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SEXUAL SELECTION ACCELERATES THE ELIMINATION OF A DELETERIOUS MUTANT IN *DROSOPHILA MELANOGASTER*

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Although theory indicates that indirect genetic benefits through mate choice should be widespread, empirical work has often either failed to detect the operation of such benefits or shown a net cost to the presence of sexual selection. We tested whether sexual selection can increase the speed with which a conditionally deleterious allele is removed from a laboratory population of *Drosophila melanogaster.* The *alcohol dehydrogenase* null allele (*Adh–*) confers slightly lower viability than wild-type alleles in the absence of ethanol but is lethal in homozygotes when ethanol comprises 6% of the medium. We tracked the frequency of this allele in artificially constructed populations reared at three different levels of ethanol (0%, 2%, and 4%) that either experienced sexual selection or did not. Loss of the deleterious *Adh–* allele was more rapid when sexual selection was allowed to act, especially in the presence of ethanol. We also quantified the strength of both nonsexual and sexual selection against the *Adh–* allele using maximum-likelihood estimation. In contrast to recent experiments employing monogamy/polygamy designs, our results demonstrate a fitness benefit to sexual selection. This is consistent with the operation of good-genes female choice.

KEY WORDS: Alcohol dehydrogenase, condition dependence, experimental evolution, good genes, indirect benefits, sexual selection.

Natural selection is often divided into selection acting on nonsexual components of fitness (e.g., viability, fecundity, and longevity) and sexual selection, which acts on the number or identity of mates (Andersson 1994). Sexual selection could enhance nonsexual selection if both types of selection push populations in the same direction, or impede adaptation if it is antagonistic to nonsexual selection. Theoretical work suggests that sexual selection can increase the rate of adaptation to novel environments (Proulx 1999; Lorch et al. 2003), speed the fixation of beneficial alleles (Whitlock 2000), lower the deleterious mutation load, and alleviate the cost of sexual reproduction (Agrawal 2001; Siller 2001). Despite the attention sexual selection has received from evolutionary biologists, the net effect of sexual selection on nonsexual fitness remains unclear in most cases.

A related idea is the "good genes" hypothesis for the evolution of female mate preferences (Fisher 1930; Williams 1966; Houle and Kondrashov 2002). If females prefer males with traits or displays that honestly reflect genetic quality, they would gain indirect benefits through higher-quality offspring. An extension of good genes models is the idea of genic capture, which proposes that sexually selected traits under strong directional selection should eventually reach a point beyond which further development requires involving genetic variation that governs both the acquisition and allocation of resources (Rowe and Houle 1996). Under this scenario, any costly, exaggerated trait will eventually become condition dependent, thus reflecting a large portion of the organism's total genetic and mutational variance. This creates the opportunity for females to use the male trait as an honest indicator of genetic quality. Through this mechanism, genetic variation that is initially responsible for unrelated phenotypic effects would become involved in the expression of sexually selected traits. There is strong evidence that many sexually selected characters are indeed condition dependent (Jennions et al. 2001).

Despite the logic of this argument, the empirical evidence that the good-genes process occurs is frequently lacking. Numerous studies have shown a phenotypic correlation between

sire attractiveness and offspring fitness (e.g., Reynolds and Gross 1992; Norris 1993; Hasselquist et al. 1996), whereas others have not (e.g., Howard et al. 1994; Brooks 2000). A meta-analysis of such studies, spanning a broad range of taxa, indicates about 1.5% of variance in viability is explained by favored male characters (Møller and Alatalo 1999). It is unclear from these single-generation studies how much of an impact on adaptation an effect of this size would have. Furthermore, the typical investigation of good genes suffers from an inability to exclude the possibility that the observed correlation between attractiveness and measures of fitness has no genetic component (e.g., Møller 1991). Females may find certain males more attractive because they are less likely to infect potential offspring with parasites, for example, or because the males provide some resource. If these features of the male are environmentally mediated rather than genetic, offspring with high measures of components of fitness result simply through the operation of female choice for direct benefits.

Several rigorous, multigeneration attempts to study the effects of sexual selection on fitness have either not shown any advantage to sexual selection or detected a cost to it (Holland and Rice 1999; Holland 2002; Radwan 2004; Rundle et al. 2006). These results are consistent with models of mate choice that predict sexual selection may lower nonsexual fitness through costly displays (Haldane 1932; Lande 1981; Price et al. 1993) or intersexual conflict (Parker 1979; Holland and Rice 1998). Sexual conflict is defined as sexually antagonistic selection on a shared trait of males and females (e.g., mating rate) (Rowe and Day 2006) and can lead to antagonistic coevolution as males and females evolve adaptations against one another (Rowe et al. 1994; Rice 1996; Holland and Rice 1998; Gavrilets et al. 2001). The possible effects of this arms race are particularly intriguing. Is it the case that sexual selection is often characterized by high levels of sexual conflict that impose a load upon populations?

Many experiments looking for indirect benefits are performed with *Drosophila*, a taxon in which conflict levels are known to be high. There is extensive sexual conflict between male and female *D. melanogaster* over mating rate (Holland and Rice 1999; Pitnick et al. 2001; Pitnick and Garcia-Gonzalez 2002; Friberg and Arnqvist 2003), and female remating reduces life span and lowers fitness (Pitnick and Garcia-Gonzalez 2002; Friberg and Arnqvist 2003). The accessory gland proteins of *D. melanogaster* male ejaculate are toxic to females (Chapman et al. 1995; Wigby and Chapman 2005) and are hypothesized to have evolved by selection on sperm competitiveness. Male–male competition is an important determinant of reproductive success in *D. melanogaster*, through both sperm competition (Clark et al. 1995; Ochando et al. 1996; Hughes 1997; Harshman and Clark 1998) and possibly the increased mating success of larger males (Partridge et al. 1987a,b). An experiment by Stewart et al. (2005), in which a trait that allowed female *Drosophila* to avoid excessive matings readily evolved, clearly demonstrates the selective pressure exerted by this sexual arms race. The lack of evidence for indirect benefits and the high level of conflict between the sexes in *Drosophila* suggest that sexual selection may generally be harmful to *D. melanogaster* populations.

It is important to recognize, however, that the strong evidence in favor of sexual conflict does not preclude the possibility of fitness benefits arising from sexual selection at other loci. The strongest evidence for sexual conflict comes from populations adapting to either increased or decreased levels of conflict. Although conflict in many of these experiments clearly has negative effects on population level fitness, it is not clear that this prevents other types of sexual selection from influencing the fate of particular alleles.

In the experiment reported here, we tested the idea that sexual selection can increase the rate of adaptation by accelerating the elimination of a deleterious allele from a population. We constructed *Drosophila melanogaster* populations segregating for a null allele at the *alcohol dehydrogenase* locus, *Adh*. The alcohol dehydrogenase enzyme, ADH, is necessary for flies to metabolize and detoxify ethanol in the medium (Daly and Clarke 1981; Oakeshott et al. 1984; Geer et al. 1985). The absence of ADH activity is slightly deleterious in the absence of ethanol in the medium (van Delden and Kamping 1988), but becomes lethal ethanol makes up 6% of the medium (Bijlsma and Bijlsma-Meeles 1991). There is also evidence for lowered levels of locomotion in flies with null alleles at the *Adh* locus when they are exposed to ethanol (Wolf et al. 2002). Populations segregating for this allele allow us to manipulate both the strength of nonsexual selection (by adjusting the amount of ethanol in the medium) and sexual selection (by adjusting the mating portion of the life cycle). We used this system to address whether sexual selection improves nonsexual fitness and, if so, the extent to which this improvement depends on the strength of nonsexual selection.

Materials and Methods

FLY STOCKS AND REARING

The experiment used a large, outbred population of flies (the IV laboratory stock) that has been adapting to the laboratory environment for more than 750 generations (Houle and Rowe 2003). Flies were maintained on standard cornmeal medium, with the appropriate ethanol concentration added just prior to dispensing food into shell vials and bottles.

The allele tracked within populations was Adh^{n1} (Grell et al. 1968), hereafter referred to as *Adh*–. We backcrossed the *Adh*– allele into the IV population for four generations to establish an *Adh*– laboratory stock that shared 94% of its genetic background

with the base population. We estimated *Adh*– homozygote frequency by exposing flies to 5 μl of 1-pentyne-3-ol on a square of filter paper (Sofer and Hatkoff 1972; O'Donnell et al. 1975; Morrison 1999) for 1 min. Wild-type flies and heterozygous *Adh*– individuals with functional alcohol dehydrogenase enzyme oxidize pentynol into a toxic ketone and are killed whereas homozygous *Adh*– flies are unharmed. This assay allowed for a large number of flies to be scored quickly (mean = 427, SD = 116 flies each generation per replicate).

MANIPULATION OF SEXUAL SELECTION

To begin the experiment, we constructed 12 replicates of 200 flies each in Hardy–Weinberg proportions with an initial *Adh*– frequency of $q = 0.6$. Each replicate therefore began with 32 wild-type flies, 96 *Adh+/Adh*– heterozygotes, and 72 *Adh*– homozygotes. This is designated generation 0. The wild-type and *Adh*– flies used to initiate each replicate were collected as virgins from bottle populations established the generation before from each respective laboratory stock. We obtained heterozygous virgins by crossing the IV laboratory stock with our *Adh*– introgressed stock the generation before the experiment began. Four replicates each were assigned to 0%, 2%, and 4% ethanol treatments. At each ethanol level, we assigned two of the replicates to a treatment that included both sexual and nonsexual selection (S+) and the remaining two replicates to a treatment allowing only nonsexual selection (S−).

At the start of each generation, virgin flies from each replicate were randomly grouped in the following manner. In each S+ replicate, 20 groups of five males and five females were transferred into polygamous mating vials. In each S– replicate, one male and one female were transferred into 100 monogamous mating vials. After two days in these mating vials, males were discarded and the 100 mated females from each replicate were transferred to four bottles containing 25 females each. The females spent three days laying eggs in these bottles before being discarded. From the 10th day after the bottles were established until the 13th day, all eclosing flies were collected twice daily from all replicates and counted. Nonvirgin flies from morning collections comprised the larger part of these collections and were used to estimate the *Adh*– frequency in each replicate. The afternoon collections provided virgin flies that were then used to establish the next generation in the same manner as above.

This design allowed both sexual selection and sexual conflict in the S+ treatments, while minimizing any differences between S+ and S− treatments outside the mating phase. The two-day mating period allowed both female choice (in both initial matings and any remating) and male–male competition, including sperm competition. Sexual selection on the *Adh*– allele occurred against the background of sexual conflict typically present in *D. melanogaster*.

After four generations, replicates in which the *Adh*– homozygote frequency dropped below 0.05 were discarded. Unfortunately, the four replicates maintained on ethanol-free medium were lost after three generations because an error in media preparation led to low fly yield. By the eighth generation, the *Adh*– homozygote frequency had dropped below 0.05 for all replicates.

ANALYSIS

We used a maximum-likelihood approach to estimate selection coefficients against the *Adh*– genotypes. Our analysis had to take into account the fact that genotype frequencies of the parents were not assayed directly, that only the frequencies of *Adh*– homozygotes were directly observed, and that Hardy–Weinberg proportions could not be assumed beyond the first generation.

Selection model

We observed the frequency of *Adh*– homozygotes midway through each generation—after viability selection, but before fecundity and sexual selection. Following Prout (1969), we termed selection acting before our frequency assay "early" selection, and selection acting after our assay "late" selection. Lifetime fitness is the product of early and late fitness. We assumed that the effects of the *Adh*– allele were recessive, so that only the early and late relative fitness of *Adh–/Adh*– homozygotes need to be estimated. We assumed that relative fitness did not change over the duration of the experiment. We estimated relative fitness for the homozygous *Adh–/Adh*– genotype during the early part of the life cycle, w_e, from the S− treatments, which did not experience late sexual selection. The late fitness for male *Adh–/Adh*– flies, w_l, was estimated under the assumption that the estimates of early fitness from the S− treatments applied to the early part of the life cycle in the S+ treatments and that all other genotypes, including female *Adh–/Adh*– flies had a late fitness of 1. The result of these assumptions is that all of the fitness differences between the S+ and S− treatments affect the male *Adh–/Adh*– late fitness term, w_l. Fecundity differences between genotypes common to all S− treatments are absorbed into the early fitness term, w_e. Thus, any fecundity differences between S+ and S− treatments or interactions between early and late fitness will be reflected in w_l, although we expect that most of the departure of w_l from 1 is due to sexual selection. We address possible departures from these assumptions in the Discussion.

Our selection model begins with the adult genotype frequencies in generation t, which are denoted $R_{a.t}$ for *Adh−/Adh−*, $H_{a.t}$ for *Adh+/Adh−*, and $D_{a.t}$ for *Adh+/Adh+*. Noting that $D = 1 - R - H$, we need only explicitly track two frequencies, R and H. The expected frequencies of genotypes in the zygote stage in generation $t + 1$ (symbolized, e.g., by R_z) is

$$R_z\,(H_{a\cdot t},\,R_{a\cdot t}) = \frac{\frac{1}{4}H_{a\cdot t}^2 + \frac{1}{2}H_{a\cdot t}R_{a\cdot t}\,(1+w_l) + R_{a\cdot t}^2\,w_l}{1 - R_{a\cdot t}\,(1-w_l)}$$

$$H_z\,(H_{a\cdot t},\,R_{a\cdot t}) = \frac{(1+w_l)\left(\frac{H_{a\cdot t}R_{a\cdot t}}{2} + R_{a\cdot t}D_{a\cdot t}\right) + \frac{H_{a\cdot t}^2}{2} + H_{a\cdot t}D_{a\cdot t}}{1 - R_{a\cdot t}\,(1-w_l)}.$$

The expected frequencies of genotypes in the adult stage of generation $t+1$ are then

$$R_a\,(H_{a\cdot t},\,R_{a\cdot t}) = \frac{R\,(H_{a\cdot t},\,R_{a\cdot t})_z\,w_e}{1 - R\,(H_{a\cdot t},\,R_{a\cdot t})_z\,(1-w_e)}$$

$$H_a\,(H_{a\cdot t},\,R_{a\cdot t}) = \frac{H\,(H_{a\cdot t},\,R_{a\cdot t})_z}{1 - R\,(H_{a\cdot t},\,R_{a\cdot t})_z\,(1-w_e)},$$

in which w_e (early fitness) is the relative fitness of *Adh*– homozygote males and females during the transition from zygotes to adults.

Sampling model

The likelihood of a particular pair of estimates of w_e and w_l is determined by how well the selection model predicts the observed frequencies of deaths in the pentynol assay. To get the likelihood, we need to calculate the probability that the data will be observed given the uncertainty caused by sampling. We deal with three sources of uncertainty. First, there is uncertainty about the frequencies of the genotypes in the sample of flies treated with pentynol. Second, a different sample is used to initiate the next generation from that exposed to pentynol. Third, the effective population size, N_e, of the breeding populations is likely to be less than the number of parents.

In general, the exact state of the population is unknown, so we work with the matrix, $\mathbf{S_t}$, of probabilities that the parental population has a particular state (*h, r*) at time *t,* where *h* is the effective number of heterozygotes, and *r* is the effective number of *Adh*−/*Adh*− homozygotes. The dimensions of the matrix $\mathbf{S_t}$ are $N_e + 1 \times N_e + 1$, so that $S_{t[h+1,r+1]}$ is the probability that the population had *h* heterozygotes and *r Adh*−/*Adh*− homozygotes at generation *t*. The first row and column hold the probability that *h* or *r* are 0. Cells in which $h + r > N_e$ have probability 0.

Given an $\mathbf{S_t}$ matrix, we then can calculate the likelihood of observing the proportion of deaths during the assay in generation $t+1$. We represent the number of surviving flies in the pentynol assay at generation *t* as r_t, the number of dead flies d_t, and that the total number of flies n_t. Then the probability that r_{t+1} deaths will be observed in a sample of n_{t+1} flies, given the full range of possible values for h_t and r_t is

$$T(r_{t+1}) = \sum_{h=0}^{N_e}\sum_{r=0}^{N_e-h} S_{t[h+1,r+1]}\mathrm{Bin}(n_{t+1},\,r_{t+1},\,R_a(h/N_e, r/N_e)),$$

where

$$\mathrm{Bin}(n_{t+1},\,r_{t+1},\,R_a(h/N_e, r/N_e)) = \binom{n_{t+1}}{r_{t+1}}(R_a(h/N_e, r/N_e))^{r_{t+1}} \times (1 - R_a(h/N_e, r/N_e))^{n_{t+1}-r_{t+1}}.$$

$\boldsymbol{S_{t+1}}$ is then calculated starting from the assumption that the genotype frequency of *Adh*– homozygotes was known without error to be $r_{t+1}/n_{t+1} = R_{t+1}$, given the large sample sizes of flies assayed. The number of heterozygotes in the assayed sample can take any value from 0 to d_{t+1}, where the probability of a particular value is

$$\mathrm{X}(i_{t+1}) = \sum_{h=0}^{N_e}\sum_{r=0}^{N_e-h} S_{t[h+1,r+1]}\mathrm{Bin}\left(d_{t+1},\,i_{t+1},\,\frac{H_a(h/N_e, r/N_e)}{1 - R_a(h/N_e, r/N_e)}\right).$$

Each element of $\boldsymbol{S_{t+1}}$ is then calculated by summing the probabilities that the count in the parents of generation $t+1$ will be effectively *h* over all possible values of *i* as

$$S_{t+1\cdot[h+1,r+1]} = \sum_{i=0}^{d_{t+1}}\mathrm{X}(i_{t+1})\frac{N_e!}{h!r!\,(N_e - r - h)!} \times \left(\frac{i}{n_{t+1}}\right)^h (R_{t+1})^r \left(1 - \frac{i}{n_{t+1}} - R_{t+1}\right)^{N_e-r-h}.$$

In the initial generation, the summation over possible values of *i* can be omitted, as the sample from which the effective parents are drawn is the constructed sample where $r = 72$, $i = 96$, and $n = 200$.

The overall likelihood of a set of early and late genotypic fitness through *t* generations is then

$$\mathrm{L}(w_e,\,w_l,\,N_e) = \prod_t T(r_t).$$

The joint likelihood of a set of early and late genotypic fitness for each treatment × ethanol level combination is given by summing the log-likelihoods of the two individual replicates for that set of fitness.

Due to computational limitations, we were only able to investigate three different values for N_e, effectively infinite, *N*/2 ($N_e = 100$), and *N*/5 ($N_e = 40$). The model with an N_e of 40 fits better than the one with an N_e of 100 (AICc [Burnham and Anderson 2002] scores of 1498.2 vs. 1526.8) and much better than an infinite population model (1755.4). We therefore report results using an effective population size of 40 individuals.

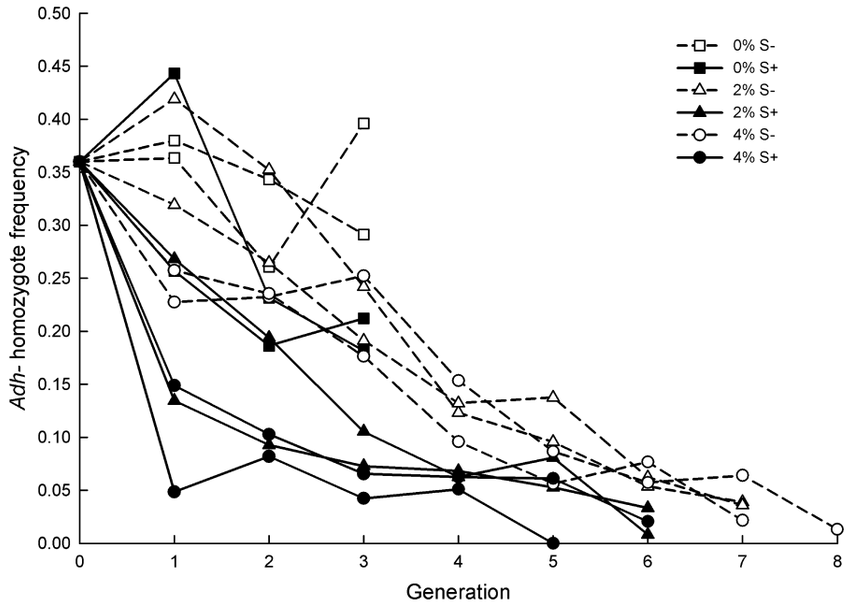

Figure 1. ***Adh*– homozygote frequency change over time. Solid lines represent treatments with sexual selection (S+) and dashed lines represent those without sexual selection (S–). Ethanol levels are indicated by square markers (0%), triangle markers (2%), and circle markers (4%). Populations maintained on 0% ethanol were lost at generation 3 due to an error.**

Results

The *Adh*– homozygote frequencies over the course of the experiment are shown in Figure 1. Not surprisingly, the *Adh*– allele was eliminated more quickly from populations maintained under higher ethanol concentrations. More importantly, the presence of sexual selection also accelerated the loss of *Adh*–. At the third generation, the last for which there are data for all replicates, S+ replicates had a lower frequency of *Adh*– homozgotes than their S− counterparts at all ethanol levels. We tested the effect of selection and ethanol treatments on the pentynol assay from the third generation using PROC Catmod in SAS (SAS Institute 2003). This categorical linear model assumes the proper binomial error variance in the dependent variable. There is a highly significant relationship between *Adh*– homozygote frequency and both ethanol level ($P < 0.0001$) and the presence of sexual selection ($P < 0.0001$). The interaction between sexual selection and ethanol level is not significant ($P = 0.5006$), providing no evidence that sexual selection differs with ethanol level after three generations of selection.

Adh– homozygote early fitness, which includes the effect of selection between the zygote and adult stages of the life cycle, as well as fecundity selection on females, was estimated for S− treatments both individually for each replicate and also by pooling replicates within an ethanol level. Late fitness, which affects only males between the adult and zygote stages of the life cycle in this model, was assumed to be equal for all genotypes in the S− treatments because the random pairing of adult flies eliminates sexual selection. Therefore, early fitness of *Adh*– homozygote genotypes in S− treatments is equivalent to net fitness in the model. Corrected Akaike Information Criterion (AICc, Burnham and Anderson 2002) scores indicated that the best model was one in which a common early fitness parameter was fit to the two S− replicates within each ethanol level (1498.2 vs. 1507.5).

Our model assumed that the S+ data can be explained by fitting one additional parameter representing "late" relative fitness of *Adh*−/*Adh*− males, during the competitive mating phase of the life cycle. AICc scores favored fitting this additional parameter for each ethanol level (609.4 vs. 612.9). Net fitness of male *Adh*– homozygotes was then determined by taking the product of early and late fitness estimates.

Net fitness estimates for homozygous *Adh*– male flies revealed differences across ethanol levels as well as treatments (Fig. 2A). In the absence of ethanol and sexual selection, *Adh*– homozygote lifetime relative fitness was estimated to be $w = 0.94$. The 2 log-likelihood support region for this estimate overlaps 1, indicating no significant difference between *Adh*– homozygote and wild-type fitness. With the addition of sexual selection in the ethanol-free treatment, the best estimate of male *Adh*– homozygote lifetime fitness dropped to $w = 0.39$. Support regions on replicates reared in the absence of ethanol were relatively large, however.

In the 2% ethanol treatment, lifetime relative fitness for homozygous *Adh*– males in the S+ treatment ($w = 0.23$) was markedly lower than S− ($w = 0.60$) and support regions for these

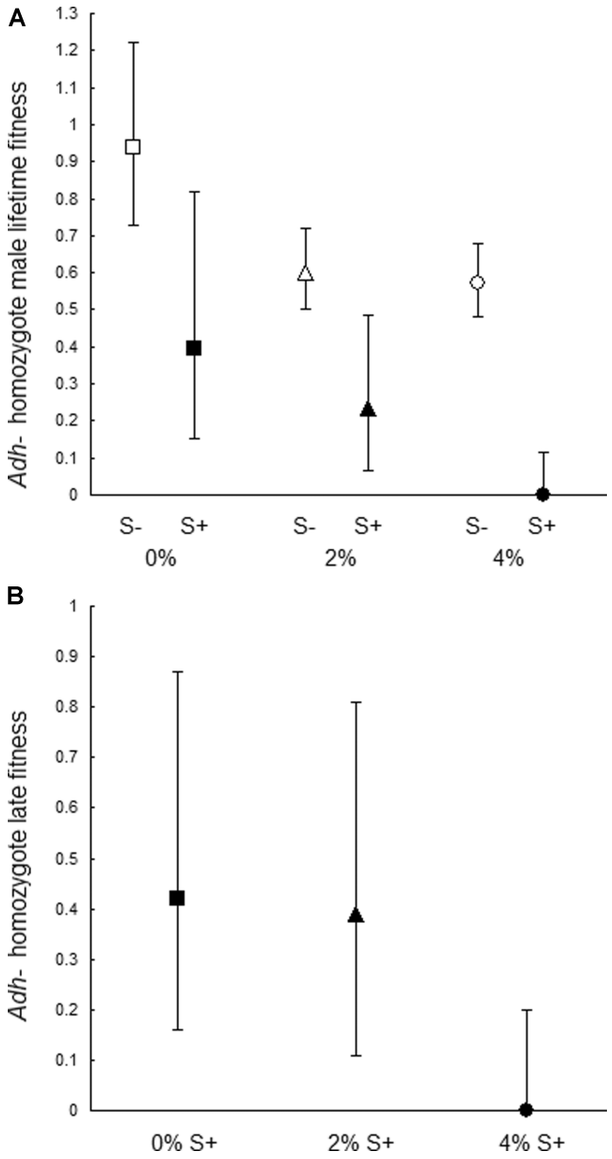

Figure 2. Maximum-likelihood estimates of (A) lifetime relative fitness and (B) late relative fitness for *Adh–* homozygote males. Open circles represent treatments without sexual selection (S–) and filled circles represent those with sexual selection (S+). Ethanol levels are indicated by square markers (0%), triangle markers (2%), and circle markers (4%), and a 2 log-likelihood support region is indicated.

estimates did not overlap. In the 4% ethanol treatments, S+ *Adh*− homozygote male lifetime fitness was estimated at $w = 0$. This estimate suggests male *Adh*− homozygotes reared at the highest level of ethanol were unable to obtain fertilizations in the polygamous mating environment. In the corresponding S− treatment, fitness was higher ($w = 0.57$). Again, the support regions for the two treatments did not overlap.

The estimates of only the late fitness component for each ethanol level are shown in Figure 2B. Increasing ethanol level increases the estimated strength of sexual selection ($w_l = 0.42$ at 0% ethanol, $w_l = 0.39$ at 2% ethanol, and $w_l = 0$ at 4% ethanol), although the support regions for all of the estimates overlap.

The results shown in Figure 2 make it clear that nonsexual relative fitness of the *Adh–* homozygotes decreases as ethanol levels increase. This is the trend for sexual selection as well, although the overlap in support regions is greater for the net fitness estimates. To test whether the trend for increasing sexual selection with ethanol stress has statistical support, we compared the likelihood of models with a common estimate for late fitness across all three ethanol levels to the likelihood of models in which late fitness are allowed to vary. The best single estimate of late fitness common to all treatments is $w = 0.39$. The AICc score for this model is 1501.1, as compared to 1498.2 for the separate treatments model. Thus, there is strong support for the idea that the strength of sexual selection changes with the strength of nonsexual selection at the *Adh* locus.

Discussion

The work reported here reveals a clear indirect benefit to populations that experienced sexual selection. The presence of sexual selection accelerated adaptation by facilitating the purging of a deleterious null allele at the *Alcohol dehydrogenase* locus. These results are consistent with a body of theory that argues that the presence of sexual selection in populations should accelerate adaptation (Proulx 1999; Whitlock 2000; Lorch et al. 2003). It also supports the idea that females can receive indirect benefits by being choosy (Fisher 1930; Williams 1966; Zahavi 1975; Rowe and Houle 1996) and therefore that the good-genes process can affect the evolution of female preferences.

The selection model that we fit to our data to obtain relative fitness estimates involves several assumptions. We assume that the *Adh–* allele is recessive and that sexual selection only acts on males. Our estimates of early selection therefore include fecundity differences that would arise after we sample adults each generation. It is possible that female fecundity is lowered in the S+ mating treatment by harassment or elevated by accessory gland proteins, but this does not affect our conclusions unless this disproportionately affects *Adh–* females. In that case, some of the fitness benefits of our S+ treatments could then be ascribed to fecundity differences. Regardless of the mechanism, our results make it clear that there is a fitness benefit to sexual selection. We have fit alternative models lumping "early" and "late" fitness into one measure of fitness and also relaxing the assumption of recessivity, and in each case the overall conclusions that sexual selection improves the elimination of *Adh–* and that the strength of

sexual selection increases with increasing ethanol concentration are unchanged.

Although some other experimental work has also shown a benefit to populations experiencing sexual selection (e.g., Promislow 1998; Dolgin et al. 2006; Fricke and Arnqvist 2007), the majority of multigeneration experiments looking at net reproductive success have not. Holland and Rice (1999) enforced monogamy in *D. melanogaster* with a design similar to this one and found lower egg production in monogamous lines than in polygamous lines but a higher net reproductive rate and faster development time. A later experiment by Holland (2002) challenged flies to adapt to thermal stress, increasing the opportunity for selection as heritable variation for fitness should be larger in a novel environment. The flies readily adapted to the increased temperature, relative to controls, but there was no difference between monogamous and polygamous treatments. Rundle et al. (2006) manipulated the presence or absence of sexual selection and followed the adaptation of *Drosophila serrata* to a novel corn-based medium. This experiment also found no difference between sexual selection treatments and was interpreted as evidence that the good-genes process was either not operating or that any benefits were exactly offset by the burdens of sexual conflict.

There are four primary reasons why researchers may not have found positive effects of sexual selection on nonsexual fitness in experiments similar to this one. First, some laboratory populations of *D. melanogaster* may simply not benefit from increased rates of adaptation through sexual selection. Some pairs of naturally selected alleles may not affect mating success, or selection during mating may actually favor the allele that has negative effects on naturally selected fitness. One possible reason for this is that the evolution of female preferences through sexual conflict may compromise the ability of females to choose mates based on genetic benefits. The chase-away model of sexual conflict (Holland and Rice 1998) proposes that female preferences evolve to avoid harmful matings. As males evolve to become more attractive to females, females simultaneously evolve resistance to the allure of male display traits to avoid suboptimal mating. This cycle continues and females better able to avoid manipulation by males are favored. Conflict between the genders—itself stemming from the mating system—would be a sufficient explanation for both the presence and behavior of sexual selection and the apparent absence of indirect benefits to female choice in enforced monogamy/polygamy experiments. Note that this scenario is inconsistent with our results; the operation of sexual selection within our populations does result in a fitness benefit.

A second reason why past work might not have revealed indirect benefits is that the negative effects of sexual conflict may obscure rates of adaptation or spread of beneficial alleles. The likelihood of this occurring is amplified by experimental designs that heighten conflict beyond a natural level. The specific details of monogamy and polygamy treatments in experiments such as this one could therefore change the relative importance of good genes and sexual conflict. For example, in Holland and Rice (1999) female flies in treatments with sexual selection are subject to a much higher level of male harassment than in this experiment. The sex ratio within vials was three males to one female and flies interacted in mating vials for five days. This results in a high level of conflict between the sexes because all males are competing for mating opportunities with just one female and females must avoid excessive mating over a much longer period of time. Holland (2002) lowered the interaction period to two days in an explicit attempt to lower the level of sexual conflict and detect any indirect benefits but still had a 4:1 male:female ratio during mating. Females in monogamous treatments in experiments like these miss out on the possible benefits of sexual selection but also avoid the potentially heavy costs of male harassment when sex ratios are skewed toward males. A monogamous individual's net reproductive success is completely contingent on their randomly selected partner, and so female-damaging males and remating-avoiding females are selected against. In the work by Stewart et al. (2005), an allele that was used to simulate resistance to remating spread rapidly through populations experiencing sexual selection. This suggests that females are unable to avoid multiple matings. In our experiment, we housed equal numbers of males and females for 2 days to present females with options and simultaneously standardize the amount of male-induced harm females are subject to. In the wild, *D. melanogaster* females typically mate once a day (Gromko and Markow 1993), so two days should allow an average of two matings per female, and possibly more if confinement in vials prevents females from resisting remating. There is evidence that female lifetime fitness drops rapidly with a higher level of mating than this (Kuijper et al. 2006).

This potential swamping of sexually selected benefits by sexual conflict is particularly likely when there is little adaptation going on in the population studied. Laboratory adaptation experiments usually try to use long-term laboratory strains in which adaptation to the laboratory environment is largely complete. In addition, laboratory stocks are insulated from ecological sources of selection generated by competitors, predators, or environmental change. This makes experiments in which adaptation is known to be taking place particularly relevant (e.g., Holland 2002).

A third explanation for the prevalence of costs in previous studies is that much of the changes in fitness come from the relaxation of the costs of sexual conflict in monogamy treatments, rather than the imposition of costs in polygamy treatments. Relieving the male–female arms race lifts a load from populations and could explain the results of many experiments that do not detect indirect benefits (e.g., Holland and Rice 1999; Holland 2002; Radwan 2004; Rundle 2006). This removal of sexual conflict may provide a one-time benefit to populations whereas indirect

benefits through good genes could accrue persistently at a low level in a population and thus assume greater importance in the long term than these conflict experiments indicate. Alternatively, there could be a lasting benefit to the relief of conflict if the sexual arms race continues to impede a population's response to nonsexual selection or if novel mutations frequently generate conflicts during sexual selection. Our results suggest that the positive effects of sexual selection on nonsexual fitness may still be present even when the net effect of mating competition and mate choice reduces fitness.

Finally, experiments like ours are more likely to detect indirect benefits than experiments in which components of fitness are only measured once, after many generations, or intermittently. This is because a measure of relative fitness (changes in genotype frequency) is obtained every generation in our experiment and others like it (Stewart et al. 2005). Theoretical work (Lorch et al. 2003) indicates the benefits to adaptation provided by sexual selection are most likely to occur in the first generations in a novel environment, prior to equilibrium, and recent experimental evolution work has supported this idea (Fricke and Arnqvist 2007). These early dynamics would not be captured by designs that wait many generations to measure fitness, as sexual selection may affect the rate of adaptation without affecting the equilibrium fitness.

An ideal experiment testing for the presence of indirect benefits would somehow isolate sexual selection from sexual conflict. This is difficult in *D. melanogaster* because most sexual selection appears to be postcopulatory and thus prevents the disentangling of these forces. Another approach would be to enhance levels of standing variation, either by constructing populations (as in this experiment) or using mutagenesis or mutation accumulation. Populations with high levels of standing variation are most likely to benefit from sexual selection, the rationale behind the work by Holland (2002) and Rundle (2006) that placed *Drosophila* in novel environments and challenged them to adapt. Although these experiments did not detect indirect benefits, it is possible that those benefits were operating in polygamous treatments and were overshadowed by the benefits received in monogamous treatments through the elimination of sexually antagonistic coevolution. The acceleration of adaptation reported here suggests the actual net effect of sexual selection may depend on the levels of both standing variation for fitness and conflict between the sexes.

Previous experiments have demonstrated that morphological mutants are frequently poor at obtaining matings in *D. melanogaster* (reviewed by Grossfield 1975; also see Merrell 1965; Whitlock and Bourguet 2000; Sharp and Agrawal 2008). These studies provide some additional evidence for good genes, although the strongly deleterious alleles used are clearly unrepresentative of variation in natural populations. The relevance of these studies is also diminished by the fact that some visible mutants are likely to directly affect the ability to detect or court mates. For example, 17 of the 20 mutants used in the experiments reviewed by Grossfield (1965) had direct effects on eyes, antennae, or wings.

A potential objection to drawing general conclusions from our experiment is that a null allele is simply not typical of the type of variation segregating in natural populations, just as the morphological mutations used in previous experiments were not. If this is the case, we would expect that the effectiveness of sexual selection at eliminating deleterious alleles would increase with nonsexually selected costs. Because the time to effect a given allele frequency change is linear in $s = 1 - w$ (Hedrick 2000, pp. 101–103), we can use the fitness reduction of the *Adh*– homozygote as a measure of the strength of selection. As shown in Figure 2B, sexual selection has a bigger proportional impact on *Adh*– fitness at 0% ethanol, when nonsexual selection is weakest. Sexual and nonsexual selection are 5.5 times as effective at removing the deleterious allele as nonsexual selection alone when ethanol is absent, but only about 1.6 times as effective at the maximum ethanol concentration. This demonstrates that sexual selection is sometimes particularly effective at removing variation with the potential to greatly reduce fitness in stressful or novel environments.

We believe that our results provide strong evidence that sexual selection can result in the fitness benefits necessary to good-genes models for the evolution of female preference. The key to this is our use of a particular deleterious allele whose frequency can be accurately tracked over multiple generations. Our use of an *Adh* null genotype with no direct morphological effect and minimal involvement with behavior or fertilization suggests that the fitness benefits to sexual selection in our study are truly indirect. Similarly, the experiment of Stewart et al. (2005), in which an allele that was used to simulate resistance to remating spread rapidly through populations experiencing sexual selection, provides the strongest, most satisfying evidence for the cost of sexual conflict. Their experiment shares with ours the use of a mutant allele to determine the direction and strength of the forces generated by sexual selection. In contrast, most previous experiments on the benefits and costs of sexual selection hinge on our ability to accurately measure fitness and fitness components, which is surely a most difficult task. We suggest that future experiments on sexual selection should be designed to take advantage of this straightforward, repeatable approach to the detection of fitness benefits.

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