

THE ROLES OF NATURAL AND SEXUAL SELECTION DURING ADAPTATION TO A NOVEL ENVIRONMENT

HOWARD D. RUNDLE,¹ STEPHEN F. CHENOWETH, AND MARK W. BLOWS

School of Integrative Biology, University of Queensland, St. Lucia, Queensland 4072, Australia

Abstract.—The net effect of sexual selection on nonsexual fitness is controversial. On one side, elaborate display traits and preferences for them can be costly, reducing the nonsexual fitness of individuals possessing them, as well as their offspring. In contrast, sexual selection may reinforce nonsexual fitness if an individual's attractiveness and quality are genetically correlated. According to recent models, such good-genes mate choice should increase both the extent and rate of adaptation. We evolved 12 replicate populations of *Drosophila serrata* in a powerful two-way factorial experimental design to test the separate and combined contributions of natural and sexual selection to adaptation to a novel larval food resource. Populations evolving in the presence of natural selection had significantly higher mean nonsexual fitness when measured over three generations (13–15) during the course of experimental evolution (16–23% increase). The effect of natural selection was even more substantial when measured in a standardized, monogamous mating environment at the end of the experiment (generation 16; 52% increase). In contrast, and despite strong sexual selection on display traits, there was no evidence from any of the four replicate fitness measures that sexual selection promoted adaptation. In addition, a comparison of fitness measures conducted under different mating environments demonstrated a significant direct cost of sexual selection to females, likely arising from some form of male-induced harm. Indirect benefits of sexual selection in promoting adaptation to this novel resource environment therefore appear to be absent in this species, despite prior evidence suggesting the operation of good-genes mate choice in their ancestral environment. How novel environments affect the operation of good-genes mate choice is a fundamental question for future sexual selection research.

Key words.—Cuticular hydrocarbons, *Drosophila serrata*, experimental evolution, fitness, good genes, indirect benefits, sexual conflict.

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The role of natural selection in adaptation is well understood. Except when the fates of different levels of biological organization (e.g., transposable elements, genes, cells, individuals, sexes, groups) are in conflict (Dawkins 1976; Keller 1999), natural selection generally acts to increase nonsexual fitness (all aspects of fitness excluding the number and quality of mates; Andersson 1994), improving the ability of organisms to live and reproduce in a given environment (Endler 1986). The role of sexual selection in affecting nonsexual fitness, and hence adaptation, is less clear. Prompted by the fact that many traits favored by sexual selection are opposed by natural selection, Darwin (1859; 1871) was careful to make a clear distinction between the two processes. Since that time, the view has arisen that sexual selection may often act in opposition to nonsexual natural selection, reducing population mean fitness by favoring the evolution of elaborate display traits (generally in males) and preferences for them (generally in females), both of which can be costly to their bearers and can reduce the nonsexual fitness of males, females, and their offspring (Lande 1980; Grafen 1990; Kirkpatrick and Ryan 1991; Price et al. 1993; Gavrilets et al. 2001; Houle and Kondrashov 2002; Lorch et al. 2003).

In contrast to the view that natural and sexual selection are opposed, good-genes models of sexual selection propose that natural and sexual selection may often act in concert, both favoring individuals of high nonsexual fitness and thus promoting adaptation. Also dating back to Darwin (1859), the basic idea is that there is a relationship between nonsexual fitness and sexual display traits, such that females preferring

extravagant males also obtain males of high genetic quality. By mating with these males, females produce high quality offspring, thereby gaining an indirect benefit from their choice of mates (Kirkpatrick and Ryan 1991). The fundamental requirement of this hypothesis is that sexual display traits are honest indicators of male genetic quality or condition (Zahavi 1975; Hamilton and Zuk 1982; Pomiankowski 1987; Iwasa and Pomiankowski 1991, 1999). In formal models of the process, this amounts to a positive genetic correlation between an individual's attractiveness and its condition. Rowe and Houle (1996) argued that this assumption is likely to be true whenever sexual display traits are costly to produce and their exaggeration increases mating success because, once genetic variation in the display traits is exhausted, variation at other loci affecting overall condition will be recruited (termed the "genetic capture hypothesis"). As higher condition individuals are better able to pay the costs of trait exaggeration, display traits become honest indicators of male condition (Pomiankowski 1988; Iwasa and Pomiankowski 1991). Condition is thought to be a large mutational target, so genetic variation in condition may be maintained and reflected in the display traits despite persistent directional selection (Rowe and Houle 1996; Kotiaho et al. 2001).

When the conditions for good-genes mate choice are met, a number of recent models have suggested that sexual selection can have beneficial effects on nonsexual fitness. These include increasing the rate and extent of adaptation (Lorch et al. 2003), increasing the spread of advantageous alleles in structured populations (Proulx 1999, 2001, 2002), and improving the purging of genetic load (Whitlock 2000). Such effects can increase population mean fitness and can even provide an advantage to sexual reproduction (Agrawal 2001; Siller 2001). How natural and sexual selection interact to

¹ Present address: Department of Biology and Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada; E-mail: hrundle@uottawa.ca.

affect nonsexual fitness in a novel or changing environment is therefore a fundamental question in evolutionary biology that has important implications for both our understanding of why mate preferences evolve and what their evolutionary consequences are for adaptation and fitness.

Results of empirical studies testing the contribution of sexual selection to adaptation are mixed. Partridge (1980) manipulated the opportunity for sexual selection in *Drosophila melanogaster* by either permitting or preventing mate choice in separate laboratory populations (+SS and -SS respectively), and then measured the consequences for larval viability. She found that larval viability was 1–2% higher in the offspring of females from the +SS treatment as compared to offspring of females from the -SS treatment, suggesting an indirect benefit of female mate choice consistent with good-genes mate choice. Results of subsequent studies have been less clear (Schaeffer et al. 1984; Promislow et al. 1998), with no consistent evidence for a benefit of sexual selection to nonsexual fitness. This may be due, in part, to a focus on individual fitness components in place of total nonsexual fitness. Trade-offs among fitness components are common, necessitating more comprehensive estimates of total lifetime fitness (Hunt et al. 2004). However, Holland and Rice (1999) showed that after 47 generations of experimental evolution in *D. melanogaster*, net reproductive rate, a close proxy of total nonsexual fitness, had increased by approximately 30% in -SS populations, suggesting instead that a cost to sexual selection, arising through sexual conflict, had been ameliorated in these populations when lifelong monogamy was enforced. (For a comprehensive review of sexual conflict, see Arnqvist and Rowe 2005.) Indirect benefits of sexual selection, which could potentially counter such direct costs, appear to be lacking in this population (Brown et al. 2004; Orteiza et al. 2005). Similar results have been found recently in experiments using the yellow dung fly, *Scathophaga stercoraria*, in which females from -SS populations had higher nonsexual fitness than their counterparts from +SS populations when each were mated only once (Martin et al. 2004).

Holland (2002) noted that the benefits of sexual selection under constant laboratory conditions may be underestimated when compared to those occurring in changing or novel environments in which heritable variation for fitness is greater. In the only experiment of its kind, Holland (2002) tested whether sexual selection improved adaptation to a novel environment. This experiment subjected two pairs of replicate experimental populations of *D. melanogaster* to 36 generations of a novel thermal stress in the presence and absence of sexual selection. Substantial adaptation was observed: net reproductive rate increased by six standard deviations in the experimental populations relative to the controls. However, there was no detectable difference in reproductive rate between the +SS and -SS experimental populations, suggesting that sexual selection did not improve adaptation. This lack of an effect of sexual selection on adaptation was not caused by the evolution of intersexual mutualism in -SS populations that, in theory, could have masked the benefits of mate choice in the +SS populations; there was no difference in male-induced harm to females between -SS and +SS populations (Holland 2002).

Here we present the results of an experiment using the

Australian fruit fly *D. serrata* in a manipulative test of the roles of natural and sexual selection during 16 generations of adaptation to a novel larval food resource. We employed 12 replicate populations in a powerful two-way factorial design that independently manipulated the opportunities for both natural and sexual selection. This resulted in three populations that experienced both natural and sexual selection, three that experienced reduced natural selection with sexual selection present, three that experienced natural selection with reduced sexual selection, and three that experienced reduced natural and reduced sexual selection. This factorial design is important because the contributions of natural and sexual selection to adaptation may be redundant over the time scales of standard laboratory evolution experiments: either process on its own may be sufficient to rapidly fix the majority of standing genetic variance in nonsexual fitness in a novel environment. Past experiments, including that of Holland (2002), are uninformative concerning this possibility because they lacked a treatment involving sexual selection alone. The factorial design employed here, however, estimates both the independent and combined roles of natural and sexual selection during adaptation, providing a more comprehensive understanding of the contribution of sexual selection to adaptation.

Drosophila serrata is an ideal candidate for testing the role of sexual selection in adaptation for three reasons. First, sexual selection arises from mutual mate choice in this species and the process is well understood. A number of manipulative experiments and quantitative genetic studies, conducted on both laboratory and field-caught populations, have revealed strong preferences for specific combinations of nonvolatile cuticular hydrocarbons (CHCs; Howard et al. 2003) by both males and females when choosing mates (Blows and Allan 1998; Hine et al. 2002, 2004; Chenoweth and Blows 2003, 2005; Petfield et al. 2005; Rundle et al. 2005). Second, the operation of good-genes mate choice is suggested in at least one laboratory population by the presence of two critical genetic correlations: one between female preferences for male CHCs and offspring fitness and the other between male attractiveness based on CHCs and offspring fitness (Hine et al. 2002). However, the latter genetic correlation between attractiveness and offspring fitness is negative, suggesting that the evolution of antagonistic pleiotropy may have occurred and that females may not currently be gaining indirect genetic benefits from their choice of mates in that environment. In addition, four individual CHCs are positively correlated with wing length (an index of body size) in a field population, suggesting that their expression may be condition dependent (Hine et al. 2004). The above results, suggestive of good-genes mate choice, contrast with those from *D. melanogaster* that suggest a mate choice system dominated by strong sexual conflict (e.g., Rice 1996; Holland and Rice 1999; Brown et al. 2004; Wigby and Chapman 2004; Friberg et al. 2005; Lew and Rice 2005; Orteiza et al. 2005; Stewart et al. 2005).

If good-genes mate choice is operating in *D. serrata*, theory predicts that it should improve both the rate and extent of adaptation to a novel environment (Lorch et al. 2003). Here we directly test this prediction using four replicated measurements of mean nonsexual fitness for each of our 12 experimental populations. Three of these measures were taken

during the course of experimental evolution (during generations 13, 14, and 15, respectively) and therefore include the possibility that any direct costs of sexual selection, including male-induced harm to females, could vary between the sexual selection treatments. The final fitness measure was larger (involving the creation of over 1100 unique male-female pairs and counting more than 26,600 offspring) and was conducted under $-SS$ conditions, therefore controlling for all aspects of environmental variation among treatment populations, including the opportunity for any direct costs of sexual selection.

MATERIALS AND METHODS

Derivation and Maintenance of Experimental Lines

A stock population of *D. serrata* was created in November 2003 by mixing six laboratory populations originally collected from different sites along the east coast of Australia (ranging from Cooktown, Queensland, in the north to Wollongong, New South Wales, in the south). This stock was maintained under constant conditions (25°C; 12L:12D photoperiod) at a large population size (16 half-pint stock bottles) with discrete, nonoverlapping generations on the same standard laboratory yeast media (Rundle et al. 2005) to which the six founding populations were long adapted. The stock was maintained under these conditions for 21 generations prior to the start of the experiment.

In September 2004, 12 replicate populations were independently derived from the stock population and maintained in the laboratory under constant conditions (25°C; 12L:12D photoperiod) with discrete, nonoverlapping generations on a novel corn-based food media (Rundle et al. 2005). These populations were assigned in a two-way factorial design to one of four experimental treatments, yielding three replicate populations within each treatment. The treatments varied the opportunities for both natural and sexual selection, following the design of Blows (2002). Three populations experienced both natural and sexual selection, three experienced reduced natural selection with sexual selection present, three experienced natural selection with reduced sexual selection, and three experienced reduced natural and reduced sexual selection.

For every population each generation, 55 virgin males and 55 virgin females were collected from an average of 45 vials (maximum of 50 randomly selected vials if more were available). How these flies were collected and then mated determined the natural and sexual selection treatments, as outlined below. Once mated, males were discarded and the 55 females were placed singly in vials for egg laying; these females were discarded after approximately 24 h. Eleven days later (at the onset of darkness for the flies), all vials were cleared of any adult offspring in preparation for virgin collection. Virgin flies were collected on the morning of day 13, repeating the above process for the next generation.

Natural selection was permitted ($+NS$ treatment) when collecting virgins by pooling the offspring from all females (vials) within a population and then randomly selecting 55 males and 55 females to form the next generation. The expected contribution of a female to the next generation in these populations was thus proportional to their number of off-

spring. In contrast, the opportunity for natural selection was greatly reduced ($-NS$ treatment) by equalizing, for each female in a population, their contribution of adult flies (males and females) to the next generation. Although this manipulation greatly weakened natural selection on female productivity and other fitness components, it did not completely eliminate all forms (e.g., alleles with large deleterious effects could still be selected against). To maintain census population sizes constant at 55 males and 55 females every generation, a second male and female were collected from randomly chosen vials when necessary.

Sexual selection was manipulated in each population, independent of natural selection, by either mixing all virgins collected every generation, allowing them to chose mates (sexual selection permitted; $+SS$ treatment), or randomly assigning individual females with a single mate, thus greatly reducing the opportunity for sexual selection ($-SS$ treatment). In the $+SS$ treatment, all 55 virgin males and 55 virgin females from a population were placed together in a half-pint bottle for three to six days for mating. In the $-SS$ treatment, 55 male-female pairs were randomly created and placed separately in vials for three to six days for mating. The time spent in these mating treatments, and the time given for subsequent egg laying, was always the same for all populations within any given generation. However, these times varied among generations, and our experimental protocol thus does not allow among-generation comparisons of the magnitude of treatment effects (natural or sexual selection).

Fitness Measures

The evolution of male and female CHCs in these populations is investigated in another manuscript (S. F. Chenoweth, H. D. Rundle, and M. W. Blows, unpubl. ms.). Here, we address the effects of natural and sexual selection on population mean fitness, measured as follows. Each generation, the number of offspring emerging from a vial between day 11 (vials cleared) and day 13 (virgins collected to create the next generation) provides a composite measure of the mating propensity, fecundity, and fertility of the female and the larval and early adult survivorship of her resulting offspring. This measure is a large component of total fitness in these populations because it is taken within the context of experimental evolution; it is these offspring that determine a female's contribution to the next generation within the design of the experiment. We counted the number of adult offspring produced by each female in every population during generations 13, 14, and 15 of the experiment.

Although the above measure is closely related to total fitness, our manipulation of sexual selection required different mating environments (i.e., 110 flies together in a bottle in the $+SS$ treatment vs. individual male-female pairs in vials in the $-SS$ treatment). The flies spent three to six days every generation in these different environments prior to the period of female egg laying that produced the next generation. Such environmental differences could alter female productivity among treatments. For example, harassment of females by males in the $+SS$ treatment could directly harm them and reduce their fecundity. For this reason, we conducted an additional fitness measure at generation 16 of the experiment.

This measure was replicated at a larger scale (approximately twice the size of the previous measures) and controlled for the above differences by removing the opportunity for mate choice and both reducing and standardizing the opportunity for male-induced harm in all populations. A comparison of the effects of our sexual selection treatment on nonsexual fitness measured during generations 13–15 of experimental evolution (in which the opportunity for direct costs and benefits of mating, including male-induced harm of females, varied between sexual selection treatments) with the effects when measured during generation 16 (in which such variation was removed and the opportunity for direct costs was minimized) therefore provides a estimate of the extent of such direct effects.

Our single, large measure was performed during generation 16 by collecting 100 virgin males and 100 virgin females from each population and creating random male-female pairs in separate vials for mating. After three days, males were discarded and each female was transferred to a new vial for egg laying. Females were discarded after 24 h, matching how they were maintained during experimental evolution. Vials were cleared of adult offspring at the onset of darkness on day 11. Live adult offspring were counted on days 13 and 14.

Statistical Analyses

In all cases, the distribution of fitness (number of adult offspring) in each population was nonnormal because some females in every population produced no offspring. Because this fraction was small (7.6% overall in the three measures taken during experimental evolution and 6.9% in the single large measure), and we did not know why these females failed to produce offspring (possibilities include infertile males and/or females, refusal to mate, and experimenter mistakes when separating the flies by sex), analyses were restricted to those females that produced offspring. Results do not change qualitatively when these data are included, although statistical tests become unreliable because the assumption of normality is violated.

Differences in fitness were tested using the following mixed linear model:

$$Y = \text{constant} + N_i + S_j + NS_{ij} + P(NS)_{k(ij)} + B_l + \text{error}, \quad (1)$$

where Y is the fitness measure (number of adult offspring) of a female belonging to population P nested within natural selection treatment N ($0 = \text{reduced}$, $1 = \text{present}$) and sexual selection treatment S ($0 = \text{reduced}$, $1 = \text{present}$). NS is the effect of the interaction of natural and sexual selection and B is the effect of the blocking of the fitness counts into three separate groups (each containing four populations, one from each treatment) that were counted in sequence. Population was modeled as a random effect, and all other terms were fixed. The model was analyzed using the MIXED procedure in SAS (ver. 8.2; SAS Institute Inc., Cary, NC), employing restricted maximum likelihood to estimate the model parameters. Analyses were performed separately for each generation because, as noted earlier, our experimental design does not permit among-generation comparisons. The presence overall of a direct cost to females of sexual selection during

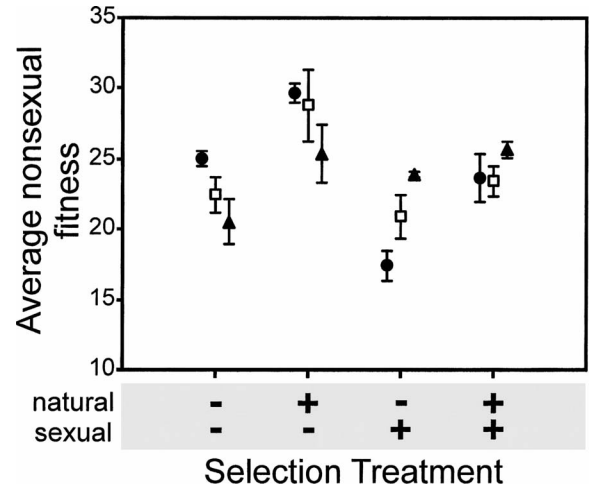


FIG. 1. Average nonsexual fitness (\pm SE) of the 12 experimental populations independently measured during the context of experimental evolution during generations 13, 14, and 15 (represented respectively by closed circles, open squares, and closed triangles). Nonsexual fitness was measured each generation by separately counting the number of adult offspring produced by an average of 49 (range 40–66) females from each population. Males and females in the +SS treatments (sexual selection present) were allowed to choose their mates, whereas individual males and females were randomly paired in –SS treatments (sexual selection reduced).

generations 13–15 was tested using both a Fisher's combined probability test and a Z-transform test (also known as Stouffer's method), the latter of which is not asymmetrically sensitive to small P -values (see Whitlock 2005). (A weighted Z-test, which weights more strongly those P -values deriving from studies having more information, is unnecessary in our case because all three experiments employed the same protocol with almost identical sample sizes.) Probabilities were converted to one-tailed directional values for a negative effect of sexual selection prior to combining them.

RESULTS

Natural Selection and Adaptation to the Novel Environment

Adaptation to the novel corn-based food occurred in the presence of natural selection. According to the three measures taken during experimental evolution, mean nonsexual fitness was 16–23% higher in populations in the +NS treatment than in those in –NS treatment (Fig. 1). These increases were significant in all three cases (Table 1A). This adaptation was confirmed by the generation 16 large fitness measure that standardized and reduced the opportunity for any direct costs of sexual selection among all treatments. The effect of natural selection was considerable and highly significant in this case, with mean nonsexual fitness being 52% higher in the +NS populations than in the –NS populations (Table 1B).

Sexual Selection and Adaptation to the Novel Environment

There was no evidence that sexual selection promoted adaptation to the novel corn-based food environment, and its presence appears to have induced significant direct costs to females (Fig. 1). Measures taken during generations 13 and 14 indicated that mean nonsexual fitness was 23% and 14%

TABLE 1. Results of the mixed linear models testing the effects of natural selection, sexual selection, their interaction, and experimental block on the nonsexual fitness (number of adult offspring) of replicate females from the 12 experimental populations of *Drosophila serrata*. Fitness measures derive from an average of 49 (range 40–66) replicate females from each population and were taken during the course of experimental evolution over each of three separate generations (A), or from an average of 92 (range 79–101) replicate females from each population that were each randomly assigned a single mate (B). Asterisks highlight *P*-values ≤ 0.05 .

	Natural selection			Sexual selection			Interaction			Block		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
(A) During experimental evolution												
Generation 13	1, 6	24.85	0.003*	1, 6	39.85	0.001*	1, 6	0.63	0.459	2, 574	1.09	0.338
Generation 14	1, 6	9.60	0.021*	1, 6	5.92	0.051	1, 6	1.83	0.225	2, 571	2.72	0.067
Generation 15	1, 6	6.60	0.042*	1, 6	2.19	0.189	1, 6	1.58	0.256	2, 580	1.56	0.211
(B) Large fitness measure												
Generation 16	1, 6	17.17	0.006*	1, 6	0.19	0.680	1, 6	0.52	0.500	2, 1086	12.00	<0.0001*

lower in the +SS treatment than in the –SS treatment respectively, with the decrease being significant in the first case and borderline in the second (Table 1A). Sexual selection had no significant effect on mean nonsexual fitness in generation 15 (Table 1A), although the estimate was slightly positive (9% increase). Combining the results from these three generations, the negative effect of sexual selection on mean nonsexual fitness was significant (Fisher's combined probability test: $\chi_6 = 23.4$, $P < 0.0007$; Z-transform test: $Z_c = 2.33$, $P = 0.010$).

The failure of sexual selection to promote adaptation was confirmed in the large, generation 16 fitness measure. In this case, sexual selection had no significant effect on mean nonsexual fitness (Table 1B) and the estimate was slightly negative (4% decrease; Fig. 2). A comparison of the effects of sexual selection in these two sets of fitness measures (gens. 13–15 vs. gen. 16) yields two conclusions. First, because mating treatments were standardized among all populations in the generation 16 measure, the lack of significant cost of sexual selection indicates that the propensity of males to harm females did not evolve between our sexual selection treatments (i.e., males from the +SS populations were not more

harmful to their females, on average, than were males from the –SS populations). Given this, the presence of a significant reduction in mean nonsexual fitness in the +SS populations in the first set of measures (gens. 13–15) must therefore be attributed to a direct cost of sexual selection occurring only in this mating environment. This is consistent with male-induced harm to females in the +SS environment.

Interaction of Natural and Sexual Selection during Adaptation

There was no indication that natural and sexual selection interacted in any way to affect adaptation to the novel corn-based food environment (Table 1). This is true for all three nonsexual fitness measures taken during experiment evolution (Fig. 1). It is also true for the large, environmentally controlled measure taken during generation 16 (Fig. 2).

This lack of an interaction indicates that the failure of sexual selection to promote adaptation was not a result of changes in effective population sizes. Although census sizes were held equal in all populations throughout the experiment, the continued operation of natural and sexual selection both necessitate reductions in effective population sizes (i.e., variance among individuals in their contribution to the next generation; Santiago and Caballero 1995). In theory, it is therefore possible that good-genes indirect benefits of mate choice could be prevented in +SS populations because sexual selection caused substantial reductions in the effective sizes of these populations that hampered selection. Our factorial experimental design, however, allows a test of this hypothesis. If sexual selection was responsible for such reductions in effective population size, the effects of natural selection should also have been hampered in the +SS populations. The absence of any significant interaction between natural and sexual selection suggests that this did not occur.

DISCUSSION

Consistent with expectation, populations rapidly adapted to the novel resource environment in the presence of natural selection. Mean nonsexual fitness was significantly higher in the presence of natural selection in all three measures taken during experimental evolution (range 16–23%), despite a significant direct cost of sexual selection in the +SS populations. When measured after 16 generations, when this direct cost

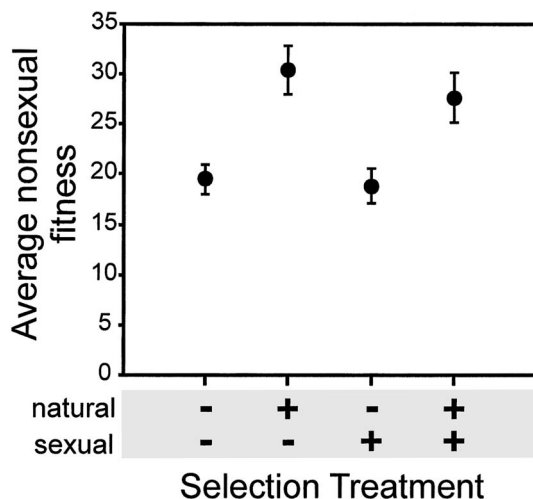


FIG. 2. Average nonsexual fitness (\pm SE) of the 12 experimental populations at generation 16. Nonsexual fitness was measured for each population by separately counting the number of adult offspring produced by an average of 92 (range 79–101) females, each mated to a single, randomly chosen male from her population.

was controlled and minimized, population mean nonsexual fitness was, on average, 52% higher in the +NS populations relative to -NS populations.

In contrast, despite clear effects of sexual selection on the evolution of male and female display traits (CHCs) in these populations during this experiment (S. F. Chenoweth, H. D. Rundle, and M. W. Blows, unpubl. ms.) and prior evidence suggesting that good-genes mate choice may be operating in this species (Hine et al. 2002, 2004), there was no evidence that sexual selection benefited population mean nonsexual fitness (Figs. 1, 2). This was true both for sexual selection in isolation and in combination with natural selection. This result is consistent with the only other test of the contribution of sexual selection to adaptation to a novel environment in another taxa (*D. melanogaster*). In this experiment, the combined presence of both natural and sexual selection also did not promote adaptation to a novel environment over that observed by natural selection alone (Holland 2002).

The absence of any benefit of sexual selection was not a consequence of our mating treatments that were designed to maximize the likelihood of sexual selection differing. When compared with our -SS (monogamy) treatment, our +SS treatment mixed 55 males and 55 females together for mating, thus providing extensive opportunities for both intrasexual competition and mutual mate choice (with sexual selection potentially altering the quantity and/or quality of an individual's mates). The success of this manipulation in altering sexual selection is confirmed by its sex-specific effects on the evolution of male and female CHCs in these same populations during this experiment (S. F. Chenoweth, H. D. Rundle, and M. W. Blows, unpubl. ms.). It is also unlikely that a lack of power was responsible for the lack of a benefit: an average difference in female productivity of only 2.2 individuals between the sexual selection treatments had a 90% chance of detection in our final fitness measure (retrospective power analysis using the actual sample sizes and root mean square error estimated from the model).

Three main explanations remain for the failure of sexual selection to promote adaptation. First, the operation of good-genes mate choice requires a genetic correlation between display traits and condition such that individuals choosing attractive mates also get mates of high genetic quality (Hamilton and Zuk 1982; Pomiankowski 1987; Iwasa and Pomiankowski 1991, 1999). If the genetic basis of the display traits used in mate choice (e.g., CHCs in *D. serrata*), or of condition, vary among environments, the genetic correlation between them that was originally present in the ancestral environment may be weakened or eliminated in a novel environment, preventing any indirect genetic benefits of mate choice in one environment from translating into another (Hunt et al. 2004). Such genotype-by-environment interactions for nonsexual fitness components, such as performance, are common (Lynch and Walsh 1998; Hunt et al. 2004). Provided the assumptions of good-genes mate choice are met in the novel environment (i.e., display traits are still costly to produce and they affect mating success), the genetic correlation between display traits and condition should re-establish itself via the process of genic capture (Rowe and Houle 1996). However, the dynamics of how this occurs have never been studied empirically. Subsequently, mate prefer-

ences may also need to evolve to find the new combination of display traits indicative of high condition in this environment. Plasticity of mate preferences could cause individuals to prefer nonoptimal display trait combinations in a novel environment, also necessitating the evolution of preferences. Little is known about how mate preferences evolve in novel environments, although selection has recently been implicated in the evolution of female mate preferences when replicate *D. serrata* populations adapted to two novel larval food environments, including corn (Rundle et al. 2005).

Second, sexual selection may have failed to promote adaptation because good-genes mate choice does not occur in *D. serrata*. In general, the extent of good-genes mate choice is unclear because: (1) experimental evidence for the condition dependence of sexual display traits is weak (Cotton et al. 2004); (2) theory suggests that the indirect selection caused by good genes may often be overwhelmed by direct selection on mating preferences (Kirkpatrick 1996; Kirkpatrick and Barton 1997; but see Houle and Kondrashov 2002; Cameron et al. 2003); (3) rigorous, manipulative tests of good-genes mate choice are generally lacking—the majority of evidence is correlational and therefore open to alternate interpretations (e.g., Sheldon 2000) and generally involves estimates of individual fitness components as opposed to total nonsexual fitness (Hunt et al. 2004; but see Head et al. 2005; Reid et al. 2005); and (4) our understanding of the genic capture hypothesis is limited (Tomkins et al. 2004), and three recent studies call into question its ability to maintain sufficient genetic variance in fitness (Blows et al. 2004; Hall et al. 2004; Hine et al. 2004). Nevertheless, the operation of good-genes mate choice in *D. serrata* is suggested by two past studies (Hine et al. 2002, 2004). It therefore appears likely that good-genes mate choice is involved in some way in the evolution of mate preferences in *D. serrata*, although a definite conclusion awaits the outcome of further manipulative tests.

Finally, sexual selection may not have promoted adaptation because indirect benefits of good-genes mate choice for some fitness components may have been offset by costs of sexual selection for others. As noted by Holland (2002), such variation among fitness components in the effects of sexual selection could yield no net cost or benefit in the end and could explain the inconsistent results observed in past studies that measured specific life-history traits (e.g., larval viability; Partridge 1980; Schaeffer et al. 1984; Promislow et al. 1998). In our experiment, the +SS mating environment had direct fitness costs for females, likely arising from some form of male-induced harm (see below). Although a net benefit of sexual selection was still absent in these populations when tested in a monogamous (-SS) mating environment, it is possible that additional forms of sexual conflict, not ameliorated in this environment, could have masked any indirect benefit to mate choice also occurring in the +SS populations. A direct test of this possibility requires independent measures of the extent of sexual conflict (i.e., male induced harm to females; Holland and Rice 1999; Holland 2002). Even if such a trade-off was occurring, however, the final outcome remains that sexual selection had no net effect in promoting adaptation.

As previously noted, sexual selection not only failed to

promote adaptation, but its presence also appeared to engender direct cost to females. Although the effect of sexual selection on nonsexual fitness varied noticeably among generations (-23% to $+9\%$), heterogeneity in trait response is a standard observation in selection experiments (Falconer and Mackay 1996) and, in our case, was almost certainly further enhanced by our failure to control among generations the time spent in the mating treatments (this time was controlled among treatments within generations). Nevertheless, combined probability tests indicated that this direct cost of sexual selection was significant. Such a cost could have arisen from any difference in the mating environments between the +SS and -SS treatments, although male-induced harm to females in the +SS treatment is a likely cause. Such harm could arise, for example, from damage to females caused by excessive male courtship, mating with multiple males, or from the action of male seminal fluid proteins. Such costs are known to occur in laboratory populations of *D. melanogaster* (e.g., Fowler and Partridge 1989; Partridge and Fowler 1990; Chapman et al. 1993, 1995) and the nature of these costs in *D. serrata* is an important topic for future research. Our +SS environment was chosen to mimic the laboratory mating conditions to which this population of *D. serrata* should be adapted. Of particular interest, however, both in *D. serrata* and other *Drosophila* species as well, is the extent of such direct costs in populations in nature where mating does not take place in a confined space at high densities of flies.

In conclusion, we used a powerful two-way factorial design to estimate the independent and combined contributions of natural and sexual selection to adaptation to a novel resource environment in a species in which prior evidence suggests the operation of good-genes mate choice (Hine et al. 2002, 2004). Natural selection had the expected effect, increasing mean nonsexual fitness by 52% on average over 16 generations when direct costs of sexual selection were controlled and minimized. Ample opportunities for mate choice by both sexes generated strong sexual selection that was responsible for the sex-specific evolution of male and female CHCs (S. F. Chenoweth, H. D. Rundle, and M. W. Blows, unpubl. ms.), the primary signal traits used in mate choice in this species. However, despite this, and as demonstrated by replicate nonsexual fitness measures taken over four subsequent generations, sexual selection did not improve adaptation. Rather, sexual selection generated significant direct costs to females in the +SS mating environment, consistent with male-induced harm arising from some form of sexual conflict. Our results imply that good-genes mate choice was not operating in this novel environment (at least during the first 16 generations), or that any indirect benefits of good genes were exactly offset in each of six replicate populations by costs of sexual selection arising from sexually antagonistic coevolution. How a change in environment affects the operation of good-genes mate choice is therefore a fundamental topic for future sexual selection research.

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LITERATURE CITED

- Agrawal, A. F. 2001. Sexual selection and the maintenance of sexual reproduction. *Nature* 411:692–695.
- Andersson, M. 1994. *Sexual selection*. Princeton Univ. Press, Princeton, NJ.
- Arnqvist, G., and L. Rowe. 2005. *Sexual conflict*. Princeton Univ. Press, Princeton, NJ.
- Blows, M. W. 2002. Interaction between natural and sexual selection during the evolution of mate recognition. *Proc. R. Soc. Lond. B* 269:1113–1118.
- Blows, M. W., and R. A. Allan. 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. *Am. Nat.* 152:826–837.
- Blows, M. W., S. F. Chenoweth, and E. Hine. 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. *Am. Nat.* 163:329–340.
- Brown, W. D., A. D. Bjork, K. Schneider, and S. Pitnick. 2004. No evidence that polyandry benefits females in *Drosophila melanogaster*. *Evolution* 58:1242–1250.
- Cameron, E., T. Day, and L. Rowe. 2003. Sexual conflict and indirect benefits. *J. Evol. Biol.* 16:1055–1060.
- Chapman, T., J. Hutchings, and L. Partridge. 1993. No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males. *Proc. R. Soc. Lond. B* 253:211–217.
- Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory-gland products. *Nature* 373:241–244.
- Chenoweth, S. F., and M. W. Blows. 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. *Evolution* 57:2326–2334.
- . 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* 165:281–289.
- Cotton, S., K. Fowler, and A. Pomiankowski. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. R. Soc. Lond. B* 271:771–783.
- Darwin, C. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London.
- . 1871. *The descent of man, and selection in relation to sex*. John Murray, London.
- Dawkins, R. 1976. *The selfish gene*. Oxford Univ. Press, Oxford, U.K.
- Endler, J. A. 1986. *Natural selection in the wild*. Princeton Univ. Press, Princeton, NJ.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to quantitative genetics*. 4th ed. Longman Group, Essex, U.K.
- Fowler, K., and L. Partridge. 1989. A cost of mating in female fruit-flies. *Nature* 338:760–761.
- Friberg, U., T. A. Lew, P. G. Byrne, and W. R. Rice. 2005. Assessing the potential for an ongoing arms race within and between the sexes: Selection and heritable variation. *Evolution* 59:1540–1551.
- Gavrillets, S., G. Arnqvist, and U. Friberg. 2001. The evolution of female mate choice by sexual conflict. *Proc. R. Soc. Lond. B* 268:531–539.
- Grafen, A. 1990. Biological signals as handicaps. *J. Theor. Biol.* 144:517–546.
- Hall, M., A. K. Lindholm, and R. Brooks. 2004. Direct selection

- on male attractiveness and female preference fails to produce a response. *BMC Evol. Biol.* 4:1.
- Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites. *Science* 218:384–387.
- Head, M. L., J. Hunt, M. D. Jennions, and R. Brooks. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol.* 3:289–294.
- Hine, E., S. F. Chenoweth, and M. W. Blows. 2004. Multivariate quantitative genetics and the lek paradox: Genetic variance in male sexually selected traits of *Drosophila serrata* under field conditions. *Evolution* 58:2754–2762.
- Hine, E., S. Lachish, M. Higgie, and M. W. Blows. 2002. Positive genetic correlation between female preference and offspring fitness. *Proc. R. Soc. Lond. B* 269:2215–2219.
- Holland, B. 2002. Sexual selection fails to promote adaptation to a new environment. *Evolution* 56:721–730.
- Holland, B., and W. R. Rice. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl. Acad. Sci. USA* 96:5083–5088.
- Houle, D., and A. S. Kondrashov. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. *Proc. R. Soc. Lond. B* 269:97–104.
- Howard, R. W., L. L. Jackson, H. Banse, and M. W. Blows. 2003. Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata*: identification and role in mate choice in *D. serrata*. *J. Chem. Ecol.* 29:961–976.
- Hunt, J., L. F. Bussiere, M. D. Jennions, and R. Brooks. 2004. What is genetic quality? *Trends Ecol. Evol.* 19:329–333.
- Iwasa, Y., and A. Pomiankowski. 1991. The evolution of costly mate preferences. 2. The handicap principle. *Evolution* 45:1431–1442.
- . 1999. Good parent and good genes models of handicap evolution. *J. Theor. Biol.* 200:97–109.
- Keller, L. 1999. Levels of selection in evolution. Pp. 318. Princeton Univ. Press, Princeton, NJ.
- Kirkpatrick, M. 1996. Good genes and direct selection in evolution of mating preferences. *Evolution* 50:2125–2140.
- Kirkpatrick, M., and N. H. Barton. 1997. The strength of indirect selection on female mating preferences. *Proc. Natl. Acad. Sci. USA* 94:1282–1286.
- Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* 350:33–38.
- Kotiaho, J. S., L. W. Simmons, and J. L. Tomkins. 2001. Towards a resolution of the lek paradox. *Nature* 410:684–686.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305.
- Lew, T. A., and W. R. Rice. 2005. Natural selection favours harmful male *Drosophila melanogaster* that reduce the survival of females. *Evol. Ecol. Res.* 7:633–641.
- Lorch, P. D., S. Proulx, L. Rowe, and T. Day. 2003. Condition-dependent sexual selection can accelerate adaptation. *Evol. Ecol. Res.* 5:867–881.
- Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.
- Martin, O. Y., D. J. Hosken, and P. I. Ward. 2004. Post-copulatory sexual selection and female fitness in *Scathophaga stercoraria*. *Proc. R. Soc. Lond. B* 271:353–359.
- Orteiza, N., J. E. Linder, and W. R. Rice. 2005. Sexy sons from re-mating do not recoup the direct costs of harmful male interactions in the *Drosophila melanogaster* laboratory model system. *J. Evol. Biol.* 18:1315–1323.
- Partridge, L. 1980. Mate choice increases a component of offspring fitness in fruit-flies. *Nature* 283:290–291.
- Partridge, L. and K. Fowler. 1990. Nonmating costs of exposure to males in female *Drosophila melanogaster*. *J. Insect Physiol.* 36:419–425.
- Petfield, D., S. F. Chenoweth, H. D. Rundle, and M. W. Blows. 2005. Genetic variance in female condition predicts indirect genetic variance in male sexual display traits. *Proc. Natl. Acad. Sci. USA* 102:6045–6050.
- Pomiankowski, A. 1987. Sexual selection: the handicap principle does work sometimes. *Proc. R. Soc. Lond. B* 231:123–145.
- Pomiankowski, A. N. 1988. The evolution of mate preferences for male genetic quality. *Oxf. Surv. Evol. Biol.* 5:136–184.
- Price, T., D. Schluter, and N. E. Heckman. 1993. Sexual selection when the female directly benefits. *Biol. J. Linn. Soc.* 48:187–211.
- Promislow, D. E. L., E. A. Smith, and L. Pearse. 1998. Adult fitness consequences of sexual selection in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 95:10687–10692.
- Proulx, S. R. 1999. Mating systems and the evolution of niche breadth. *Am. Nat.* 154:89–98.
- . 2001. Female choice via indicator traits easily evolves in the face of recombination and migration. *Evolution* 55:2401–2411.
- . 2002. Niche shifts and expansion due to sexual selection. *Evol. Ecol. Res.* 4:351–369.
- Reid, J. M., P. Arcese, A. Cassidy, S. M. Hiebert, J. N. M. Smith, P. K. Stoddard, A. B. Marr, and L. F. Keller. 2005. Fitness correlates of song repertoire size in free-living song sparrows (*Melospiza melodia*). *Am. Nat.* 165:299–310.
- Rice, W. R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* 381:232–234.
- Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* 263:1415–1421.
- Rundle, H. D., S. F. Chenoweth, P. Doughty, and M. W. Blows. 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biol.* 3:1988–1995.
- Santiago, E., and A. Caballero. 1995. Effective size of populations under selection. *Genetics* 139:1013–1030.
- Schaeffer, S. W., C. J. Brown, and W. W. Anderson. 1984. Does mate choice affect fitness? *Genetics* 107:S94.
- Sheldon, B. C. 2000. Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* 15:397–402.
- Siller, S. 2001. Sexual selection and the maintenance of sex. *Nature* 411:689–692.
- Stewart, A. D., E. H. Morrow, and W. R. Rice. 2005. Assessing putative interlocus sexual conflict in *Drosophila melanogaster* using experimental evolution. *Proc. R. Soc. Lond. B* 272:2029–2035.
- Tomkins, J. L., J. Radwan, J. S. Kotiaho, and T. Tregenza. 2004. Genic capture and resolving the lek paradox. *Trends Ecol. Evol.* 19:323–328.
- Whitlock, M. C. 2000. Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution* 54:1855–1861.
- . 2005. Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. *J. Evol. Biol.* 18:1368–1373.
- Wigby, S., and T. Chapman. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution* 58:1028–1037.
- Zahavi, A. 1975. Mate selection: selection for a handicap. *J. Theor. Biol.* 53:205–214.