.494
U	0.600 (0.054)	0.596 (0.049)	0.02	0.882

Table 3. Estimates of selection against mutant alleles.

Gene	Selection estimate (95% confidence interval)			
	Viability $\hat{s}_v$	Fecundity $\hat{s}_f$	Sexual selection	
			Low density, $\hat{s}_{s,low}$	High density, $\hat{s}_{s,high}$
Dr	0.25 (0.18, 0.31)	−0.16 (−0.39, 0.03)	0.73 (0.63, 0.80)	0.67 (0.59, 0.73)
Frd	0.16 (0.13, 0.20)	0.07 (0.03, 0.32)	0.05 (−0.30, 0.29)	0.07 (−0.15, 0.24)
Gla	0.48 (0.40, 0.54)	0.10 (−0.06, 0.24)	0.69 (0.57, 0.78)	0.57 (0.41, 0.68)
L	0.22 (0.12, 0.31)	0.05 (−0.08, 0.17)	0.59 (0.44, 0.69)	0.51 (0.36, 0.61)
Ly	0.40 (0.31, 0.48)	0.21 (0.07, 0.33)	0.66 (0.52, 0.76)	0.80 (0.75, 0.85)
Pin	0.18 (0.12, 0.24)	0.05 (−0.11, 0.17)	−0.17 (−0.62, 0.11)	−0.15 (−0.42, 0.05)
Sb	0.13 (0.07, 0.19)	0.07 (−0.08, 0.20)	0.40 (0.20, 0.54)	0.45 (0.32, 0.54)
U	0.23 (0.19, 0.28)	−0.04 (−0.17, 0.07)	−0.47 (−1.12, −0.08)	−0.61 (−1.03, −0.34)

sexual selection on different genes is affected differentially by mating density. The *P*-values from our contrasts suggest that although we do not have the statistical power to determine with high confidence which genes are responsible for this interaction, three genes stand out as the best candidates (*Dr, Gla, Ly*).

SELECTION ESTIMATES FOR DIFFERENT FITNESS COMPONENTS

Selection estimates and their 95% confidence intervals for each mutation are shown in Table 3 and Figure 2. We considered selection to be statistically significant if 95% confidence intervals did not overlap zero. There was significant viability selection against all eight mutations. Point estimates of viability selection were fairly high, $\hat{s}_v = 0.13 - 0.48$. There was significant sexual selection (at both densities) against five of the mutations: *Dr*, *Gla*, *L*, *Ly*, and *Sb*. In contrast, there was significant sexual selection favoring the *U* mutation. For the remaining two loci the confidence intervals overlapped zero. Fecundity selection against all eight mutations tended to be weaker than the other forms of selection. We were able to detect significant fecundity selection against *Frd*

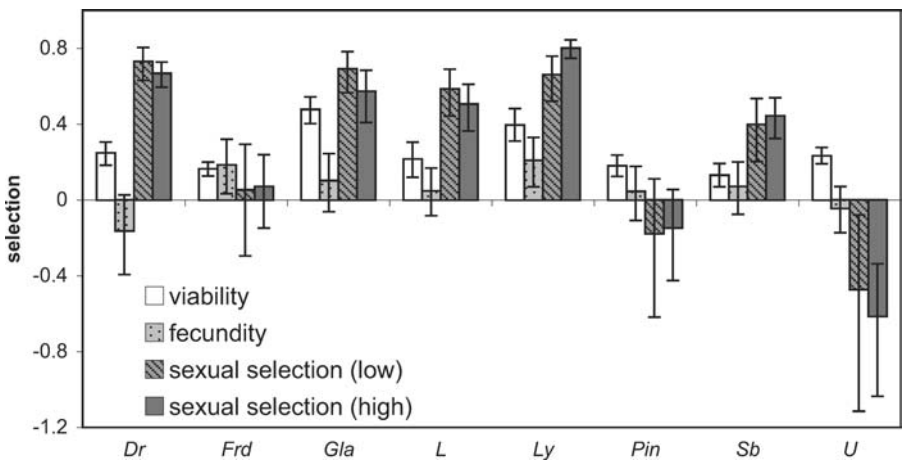

Figure 2. Selection estimates (with bootstrap 95% confidence intervals) for different fitness components. Positive values of selection indicate selection against the mutant allele; negative values of selection indicate selection favoring the mutant allele. See Methods for details on calculations and Table 3 for estimate values.

and *Ly* only. Analysis of variance on the fecundity data for each of these markers confirmed these results (not shown).

Discussion

Theory predicts that deleterious mutations can have important effects on populations. Most mutation load theory considers only total selection (rather than selection components) and measures load as the reduction in mean fitness relative to an ideal, mutation-free, individual. Although mathematically convenient, this simplified perspective can be misleading in those contexts in which we are interested in the consequences of load with respect to aspects of population productivity (e.g., population persistence, the outcome of competition between obligately sexual and asexual forms).

In such cases, we need to consider how deleterious alleles affect females separately from how they affect males because population-level performance is thought to be determined primarily by female fitness in most species. Selection in both sexes is equally important for determining the equilibrium frequency of a deleterious allele, but the consequences of this equilibrium frequency on population performance is mediated mostly by the allele's effect on females. This logic leads to the idea that population productivity will not be as strongly affected by deleterious mutations as implied by Haldane's classic theory (1937) if deleterious mutations are held at low frequency because of strong selection on males. (Conversely, the effects will be bigger than expected if selection on males is weaker than on females).

Sexual selection is the most obvious reason that selection would be stronger on males than females. Because sexual selection can help to reduce the mutation load of females and thereby affect population productivity, we are particularly interested in whether demographic properties of the population (specifically, density) feed back to affect the strength of sexual selection. These simple but important ideas about the role of sexual selection in eliminating deleterious alleles have received little empirical attention to date.

For each of eight mutations in *D. melanogaster*, we compared sexual selection at two mating densities. We found evidence for sexual selection on six of the eight mutations (Fig. 2, Table 3). For five of these six loci, sexual selection acted against the mutant allele whereas selection favored the mutant *U* allele. There was some evidence that sexual selection was different between the two mating densities for three of these loci. Selection appeared to be stronger at high density for *Ly* but stronger at low density for *Dr* and *Gla*. Although half of the mutations for which we could detect sexual selection (i.e., three of six) showed changes in the strength of sexual selection across the two densities, our results provide no obvious support for the notion that sexual selection typically increases (or decreases) at higher density—there was no main effect of mating density but there was a significant interaction between gene and mating density. From these limited results we can tentatively conclude that there would be no net feedback effect of mating density on the average strength of sexual selection that might accelerate or retard the rate of mutational meltdown.

Our high mating density treatment was three times as dense as our low mating density treatment. These density treatments were designed to roughly mimic high and low densities seen within the range of typical laboratory fly cultures. However, even our low-density treatment had 12 males and 12 females per 13.5-dram vial and, as such, it represents a reasonably high density relative to what might be encountered in nature. Sexual selection on some loci might differ considerably at much lower densities than examined here. For example, we found that sexual selection favored the mutant *U* allele at both densities we tested. This mutation causes wings to curve upwards, reducing flight capacity. We suspect that at very low densities, males with this mutation would have very low mating success simply because they would have difficulty finding or pursuing females. Although sexual selection at much lower densities might differ dramatically for some loci, we have no reason to believe that there would be any general pattern different from what we observed.

Sexual selection is only relevant to reducing the effect of mutation load on population-level performance if it acts on mutations that also affect traits relevant to total female fitness such as juvenile survival and female fecundity. We measured significant viability selection against all eight of the mutations and significant fecundity selection against two of these mutations (*Frd* and *Ly*). For five of the eight (62.5%) mutations (*Dr, Gla, L, Ly, Sb*), our data seem to indicate that selection on males is more important than selection on females in eliminating these deleterious alleles (see Fig. 2). Of the remaining three loci, two are ambiguous (*Frd* and *Pin*). For the final locus, *U*, point estimates of both fecundity and sexual selection (but not viability selection) were negative, indicating that the mutant allele was favored over the wild-type allele. However, as previously noted, we suspect that this pattern would be reversed in larger arenas.

Unfortunately, there are only a few other examples in which individual mutations have been examined with respect to both female and male fitness components. All of the examples of which we are aware come from *D. melanogaster.* Whitlock and Bourguet (2000) measured the effects of five recessive phenotypic mutations on male mating success and on productivity (a joint measure of female fecundity and larval survival). The three markers that had significant negative effects on male mating success (*b, ca, e/sr*) also had significant negative effects on productivity. Moreover, the effects of these mutations on male mating success appear to be stronger than those on productivity. However, the remaining two markers (*ps/sp, h*) do not fit this pattern. Stewart et al. (2005) measured selection against an eye-color mutation (*bw*) in *D. melanogaster* and found selection against this mutation in males but not females. Pischedda and Chippindale (2005) found

that a mutant allele of *nub* caused a much larger reduction in male mating success than in either larval viability or female fecundity. Older studies involving phenotypic mutations are somewhat difficult to interpret because they typically involve specially constructed genotypes that result in heterosis. Nonetheless, several of these studies indicate that selection on male mating success may be more important than selection on female fecundity (Prout 1971; Bundgaard and Christiansen 1972).

Although our marker mutations were chosen somewhat haphazardly, they have characteristics that are not typical of most new mutations. Most importantly, all of the tested mutations (and those in examples cited above) have fairly obvious dominant phenotypic effects on adult morphology, affecting traits such as eye shape, bristle distribution, wing shape, or body color. Nonetheless, these are mutations that can occur in nature and there are many other mutations that have similar characteristics (although there are many more mutations which have different characteristics).

In attempting to generalize our results to typical random mutations we should consider which of our results are likely to be biased by our choice of mutations for this study. All of our mutations have obvious dominant phenotypic effects whereas most random mutations are not visually detectable, especially in heterozygotes. Given their large phenotypic effects, one might expect that selection would be stronger on our mutations than on typical random mutations. Indeed, the magnitude of selection is weaker for typical mutations ($s \ll 0.1$, Lynch et al. 1999; Eyre-Walker and Keightley 2007) but we are more interested in patterns of selection rather than absolute strengths. Our goal was to study how selection changes across mating densities and across life-history stages (juvenile survival vs. female fecundity vs. male mating success). There is no obvious reason why using genes with large effects should bias these patterns.

By studying alleles with known phenotypic effects on adult morphology, we may be biased toward finding that selection is stronger on adults than on juveniles. This might be true but it is worth noting that we did find strong selection on juvenile viability for all of these mutations. With respect to fecundity and mating success it is possible that mutations with effects on adult morphology are more likely than typical mutations to experience strong sexual selection rather than fecundity selection, although it is not obvious why this should be so. Finally, with respect to the effect of density on the strength of sexual selection, there is no obvious reason why our choice of mutations should lead to a strongly biased result. In sum, it is important to recognize that the mutations we studied differ from typical mutations in some obvious ways and one should use caution in extrapolating our results too broadly. Of course, it would be ideal to perform these kinds of experiments on an unbiased, randomly chosen set of mutations but it would be extremely difficult to do so (i.e., requiring genotyping of hundreds of thousands of individuals; we phenotypically scored over 420,000 individuals in this study of eight genes).

A primary motivation for our work was to investigate how changes in density alter the strength of sexual selection against deleterious alleles. Although the study of sexual selection on specific mutations may be limited to model organisms, the study of sexual selection in general is not. A number of other authors, using a wide variety of taxa, have investigated how density affects sexual selection on particular phenotypic traits. Most studies examine morphological traits, whereas some examine behavioral traits. In summarizing these data it is worth noting some other important differences between this body of work and our study.

First, our study differs from previous work in how the strength of sexual selection was compared across densities. In most previous studies sexual selection was found to be significant at some densities but not at others (i.e., selection estimates at different densities were tested against zero rather than against one another, Arnqvist (1992) and Fleming and Gross (1994) are notable exceptions). If selection was significantly different than zero at one density but not at another, selection was usually assumed to differ between the two densities. However, finding significant selection at one density but not at another does not necessarily mean that selection differs between densities, especially given that confidence intervals on selection estimates tend to be large. Our study directly compares the strength of sexual selection at different densities in a single statistical model.

Another major point of distinction among studies concerns the sex ratio of the study population. In our study, sex ratio was even, and was held constant across density treatments. In some previous studies sex ratio was held constant across densities (Conner 1989; French and Cade 1989; Jirotkul 1999; Bertin and Cezilly 2005), or sex ratio remained approximately constant in unmanipulated field populations of varying density (Conner 1989; Arnqvist 1992; McLain 1992), or was not investigated at all (McLain 1982). In contrast, many studies have considered how sexual selection changes with sex ratio itself, such that the density of one sex relative to the other changes with total density (Fleming and Gross 1994; Berglund 1995; Carroll and Salamon 1995; Lauer et al. 1996; Jann et al. 2000). The importance of this distinction likely depends on the ecology of the study organism and the question of interest. In theory, mate density is thought to be more important than absolute density (Kokko and Rankin 2006), but it is clear that both aspects of density can affect the strength of sexual selection.

Table 4 summarizes the studies listed above for all of the traits that were found to be under significant sexual selection at one or more density levels. Note that some studies examined several different sexually selected traits, and found density dependence

Table 4. Summary of previous studies that examined the effect of density on sexual selection. Sex ratio (male:female) was either constant, or covaried positively with density. The last column shows the result for a particular trait: sexual selection was stronger at low, high, or neither density. See text for details.

Reference	Species	Population	Sex ratio	Trait	Result
Arnqvist 1992	*Gerris odontogaster*	field	constant	abdominal process length	low
				weight	neither
				parasite load	low
				pronotum length	low
Bertin and Cezilly 2005	*Asellus aquaticus*	laboratory	constant	body size	high
				antennae length	low
Berglund 1995*	*Syngnathus typhle*	laboratory	covaried	body size	high
Carroll and Salamon 1995	*Jadera haematoloma*	field, laboratory	covaried	body size	high
Conner 1989	*Bolitotherus cornutus*	field	constant	horn size	low
Fleming and Gross 1994	*Oncorhnchus kisutch*	mesocosm	covaried	body size	low
				snout length	neither
French and Cade 1989	*Gryllus veletis*	mesocosm	constant	calling duration	neither
				movement rate	high
	Gryllus pennsylvanicus	mesocosm	constant	calling duration	low
				movement rate	neither
Jann et al. 2000	*Scathophaga stercoraria*	field	covaried	body size	high
Jirotkul 1999	*Poecilia reticulata*	laboratory	constant	coloration	neither
Lauer et al. 1996	*Aquarius remigis*	laboratory	constant	body size	low
McLain 1982	*Chauliognathus pennsylvanicus*	field	NA	antennae size	low
				weight	neither
				elytra length	neither
McLain 1992	*Neacoryphus bicrucis*	field	constant	body size	low

*This study deals with a sex-role reversed species, hence density refers to female density.

for some traits and not for others (McLain 1982; French and Cade 1989; Arnqvist 1992; Fleming and Gross 1994). Overall, these studies show a mix of results. Eight of the 12 studies listed found that sexual selection was stronger at low absolute density or low male density for at least one of the traits studied. Five of the 12 studies found that sexual selection was stronger at high absolute density or high male density for at least one of the traits studied. These studies focus on phenotypes rather than specific alleles and differ from our own study (and from each other) in numerous other ways as described above. Nonetheless, this informal survey of literature is consistent with our finding that there is no strong trend for the strength of sexual selection to consistently increase or decrease with density.

Sexual selection may play an important role in eliminating deleterious alleles from populations, irrespective of density. For sexual selection to be relevant in this way, sexual selection must act not only against mutations that directly affect secondary sexual characters but also against almost any mutation that affects other components of fitness (e.g., juvenile survival and female fecundity). The data presented here add considerably to the very limited number of examples indicating that this is so. We found little evidence that the average strength of sexual selection changes with density implying that the importance of sexual selection does not change with demography. This latter inference is only strictly true if the average strengths of viability and fecundity selection also remain reasonably constant across variable densities. This is because the relevant issue is the total selection against mutations in males relative to the total selection against mutations in females. In principle, the realized importance of sexual selection could increase if either the strength of sexual selection increases or if the strengths of viability and/or fecundity selection decrease. Future work is needed to measure whether these other components of selection change with density. It is worth noting that a recent analysis of published studies suggests that the average strength of selection on fitness components such as viability and fecundity does not seem to change with stress (Martin and Lenormand 2006).

[Correction added after publication March 3, 2008: In the Abstract "Tjuvenile" changed to "juvenile."]

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