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Adult fitness consequences of sexual selection in *Drosophila melanogaster*

DANIEL E. L. PROMISLOW*, EMILY A. SMITH, AND LOUISE PEARSE

Department of Genetics, University of Georgia, Athens, GA 30602-7223

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ABSTRACT **Few experiments have demonstrated a genetic correlation between the process of sexual selection and fitness benefits in offspring, either through female choice or male competition. Those that have looked at the relationship between female choice and offspring fitness have focused on juvenile fitness components, rather than fitness at later stages in the life cycle. In addition, many of these studies have not controlled for possible maternal effects. To test for a relationship between sexual selection and adult fitness, we carried out an artificial selection experiment in the fruit fly, *Drosophila melanogaster*. We created two treatments that varied in the level of opportunity for sexual selection. Increased opportunity for female choice and male competition was genetically correlated with an increase in adult survivorship, as well as an increase in male and female body size. Contrary to previous, single-generation studies, we did not find an increase in larval competitive ability. This study demonstrates that mate choice and/or male–male competition are correlated with an increase in at least one adult fitness component of offspring.**

Four basic models have been proposed to explain the existence of female preference for elaborate male traits in animals. "Good-genes" models suggest that female preference for elaborate male traits evolves because the male trait is an indicator of genetic quality (1–3). By choosing a particular male, a female may gain indirect genetic benefits, such as an increase in the survival of her offspring (4, 5). Under the runaway or Fisherian model, an initially arbitrary female preference leads to an elaborate male trait and generates a genetic correlation between preference and trait (reviewed in refs. 6 and 7). The female does not obtain benefits from the male. A third model suggests that females may choose particular males because of some direct benefit (6, 8), such as a courtship or nuptial gift from the male (9). Finally, sensory exploitation models hypothesize that elaborate male traits evolve to take advantage of a preexisting sensory bias in females (10, 11).

Each model makes certain assumptions about the genetic and environmental variances and covariances for female choice, male display traits, paternal fitness, and offspring fitness. To understand the potential role that one or more of these models might play in the process of sexual selection, selection experiments have been carried out to measure genetic correlations between male traits and female preference. In two separate studies on guppies, researchers selected on bright male coloration to test for a correlated response of female preference (12, 13), with mixed results. In a similar experiment, Wilkinson and Reillo (14) found that in stalk-eyed flies selected for large eye span, females preferred males with large eye span, whereas females from lines selected for short eye span preferred males with short eye span.

Our primary focus is to test the basic assumption that the process of sexual selection can enhance offspring genetic quality (6). Though generally expected under good-genes scenarios, we emphasize that such an effect is not necessarily inconsistent with Fisherian models of sexual selection. In support of the good-genes model, some studies have found correlations between dimorphic male traits and offspring fitness (15–19). However, these studies are limited in their scope in several ways.

First, in most cases the experimenter has chosen to focus on one particular trait, which may or may not be of particular importance to the female (20). In fact, many, if not most, animal signals may fall outside the range of unassisted human perception (21).

Second, previous studies have analyzed only a limited number of fitness traits in offspring and usually have focused on juvenile survival. However, good-genes models do not tell us which fitness components will correlate the male trait—only that one or more fitness components should be correlated (22). Very little is known about the adult fitness components of offspring born to males with high or low average reproductive success, though females may discriminate among males on the basis of adult fitness components.

Third, many of these studies have looked only at phenotypic correlations between female choosiness or male attractiveness and offspring quality, and not examined the underlying genetic basis of these correlations (15). As pointed out previously (23), while genetic approaches are critical to understanding the evolution of sexual selection, responses that appear to be genetic may be confounded with a female's facultative response. For example, a female that mates with a high-quality male may respond facultatively by increasing investment in her offspring (24, 25). This response could be interpreted erroneously as a genetic effect.

We present results from an artificial selection experiment in *Drosophila melanogaster*. We designed the experiment to determine how variation in the opportunity for sexual selection affects larval and adult fitness components. We attempted to avoid each of the potential pitfalls mentioned above. We allowed the animals themselves to determine which traits were relevant to the sexual selection process. We analyzed adult survival rates, in addition to larval viability and several morphological traits. We also used an artificial selection approach in which we varied levels of sexual selection over multiple generations, followed by a period of relaxed selection before we assayed for differences among lines. This allowed us to eliminate the potential confounding effects of nongenetic factors.

Our results show that the process of sexual selection cannot only alter morphological traits, but also can lead to a genetically related increase in adult fitness components.

METHODS

In this experiment, we created two sets of artificial selection lines of *Drosophila melanogaster* that differed in their oppor-

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Abbreviation: FA, fluctuating asymmetry.

*To whom reprint requests should be addressed. e-mail: promislo@arches.uga.edu.

tunity for sexual selection, with three replicates per treatment. After carrying out artificial selection, we compared the two treatments with respect to age-specific adult survival, larval competitive ability, wing size, sex-comb tooth number, and fluctuating asymmetry for both wing size and sex-comb tooth number. Studies on the fitness consequences of sexual selection need to consider multiple traits, since sexual selection may influence some fitness components but not others (22). Larval competitive ability has been the standard estimate of fitness in previous sexual selection studies (e.g., 15, 16–18). Body mass and wing size were measured, as previous studies have demonstrated a correlation between male mating success and body size (26). Sex combs are an obvious sexually dimorphic trait in certain species of *Drosophila* and are thought to play an important role in mating behavior (27). We examined the possible effect of sexual selection on fluctuating asymmetry, as recent work suggests that it may play a critical role in female choice (28, 29). Finally, we tested the effect of sexual selection on adult mortality, as it is a major component of fitness, yet one that usually has been ignored.

Altering the Opportunity for Sexual Selection. The experiment consisted of two treatments. In one, we reduced the opportunity for sexual selection by mating one virgin male and female in each vial (this treatment is referred to hereafter as the "S" lines, for Single male). In the other treatment, we placed one female with five males (hereafter referred to as the "M" lines, for Multiple male). In M lines, both female choice and male–male competition might influence mating behavior and subsequent fitness. In each case, we set up matings between virgin flies in 8-dram vials with 5 ml of standard yeast/agar/molasses medium at room temperature for 2.5–3.5 h. Each treatment had three replicates, with 50 vials per replicate. We assigned the numbers 1, 2, and 3 arbitrarily to each replicate within a treatment and labeled the lines S_1–S_3 and M_1–M_3 for the three single-male and three multimale lines, respectively.

We initiated the experiment with the Dahomey line of flies collected in 1980 by L. Partridge. The flies were kept in our lab for approximately 1 year in a population cage at large numbers with overlapping generations, and then in 6-oz bottles for two generations, before beginning the experiment.

At the beginning of each generation, we collected several hundred virgin males and females from each replicate over 2–3 days by using light CO_2 anesthesia. Flies were maintained at a density of 20 flies per 8-dram vial on 5–10 ml of fly medium at 24°C, 75% relative humidity (R.H.) and 12-h light/12-h dark cycle. When the flies were 3–5 days old, in each replicate males were anaesthetized by cooling, and one (S line) or five (M line) males were placed in each of 50 vials by using an aspirator. After all vials contained males, females from the appropriate replicate were then cooled on ice and placed singly in each vial. Mating vials were kept at room temperature for approximately 3 h. This time was of short enough duration to prevent multiple mating (personal observation). To determine what proportion of females were fertilized, for each generation we took 10 females from each laying chamber (see below) and placed them singly in vials. On average, 92% of females laid fertilized eggs 2 days after mating, and there was no difference in percentage of mated females between treatments.

After the mating period, from each replicate line, we placed 25 females in each of two egg-laying chambers. The chambers were clear, plastic 75-mm internal diameter pipe cut into 75-mm long sections, with a clear plastic lid glued to one end, a 25-mm hole cut with a mesh screen in one side, and a 10-mm hole on the opposite side to insert flies. The bottom of each chamber consisted of a Petri dish with 45-ml of yeasted fly medium.

After approximately 18 h, the Petri dish of food was removed for egg collection and replaced with fresh yeasted food for a further 24 h. For each of the 2 days, we collected between 400 (S lines) and 600 (M lines) eggs from each Petri dish and placed the eggs in 8-dram vials at a density of 100 eggs per vial to ensure constant larval density between treatments.

Between 10 and 12 days later, we collected virgin flies for the subsequent generation, pooling individuals that were obtained from each of the two egg-laying chambers per replicate. We repeated this entire process for 17 generations. Ideally, with 50 males and 50 females per line, effective population size for each replicate would be $N_e = 100$. However, not all females were mated, and family size among females probably varied. This latter factor can dramatically reduce effective population size (30).

The selection regimes were designed to maximize the difference between the treatments in the opportunity for sexual selection. We should point out here that we cannot completely eliminate the possibility for sexual selection in the enforced monogamy treatment. Females may still choose not to mate with the male they are given and may also vary in fecundity or offspring viability because of the quality of the male with which they are paired, via cryptic female choice (see *Discussion*, below).

Age-Specific Mortality Rate. Age-specific adult mortality rates were determined for each line after 10 generations of selection. At generation 10, we relaxed selection in a subset of each replicate for two generations and expanded population size by breeding in 6-oz Drosophila stock bottles (Applied Scientific). After two generations of population expansion, we collected an average of 831 ± 99 virgin males and 773 ± 35 virgin females (mean ± SD) from each of the six lines.

We then placed virgin flies in Drosophila mortality cages (for details, see refs. 31 and 32). Each cage contained an average of 267 ± 44 flies. We placed cages at random in a 24°C, 75% R.H. incubator on a 12-h light/12-h dark cycle. Dead flies were removed, sexed, and counted from each cage once per day. We changed food every other day and changed wire-mesh screens on cages once per week or more often if necessary.

We placed virgin flies from each line in cages in one of four configurations: (*i*) single sex cages; (*ii*) males and females from the same line; (*iii*) males from one treatment with females from the opposite treatment (e.g., S_1 males with M_2 females, S_1 females with M_2 males, etc.); and (*iv*) males from one replicate with females from another replicate within the same treatment (e.g., S_1 males with S_2 females, M_1 males with M_2 females, etc.). For comparisons (*i*) and (*ii*), the statistical model pools data from all three cohorts within a treatment and assumes that each of the three cohorts has the same underlying distribution of ages at death. We set up cases (*iii*) and (*iv*) to provide independent, pairwise comparisons of the null hypotheses that M lines and S lines do not differ in age-specific mortality rates. With data from these two cases, we could obtain three statistically independent tests of a difference in mortality rates between S_i males and M_i males, when either of these shares a cage with M_j females (i.e., S_1 vs. M_1 males with M_2 females, S_2 vs. M_2 males with M_3 females, and S_3 vs. M_3 males with M_3 females). Additionally, we compared differences between males in a common S female background and differences between females in a common S or M male background.

We compared mortality rates by using the "Actuarial" nonparametric survival analysis model in STATVIEW 4.0 (33) with 10 time intervals per cohort. This survival analysis, common to medical statistics, allows us to compare the mortality among populations based on the distribution of ages at death, does not require fitting a specific parametric model, and incorporates any censored data (e.g., flies escape before dying). We stopped the mortality experiment at day 63, by which time only 3.9% of the original 10,031 flies were still alive. The remaining flies (390 in total) were censored in the survival analysis. To test for significance, we used a Logrank (Mantel–Cox) test, which gives equal weight to observations at every age. *P* values for this and all other comparisons are based on

two-tailed tests of a χ^2 value with 1 df (for further details, see ref. 34).

Body Mass. To obtain average dry mass at each generation, 25 virgin males and 25 virgin females from each replicate line were dried at 60°C overnight and then weighed in groups of five to the nearest 0.01 mg on a Mettler AT261 analytical balance. During the first five generations, males and females from the mating vials were weighed. However, only one-fifth of the M line males mate, whereas all the S line males mate. Thus, if we find a lower weight among S line than M line males, it might be because of greater loss of sperm and accessory gland fluid in the average S line male (each of whom had mated) versus the average M line male. Accordingly, after generation 5, only virgin males and females were used to obtain body mass.

To determine the effect of treatment on body size, we plotted the relative body mass (difference between line mean and grand mean) for each of the six lines as a function of generation time.

Wing Size and Sex-Comb Tooth Number. At generation 13, we stored 20 males and females from each of the six lines at −80°C for later analysis. After thawing flies to room temperature, we mounted left and right wings on slides and digitized them on a Macintosh computer connected to a Leica MZ8 dissecting scope using NIH IMAGE (developed at the National Institutes of Health, available on the Internet at http://rsb.info.nih.gov/nih-image/). We measured components of wing width and wing length (segments LC and HI from figure 1 in ref. 35).

For males, we removed and mounted both front legs and counted the number of teeth on each sex comb on a compound microscope. Sex-comb analysis was repeated on flies from generation 14.

We compared size differences for average wing width, wing length, and sex-comb tooth number between treatments. We also compared fluctuating asymmetry (FA) among lines. We measured the difference between L and R (left and right sides for each trait) and defined FA as the variance among flies within a replicate of (L − R) (36). We used a second measure of FA to correct for any potential scale effects, with FA defined as the variance of (L − R)/(L + R). Tests of directional asymmetry were all nonsignificant.

To avoid problems of nonnormal distribution and unequal sample sizes among groups, we used a randomization approach (37) to test for significant differences between treatments in mean and FA for sex-comb tooth number and wing size. For mean and FA values, we first calculated the average difference between flies in the M treatment (the average of the three M lines) and the S treatment (the average of the three S lines). We then randomly assigned flies among lines and recalculated the difference between treatment averages. We repeated this process 1,000 times for each analysis. *P* values were calculated as the number of iterations equal to or greater than the actual value.

Larval Competitive Ability. At generations 9 and 17, we compared larval competitive ability by placing a constant number of embryos from the wild-type, selected lines with an equal number of a marker strain, *ebony*. For this test, to eliminate possible maternal effects in comparing treatments, we placed males and females from each of the six lines in single-pair vials for mating. To collect *ebony* eggs, we placed 25 male and 25 female *ebony* virgin flies in egg-laying chambers. As with the selection lines, we collected fertilized eggs over 2 subsequent days.

In the first experiment, we placed 200 wild-type embryos from the selected lines with 200 *ebony* embryos in each of four vials per replicate line with 10 g of standard fly medium and counted emerging offspring over a 6-day period from 11 to 17 days after setup. With 400 larvae per vial, very few flies emerged and those that did emerge were very small because of intense competition. Accordingly, for the second test, we placed 150 wild-type embryos from generation 17 of the selected lines and 150 *ebony* embryos in each of 13 vials for each of the six replicate lines. We then collected emerging adults from each vial at 2-day intervals between 12 and 19 days after setup. For each vial, we defined larval-competitive ability as the fraction of all flies that emerged that were wild type.

RESULTS

Age-Specific Mortality Rate. We compared survival between M and S lines for three situations, including (*i*) single-sex cages with virgin males or females; (*ii*) mixed-sex cages within lines; and (*iii*) mixed-sex cages with one sex from one treatment line and the opposite sex from a line from a different treatment. In all cases, χ^2 values are obtained from the Mantel–Cox survival test, with 1 df (34). Results are summarized in Table 1.

(*i*) Virgin males from the multimale (M) lines lived significantly longer than virgin males from the single-male (S) lines. However, the effect primarily was a result of an increase in mortality among S lines relative to M lines after day 30. Before this, S males had lower mortality than M males. This may be due, in part, to selection for more vigorous behavior among M line males versus S line males, which would lead to higher mortality early in life. Effects of heterogeneity in quality among individuals then could give rise to the observed mortality cross-over late in life (38–40).

Virgin females from S lines, where there was reduced opportunity for sexual selection, showed higher survival than for M lines. However, the significant result may have been an artifact of poor survival among virgin females of the M2 line.

(*ii*) Among cages with males and females from the same line, M line males and females lived significantly longer than S line males and females. As with the single-sex cage, the difference primarily was a result of greater mortality in the S lines relative to the M lines after day 30. Before this time, mortality was slightly higher in the M lines. As with test (*i*) above, this pattern may arise because of higher costs of reproductive activity among M lines, both in terms of male–male competition and the cost of females trying to evade males early in life. After day 30, when reproductive activity is reduced substantially, we may again expect a reversal in mortality rates.

(*iii*) In analyses (*i*) and (*ii*), statistical survival analysis does not allow us to nest replicate within treatment effects, and so we had to pool the data from each of the replicates. For the following results, we did not have to pool data from different lines, and so we gain substantial statistical power. For each comparison, the survival model provides a summary statistic of the three independent, pairwise tests.

In the presence of M females, M males had significantly lower mortality than S males (Fig. 1). Similarly, in the presence of M males, M females had lower mortality than S females.

Table 1. Effect of treatment on survivorship

Treatment	Survival	χ^{2*}	*P* value
Virgins			
M vs. S males	M > S	213.3	<0.001
M vs. S females	S > M	6.5	0.011
Within-treatment mixed sex			
M vs. S males	M > S	38.0	<0.0001
M vs. S females	M > S	30.5	<0.0001
Cross-treatment mixed sex			
M_i vs. S_i males (with M_j females)	M > S	28.1	<0.0001
M_i vs. S_i females (with M_j males)	M > S	52.2	<0.0001
M_i vs. S_i males (with S_j females)	M = S	0.01	0.9
M_i vs. S_i females (with S_j males)	M = S	0.2	0.7

*Based on a Mantel–Cox survival test (34), with 1 df. See details in text.

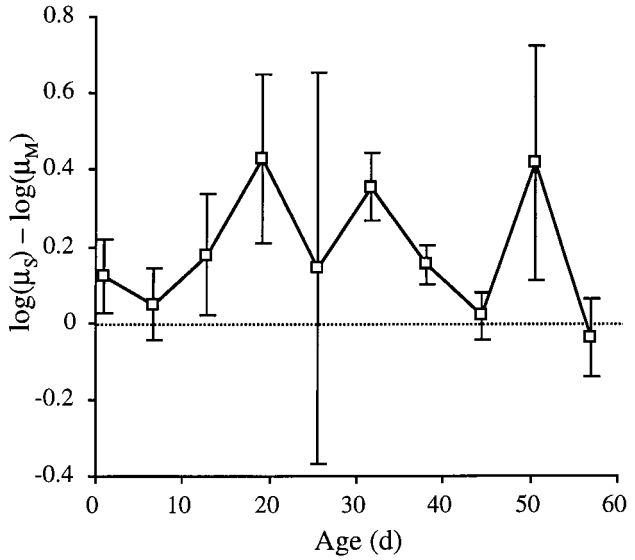

FIG. 1. Average difference between S line log (mortality) and M line log (mortality) [log (μ_S) − log(μ_M)] in adult males at different ages. This figure shows the difference between M_i males and S_i males in a common M_j female background (see text for further details). Error bars are ±1 SE.

Thus, M line individuals have higher fitness than S line individuals, at least in terms of age-specific adult mortality.

In contrast, we found no significant difference between M versus S males or M versus S females when in the presence of S line individuals of the opposite sex. This lack of difference

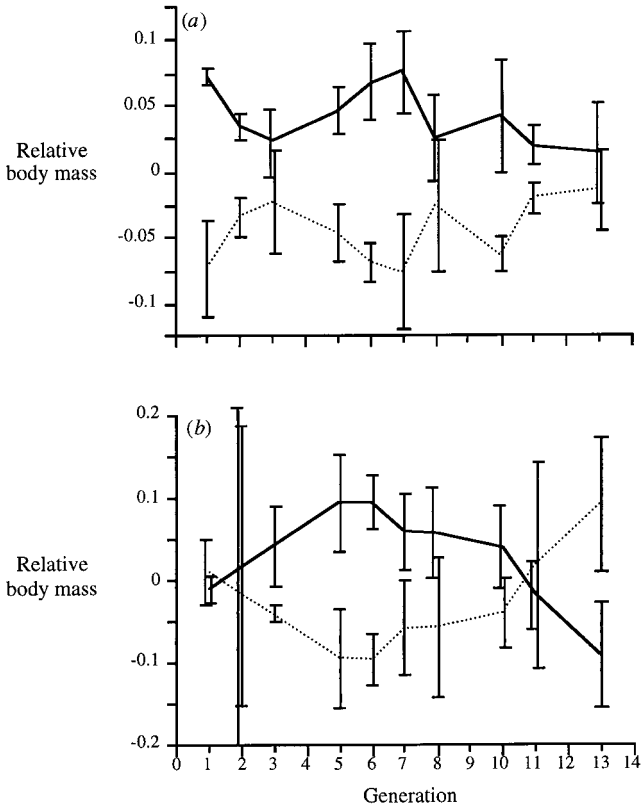

FIG. 2. Body mass in M line (solid line) and S line (dashed line) males (*a*) and females (*b*). The lines illustrate the difference between the mean for the three lines within a treatment and the mean for all six replicates. Error bars are ±1 SE for mean of the three replicates within each treatment.

Table 2. Effect of treatment on male sex-comb tooth number

	Generation 13			Generation 14		
Line	Average	FA [var(L − R)]	N	Average	FA [var(L − R)]	N
S_1	10.33	1.32	18	10.47	0.86	16
S_2	11.05	1.69	19	10.25	0.86	14
S_3	11.09	2.36	17	10.50	0.70	5
M_1	10.93	0.77	20	10.88	1.66	12
M_2	10.81	1.94	21	10.97	1.80	16
M_3	11.09	1.99	17	10.75	1.16	20

may be because S line individuals have been under weaker selection for competitive ability. Differences between males from M lines and S lines may be apparent only under relatively stressful social conditions (i.e., in the presence of M line females), but not in more benign social conditions (i.e., in the presence of S line females).

Body Mass. Dry body mass was greater in the M lines than in the S lines for both males and females (Fig. 2 *a* and *b*). However, the difference in body mass declined after approximately 11 generations.

Wing Size. Wing size was measured in arbitrary units (pixels on a computer screen), so we have not included a table with the original data. Our randomization tests showed weak evidence for slightly greater wing length in the S lines ($P = 0.058$ and $P = 0.068$ for females and males, respectively). We found no differences for either wing width ($P > 0.15$ for both males and females) or fluctuating asymmetry in wing width and length ($P > 0.15$ in all cases).

Sex-Comb Tooth Number. In the first sample (generation 13), the average sex-comb tooth number for flies used in this study was 10.9 ± 1.0 (mean ± SD, $n = 116$ males) and did not differ between lines (M line − S line average = 0.11, 1,000 randomizations, $P = 0.23$) (Table 2). Fluctuating asymmetry for sex-comb tooth number was slightly but nonsignificantly higher in S lines than in M lines, ($FA_M - FA_S = -0.252$, $P = 0.264$, based on 1,000 randomizations). In the second analysis on flies from generation 14, the two treatments differed significantly in average comb tooth number (M line average = 10.87, S line average = 10.41, $P = 0.005$, 1,000 randomizations). While there was a slight increase in FA in the M lines where FA was defined as Var(L − R) ($FA_M - FA_S = 0.72$, $P = 0.052$), the result appeared at least in part to be from a mean-variance correlation (for FA′ = Var (L − R)/(L + R), $FA'_M - FA'_S = 0.0013$, $P = 0.072$, 3,500 randomizations).

Larval Competitive Ability. In the first test of larval competitive ability, the percentage of wild-type flies emerging differed slightly but nonsignificantly (nested ANOVA, $F_{1,4} = 2.02$, $P = 0.23$) between M lines and S lines (45.5 ± 10.8% versus 34.7 ± 13.6%, respectively). A second, larger analysis (13 vials per line, collected on four subsequent occasions at 2-day intervals) also was nonsignificant (S line wild-type flies, 67.2%; M line wild-type flies, 67.8%; nested ANOVA, $F_{1,4} = 0.08$, $P = 0.79$) (Table 3).

Table 3. Effect of treatment on larval competitive ability

	Generation 9			Generation 17		
Line	Ebony	WT	% WT	Ebony	WT	% WT
S_1	219	70	0.242	418	1,078	0.721
S_2	119	102	0.461	501	1,192	0.704
S_3	165	96	0.368	670	1,119	0.625
M_1	129	137	0.515	533	1,121	0.678
M_2	187	99	0.346	709	1,206	0.630
M_3	80	86	0.518	482	1,153	0.705

WT, wild type.

DISCUSSION

Previous studies have found evidence for a benefit of sexual selection in terms of increased preadult viability (e.g., refs. 15 and 16–18), but usually have not analyzed the effect of sexual selection on benefits of adult survival (but see ref. 19). Our results demonstrate that an increase in the opportunity for sexual selection (through female choice or male–male competition) is genetically correlated with increased adult survival rates and may also be genetically correlated with an increase in body size. In addition, we found evidence for an increase in comb tooth number in males from lines with high opportunity for sexual selection, but no evidence for a treatment effect on wing size or on fluctuating asymmetry of either comb tooth number or wing size.

Our experiment differs from previous studies of correlated responses to sexual selection not only in its focus on an adult fitness trait, but also in several methodological respects. Within a single generation, we may have a low likelihood of finding what may be weak genetic effects due to low heritability of traits correlated with fitness. This is particularly important for traits such as mortality, for which estimates are prone to large sampling error (41). Accordingly, by carrying out a selection experiment over multiple generations, we increase the likelihood of identifying small genetic effects. Furthermore, by breeding flies for two generations in the absence of selection, we controlled for environmental and maternal effects. Finally, to avoid experimental bias, we allowed the flies to impose selection, so that flies determined which traits would be relevant for female choice or male competitive ability.

Our experiment provided a broad analysis of how fitness components responded to variation in the opportunity for sexual selection, though future studies need to examine even more traits. Although the survival results are consistent with good-genes models of sexual selection, they need to be interpreted with caution. First, as in previous experiments (e.g., ref. 15), we cannot distinguish between the effects of female choice and male competition in the multimale lines. The increase in fitness may have been a result of females choosing high-quality males, consistent with good-genes models. But previous studies have demonstrated genetic variation for male competitive ability (42, 43). Our results could have been due to positive genetic correlations between male competitive ability and male survival.

Second, we have not eliminated the possibility for selection via cryptic female choice (44, 45) in the S lines. In single-male lines, females that mated with high-quality males may have contributed a disproportionate share of offspring to the next generation. To control for any effect of cryptic choice, subsequent experiments should control for variation in family size among females.

Third, we cannot rule out the possibility of sexual selection through direct benefits in our experiment. Direct benefit models do not make any predictions about the *genetic* correlations between viability and the opportunity for sexual selection (6). We do know, however, that *Drosophila* males contribute not only sperm in their ejaculate, but also a large number of accessory gland proteins (ACPs). Some of these proteins have direct and substantial effects on female fitness (46, 47). Though M line males may have been selected to provide beneficial substances to females, there is nothing inherent in the selection regime that would lead us to expect this response, especially given the absence of multiple mating here.

Finally, although nonadaptive, Fisherian models of sexual selection do not predict a correlation between female choice and fitness traits, the existence of such a correlation does not preclude the possibility of runaway sexual selection. Future studies may benefit from artificial selection approaches such as the one we have used here to help elucidate the relative contribution of these other mechanisms to the evolution of secondary sexual characteristics.

Survival Rates and Good-Genes Models. The most salient finding of this study to tests of sexual selection theory is that adult survival rates increased in populations with an increased opportunity for sexual selection. The result is consistent with the idea that females choose males on the basis of relatively high genetic quality. Selection could also operate via male–male competition, whereby relatively high-quality males compete more successfully for access to females in the multiple-male lines.

After 10 generations of selection, we found that lines with the opportunity for sexual selection had significantly greater adult survival than those lines with enforced monogamy. Although the effect was statistically significant, it was not as strong as survival effects that have been found previously in response either to direct selection on survival (48–50) or phenotypic manipulation on reproductive effort (46, 51, 52). Several factors may have led to a relatively weak response to selection in this experiment and make the significant results all the more impressive. First, in our experiment, any selection on survival was necessarily indirect. Second, the increase in survival that we observed may have been counter-balanced, in part, by costs of sexual selection (53–55). Third, by allowing the flies to impose their own selection, the selection pressure may have changed from one generation to the next (because of gene–environment interactions) and from one vial to the next [because of variation in female preference (e.g., ref. 56)]. Fourth, variation among females in offspring number may have reduced the selective distinction between "opportunity for sexual selection" and "no opportunity for sexual selection" treatments. Among females paired with a single male, those with "low-quality" males simply may not mate with him, and those with "high-quality" males may respond facultatively by producing relatively more eggs in the egg-laying chamber. Finally, flies in the mortality cages were kept in relatively benign, low-density conditions. Penetrance of genes that influence longevity is greater under harsh conditions (57, 58).

Larval Viability. A previous study (15) found that with increased opportunity for sexual selection, females produce offspring with a small but significant increase in viability relative to monogamously mated females. Several later studies have not found a correlation between female preference and offspring fitness for specific males (e.g., refs. 59–61), though alternative experimental designs have lent support to Partridge's original claim (e.g., refs. 62–66). In our analysis of flies from generation 9, larval viability was greater in M lines than in S lines, as predicted, but the result was not significant. Although the first experiment was nonsignificant, the power was weak because of small sample size (four vials per replicate $\times$ six replicates). In the second experiment, we increased statistical power by increasing the sample size (77 vials in total). However, in the second experiment, in which each vial had 300 embryos, approximately 68% of the adult flies emerging were wild type, as compared with approximately 40% of the flies in the first experiment, and there was no significant difference between treatments. Previous studies on fitness have shown that certain genetically determined differences in fitness may be expressed only under highly competitive conditions (e.g., refs. 57 and 58). Thus, in the second larval viability assay, we may have not seen a difference among lines because of insufficient levels of competition.

Morphological Traits. Our primary interest in this experiment was to measure the effect of sexual selection on an adult fitness trait. However, in the course of the experiment, we were also able to estimate the effect of sexual selection on three morphological traits. The M lines were heavier than S lines for both males and females, supporting previous findings that sexual selection increases dry body mass in *D. melanogaster* (26). However, the difference in mass disappeared by generation 11. No differences

were detected for wing size. The lack of response in wing size may be due to opposing forces of selection. Wilkinson (67) showed that sexual selection increases wing size, while selection on larval viability decreases wing size.

Analyses of sex-comb tooth number provided evidence for a difference between treatments, though the effects were not consistent between generations. In generation 13 flies, there was no treatment effect on comb tooth number, and only a slight but nonsignificant increase in FA in S lines. In generation 14, however, M lines had significantly higher comb tooth number, and a slight, marginally significant increase in FA in the M lines. This change in result between generations also was observed in a similar study on guppies (12). The increase in comb tooth number in the M line males contrasts with a previous study of *D. simulans*, in which males caught in the field during copulation had significantly fewer sex-comb teeth than unmated males (68).

Maintenance of Genetic Variation for Female Choice. If mate choice leads to fitness benefits for both males and females, we are left with the question of how variation for female preference or male traits is maintained. Several factors may be involved. First, not all fitness traits necessarily will benefit from sexual selection (22). Second, gene–environment interaction could lead to environmental shifts in optimum preference and trait genotypes (19). Third, variation in fitness traits related to sexual selection may be maintained by mutation–selection balance (69). Finally, genetic benefits to one sex may prove detrimental to the other sex, as has been shown for survival in *Drosophila* (47, 70). Future "artificial selection on sexual selection" experiments may serve as a powerful tool to clarify these issues.

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