Evolution, 56(4), 2002, pp. 721–730

SEXUAL SELECTION FAILS TO PROMOTE ADAPTATION TO A NEW ENVIRONMENT

BRETT HOLLAND[1]
Department of Biology at Natural Sciences IV, 1156 High Street, University of California, Santa Cruz, California 95064

Abstract.—Selection can be divided into sexual and nonsexual components. Some work finds that a component of sexual selection, adaptive female selection for good genes, can promote nonsexual fitness. Less studied is the benefit from sexual selection in toto, that is, when intra- and intersexual selection are both present and able to affect females directly and indirectly. Here an upper bound for the net benefit of sexual selection is estimated for *Drosophila melanogaster*. Replicate populations were allowed to adapt to low-grade thermal stress, with or with out the operation of sexual selection. Because proteins and lipids are highly sensitive to temperature, low-grade thermal stress will select broadly across the genome for alternative alleles. Such broad, directional selection for thermal tolerance should increase the measurable benefits of sexual selection far beyond that available under stabilizing selection. Sexual selection was removed by enforced monogamy without mate choice and retained by enforced polyandry (four males per female). After 36 generations of thermal stress exposure, there was substantial adaptation to the new environment (the net reproductive rate increased six standard deviations relative to thermal controls). However, sexual selection did not affect the rate of adaptation. Therefore, adaptive female selection for thermal tolerance either was insignificant or negated by other aspects of sexual selection, for example, male-induced female harm, which has been shown to diminish under monogamy. This experiment employed two parameters that reduced the opportunity for divergence in such harm: a truncated intersexual interaction period and strong directional selection for thermal tolerance. No divergence in male-induced harm was observed.

Key words.—Adaptation, antagonistic seduction, good genes, intersexual conflict, sexual selection, thermal stress.

Received May 23, 2001. Accepted December 11, 2001.

There is a long-standing dichotomy between components of male sexual fitness (mating and sperm success) and fitness outside of the context of sexual selection (i.e., survival prior to sexual maturity, development rate, and female fecundity), hereafter referred to as ''nonsexual fitness.'' Darwin noted that sexual selection can improve nonsexual fitness: ''The advantage thus gained by the more vigorous pairs in rearing a larger number of offspring has apparently sufficed to render sexual selection efficient'' and ''it appears that the strongest and most vigorous males, or those provided with the best weapons, have prevailed under nature and have led to the improvement of the natural breed or species'' (Darwin 1871, pp. 271 and 258, respectively). Herein, ''benefit'' always refers to net nonsexual fitness and is never used in the self-reinforcing sense, for example, the benefit of producing sexier sons.

Darwin's hypothesis has not been tested previously, but the relationship between components of female selection and components of nonsexual fitness have been addressed in several experiments (reviewed in Andersson 1994). Importantly, those published studies, along with more recently published reports (e.g., Petrie 1994; Welch et al. 1998) sometimes find positive correlations between specific female-selected traits and specific components of nonsexual fitness (e.g., peacock eyespot number and juvenile peacock, but not peahen, survival; Petrie 1994). Sexual selection also acts through intermale competition, which may limit, or reinforce, adaptive female selection. The benefits of, for instance, mate selection for alleles that confer increased offspring survival can be negated through decreased survival or fecundity of females as a by-product of male competition (see reviews by Parker 1979; Rowe et al. 1994; Stockley 1997; Holland and Rice 1998; Partridge and Hurst 1999; Civetta and Clark 2000). Therefore, an issue to be resolved experimentally is whether sexual selection, in its entirety, reinforces nonsexual fitness and, if so, to what extent?

Several studies have isolated net sexual selection as an experimental treatment, measuring its effects on components of nonsexual fitness. Partridge (1980) used *Drosophila melanogaster* as a model system (males court vigorously with dance and wing song [Hall 1994] and provide no measurable resources to females; Markow and Ankney 1984). In one treatment, Partridge enforced life-long monogamy and random mate assignment; in a second group in which she allowed sexual selection, the progeny showed a 1–2% elevation in juvenile survival.

The positive association between sexual selection and juvenile survival found by Partridge (1980) was not repeatable with either *D. melanogaster* or *D. pseudoobscura* (Schaeffer et al. 1984). Promislow et al. (1998) increased the power of Partridge's (1980) experiment by maintaining the mating treatments for nine to 17 generations before taking measurements. Again, the mating treatments produced no significant difference in survival under the environmental conditions to which they had been selected; that is, at less than 30 days of age (Promislow et al. 1998).

Holland and Rice (1999) used a design similar to that of Promislow et al. (1998), but compared the net reproductive rate (i.e., all nonsexual fitness components) of populations with and without sexual selection. After 47 generations, the net reproductive rate was approximately 30% greater in populations in which sexual selection was removed. The increased net reproductive rate of the purely monogamous and randomly mating populations apparently accrued due to the reversal of antagonistic coevolution between the sexes (see Discussion). Because net reproductive rate includes all components of nonsexual fitness, this demonstrates a cost of sexual selection.

[1] Present address: Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Mailstop 84-171, Berkeley, California 94720; E-mail: btholland@lbl.gov.

Why are the experimental results described above inconclusive and even contradictory? Low experimental power may be one contributing factor. Previous studies of benefits have generally been conducted on populations that have been under stabilizing conditions, where the variation in fitness is primarily due to mutation and selection balance. Such experiments underestimate the potential benefits of sexual selection that might arise when heritable variation in phenotypic condition is larger, as would occur during episodes of adaptation to a changing environment.

The approach here was to take the original design of Partridge (1980) and increase experimental power in three ways: (1) increase the opportunity for selection; (2) increase the number of generations over which the selection acts; and (3) measure net nonsexual fitness rather than its components. Because components (i.e., juvenile survival prior to sexual maturity, development rate, and fecundity of females) may negatively covary, they, or a composite, must be included when assessing the role of sexual selection.

The opportunity for selection was increased by challenging populations with a thermally stressful environment. Low-grade thermal stress was chosen for two reasons. First, studies of natural populations indicate that it is a common source of selection (James and Partridge 1995; Feder et al. 1997). Second, a large number of loci are simultaneously selected for alternative alleles, thereby substantially increasing the heritable variation in condition. Membrane fluidity, enzyme catalytic function, and heat shock response are expected to change importantly in response to an elevation of a few degrees (see White and Somero 1982). The populations were allowed to adapt to the new environment for 35 generations and then their net reproductive rate was measured on six occasions over the following 11 generations.

Materials and Methods

Ancestral Conditions

The experiments were carried out with a large, outbred population of flies that had adapted to a controlled laboratory environment for more than 200 generations. This population was established in 1988 from 400 mated females that were collected in central California by L. Harshman. It has subsequently been maintained at $N_e > 5000$, at 25°C, on cornmeal/molasses/killed-yeast medium (hereafter referred to as ''medium''), seeded with live yeast, with a 12:12 L:D diurnal cycle and a 14-day generation cycle. The experimental protocol maintains these conditions except as noted otherwise.

Beginning the Experiment

To start the experiments, replicated samples of flies were taken from the base population to form paired thermal treatment and control lines. The thermal treatment lines were then further divided into lines with and without sexual selection (polyandry and monogamy treatments, respectively). In total there were two replicate sets of these triads. More specifically, initially 400 females and 400 males were sampled from the ancestral population. The sample was equally divided into replicates (A and B) and cultured in large vials (95 × 27.5 mm) with 10 ml of medium with live yeast ($n = 40$ vials/replicate). After being cultured overnight, the adults were transferred without anesthesia to fresh vials for a second night of culture, after which the adults were discarded. The eggs from the first culture were used to start the control populations that were thereafter maintained under ancestral conditions ($n = 133$ females and males/replicate/generation), and at the same density as the experimental populations described below.

The eggs from the second night of culture were used to initiate the two mating treatments. Beginning on day 8 (i.e., the eighth day since eggs were laid) virgin progeny were collected. Virgin collection occurred twice daily from each population within an 8-h period from eclosion (emergence of adults from their pupal cases). Progeny emerging more than 12 h before a virgin collection (e.g., in the evening) were discarded. Flies were initially collected without anesthesia and then held for 2 h before being anesthetized with CO_2, separated by sex, and placed into holding vials (95 × 27.5 mm with 10 ml of medium; $n = 50$ adults/vial). Virgins were collected over two to three days, and this continued until 800 males and 400 females were collected. The virgin adults were maintained by transferring them, every other day, to fresh holding vials until they were mated on day 12 of the 14-day cycle.

Before mating the flies, all of the previously collected virgins were mixed (by sex) and then mates were assigned randomly to individual vials: one male and one female in the monogamy treatment and four males and one female in the polyandry treatment. More specifically, all previously collected females were first combined without anesthesia, and then divided into 14 aliquots using light anesthesia (30 sec of CO_2). The division into 14 smaller aliquots permitted shorter periods of anesthesia during the next step in the mate assignment process. After a 5-min recovery period, the 14 aliquots were sequentially processed to produce 133 individually cultured females per replicate per treatment. The processing of aliquots included 3 min of CO_2 anesthesia during which 19 females were each randomly assigned to a mating vial (100 × 13 mm; containing 3 ml of medium).

Males were next randomly assigned to the individually cultured females. As described for the females above, all previously collected males were first combined, then split into 14 aliquots, and finally individual males were separated from each aliquot. The anesthetized males were placed into recovery vials (one male per vial in the monogamy treatment and four males per vial in the polyandry treatment) for 30–60 min before being combined, without additional anesthesia, with the individually cultured females. At this point in generation 1, all treatment and control populations had been constructed. The mating treatment populations were maintained for 2 days in interaction vials (100 × 13 mm) containing 3 ml of medium. The difference in sex ratio between treatments is a natural aspect of sexual selection in this species: Mating takes place at the feeding site, where arriving females are courted by an average of five wild-type males (Markow and Sawka 1992).

Although the flies courted and mated in the interaction vials, no progeny were retained from this time interval. To begin the next generation, on day 0 of the 14-day cycle, all flies were transferred, without anesthesia, to fresh culture

vials (identical to interaction vials but seeded with live yeast), where eggs were laid overnight and used to produce the next generation.

Subsequent Generations

During day 1 of each new generation, the adults from the previous generation were discarded from the culture vials and their progeny were allowed to develop. Beginning on day 8, virgin progeny were collected from an identical number of productive culture vials (i.e., those producing one or more adult offspring) per population (details given below). Virgin collection followed the protocol described above except that no more virgins were taken once a total of 600 males and 200 females were collected from each population (which occurred by day 9 or 10). Surplus adults were discarded after assignment to mating vials.

The above protocol was reiterated in each successive generation. No manipulation of family size or other aspect of artificial (truncation) selection was imposed. The number of productive culture vials from which virgins were collected varied between generations (100–125 per generation), but in every generation an identical number of productive vials were used from each population.

There were two advantages to housing only a single female within each mating and culture container: (1) individuals from both mating treatments were maintained within identical containers, so there was no possibility that adults experienced different thermal microenvironments (e.g., due to evaporative cooling) caused by different container dimensions; and (2) because nonproductive females were detectable, an identical number of productive females could be used in each population, each generation. Under the conditions of this experiment the effective size of the sexually selected populations was necessarily equal to, or slightly greater than, that of the monogamy populations, owing to the opportunity for multiply sired broods under polyandry.

Thermal Regime

The sensitivity of *D. melanogaster* to thermal stress varies with developmental stage, with more mobile life stages exhibiting decreased tolerance (reviewed in Ashburner 1989). The thermal treatment reflects this variation (Fig. 1a). Egg deposition by adult females and early embryo development (day 0) occurred at 32°C; larval development and early pupation (days 1–3) occurred at 33°C; later pupal development and early adult stages (days 4–11) occurred at 28°C (males are sterile when developing above 28.5°C); courtship and mating (days 12–13) occurred at 31°C (courtship and mating could also occur on day 0 at 32°C). The experimental populations were maintained in a Percival incubator (model I-35VL, Perry, IA). The thermal treatment was controlled with a Watlow (model 942, St. Louis, MO) microprocessor, and measured with several Fisher Scientific precision thermometers (no. 15–041–13A, Houston, TX) distributed across the shelf that held the populations. The vials from the two mating treatments were interspersed to block on any spatial heterogeneity in temperature.

Previous studies of *D. melanogaster* (reviewed in Ashburner 1989) found that exposure to 30°C depresses a variety

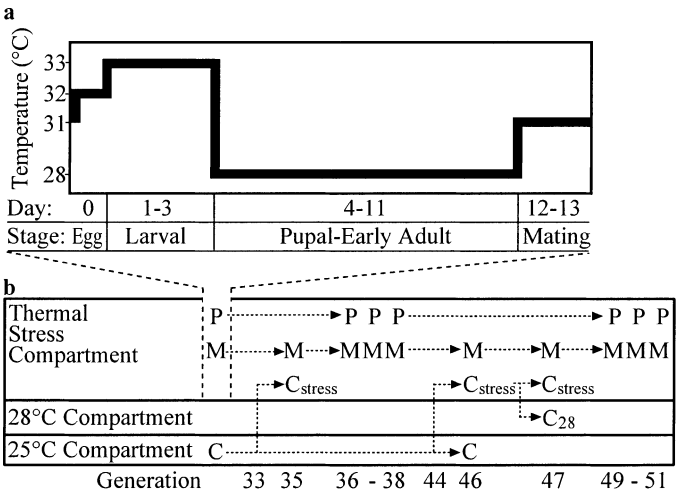

FIG. 1. Overview of thermal regime and assays. (a) Temperature across the life cycle within thermal stress compartment. (b) Thermal stress and adaptation assays. Assayed populations are shown directly above the generation at which comparisons were made. Thermal stress was measured in generation 46 through comparison of thermal controls (C) at 25°C and after acclimation to the thermal stress regime (C_{stress}). Initiation of acclimation is indicated by the point of bifurcation (e.g., generation 44). The effect of thermal regime on development rate was measured in generation 47 by comparison of C_{stress} reared under thermal stress and at 28°C (C_{28}) at which point development rate is nearly maximized. Net thermal adaptation was evaluated in generations 35 and 46 and development rate in generation 47 through comparison of C_{stress} and monogamous (M) populations. The effect of sexual selection on thermal adaptation was measured in generations 36–38 and 49–51 by comparison of M and polyandrous (P) populations.

of fitness components. It reduces egg-to-adult mortality, fecundity rate, and development rate. Because thermal stress depresses these fitness components, the evolution of thermal tolerance should restore these same parameters.

Measuring Thermal Stress

Overview

To measure the extent to which the thermal treatment was stressful, two assays were performed on the control populations in generations 46 and 47 (Fig. 1b). To initiate the assays, from each of the two control replicates, additional progeny were collected in generation 44 (350 adults of each sex; 50 adults/holding vial) and divided into two subpopulations; one subpopulation continued to be maintained as a thermal control, whereas the other subpopulation experienced the thermal stress regime (hereafter referred to as the ''stressed control''). The assay populations were allowed to equilibrate for two generations under their respective temperature regimes, during which time they were otherwise maintained identically to the monogamy populations (n = 133 females/population). Two generations of thermal equilibration were used to fully dissipate the effect of nongenetic factors on the fitness measures. Net reproductive rate could not be measured directly to demonstrate thermal stress because the ancestral temperature of 25°C induces slower development than the thermal treatment (owing to the latter's use of 28°C for several days, at which temperature devel-

opment rate is very nearly maximal). Therefore, the components of net reproductive rate, total surviving adult progeny and development rate, were measured separately.

Measure 1 of thermal stress

Number of surviving progeny was measured in the control and the stressed control populations after the two-generation equilibration period. This was measured as the total number of adult progeny produced per fertile female. This measure includes both maternal fecundity and offspring viability. To make the measure independent of development time, all progeny that survived to maturity were counted.

Measure 2 of thermal stress

Development rate in *D. melanogaster* increases with temperature until approximately 28°C, after which point it begins to slow due to the more rapidly increasing physiological stress (David et al. 1983). Development rate was measured in the stressed control populations that were exposed to either 28°C or the thermal stress regime. Specifically, in generation 46, additional adults were collected from the stressed control populations following the experimental protocol described in the section Beginning the Experiment. Adults were mated in holding vials (10 males and 10 females per vial; $n = 10$ vials/population). One-half of the adults from the stressed control lines were randomly assigned to 28°C (C_{28} treatment lines) but were otherwise treated identically to stressed control treatment lines. On day 0 the assay adults were cultured in 250-ml containers with live yeast (50 males and 50 females/container; $n = 2$ containers/population). Ten hours after culturing, eggs were transferred (using a small damp paintbrush and spatula) into fresh culture vials; 20 eggs/vial; $n = 5$ vials/population). The eggs were returned to their respective temperature regime and allowed to develop. Egg-to-adult development time of assay individuals was measured by collecting all adults on nine occasions during days 8–11, during which period all adults emerged. Relative weighted mean development time was calculated as the fraction of adults that emerged at each collection multiplied by the time elapsed since the onset of emergence.

Measuring Thermal Adaptation

Overview

After 35 generations of exposure to the thermal treatment, three types of assays were performed: (1) net reproductive rate; (2) total surviving adult progeny; and (3) development rate. To maximize experimental power, these assays compared thermal control populations only to the monogamy populations. (A subsequent evaluation of the relative adaptation of the polyandry populations was made by comparison to the monogamy populations.) To avoid nongenetic factors, the stressed control populations were reared under the monogamy treatment for at least two generations before measurements were taken.

Measure 1 of thermal adaptation: net reproductive rate

To measure the extent of adaptation, two assays were performed on the monogamy and control populations in generations 35 and 46. To initiate the assays, stressed controls were generated from each of the two control replicates two generations prior to the assay. Stressed controls were generated by collecting additional control progeny, following the protocol for the monogamy treatment that thereafter experienced the thermal stress regime. The stressed control lines were allowed to equilibrate for two generations under the thermal treatment, during which time they were maintained identically to the monogamy populations ($n = 133$ females/population). After the two-generation equilibration period, net reproductive rate was measured for each population. Progeny that were available for collection during the normal collection period were counted (i.e., those emerging before day 10). This measure includes the fecundity of the females and the viability and development rate of their offspring. Note that the stressed control lines used in generation 46 were simultaneously used in Measure 1 of Thermal Stress described above (i.e., their number of surviving progeny were compared to the thermal control lines to measure thermal stress, whereas their net reproductive rate was compared to the monogamy lines to measure adaptation).

Measure 2 of thermal adaptation: surviving progeny

This measure was conducted during the same assay described above in Measure 1 of Thermal Adaptation, by continuing the progeny counts until all progeny had emerged (i.e., by not terminating the assay at the beginning of day 10, which defined the end of measure 1). As a result, development time, which is substantially extended during thermal stress (see below) was not a component of this measure.

Measure 3 of thermal adaptation: development rate

In generation 47, the development rate of the monogamy lines was compared to that of the stressed controls. Stressed control flies were preconditioned to the monogamy thermal treatment for two generations as described above in Measure 1 of Thermal Adaptation. During generation 47, surplus adults were collected from the stressed controls and monogamy populations following the monogamy protocol. Adults were mated in holding vials (10 males and 10 females/vial; $n = 10$ vials/population). On day 0 the assay adults were cultured in 250-ml containers with live yeast (50 males and 50 females/container; $n = 2$ containers/population). Ten hours after culturing, eggs were transferred (using a small damp paintbrush and spatula) into fresh culture vials (see Standard Protocol; 20 eggs/vial; $n = 5$ vials/population). The assay eggs were then allowed to develop under the thermal treatment. Egg-to-adult development time of assay individuals was measured by collecting all adults on nine occasions during days 8–11, during which period all adults emerged. The relative weighted mean development time was calculated as the fraction of adults that emerged at each collection multiplied by the time elapsed since the onset of emergence. Note that the stressed control populations used in this development rate assay and the development rate assay described under Measuring Thermal Stress were one and the same (i.e., the stressed control lines were simultaneously used to detect thermal stress against the C_{28} lines and adaptation against the monogamy lines).

Sexual Selection and Thermal Adaptation

To evaluate the effect of sexual selection on the rate of adaptation, the net reproductive rate of monogamous and polyandrous populations was compared on six occasions. To compare the populations, it was necessary to control for any subtle effects due to differences in the social environment of the monogamy and polyandry treatments. To do this, polyandry assay lines were constructed from individuals of each polyandry line that were then reared under the monogamy protocol (in all respects, including population size). Once formed in generation 35, the net reproductive rates of the polyandry assay lines were compared to that of the two monogamy lines in generations 36–38.

Next, a reciprocal assay was prepared in generation 48. Monogamy assay lines were constructed from individuals from each monogamy line that were then reared under the polyandry protocol (in all respects, including population size). Once formed in generation 48, their net reproductive rates were compared to that of the two polyandry lines in generations 49–51.

Intersexual Mutualism

Overview

After 55 generations of selection, male-induced harm to females was compared between the monogamy and polyandry populations. Male-induced harm to females has generally been found to be minor in the short term, but it culminates over long periods to be substantial (Chapman et al. 1995). To increase the sensitivity of the assay of male-induced harm, I used genetically weakened females that are known to be especially susceptible to male induced harm (see Holland and Rice 1999). These test females had been repeatedly backcrossed through the base population used to begin the experiments and carried multiple genotypic and karyotypic mutations, with genotype [C(1)DX *y f;* T(2.3)*rdgC st ri* p^p bw^D].

To generate sufficient males to carry out the assay, surplus males and females were collected in generation 54 and mated (10 males and 10 females/vial; $n = 10$ vials/population) to produce replicate offspring from each population. To maintain a density matching that of the experimental lines, eggs (< 10 h old) were transferred to large culture vials (100 eggs/vial; $n = 4$). Males were collected from these culture vials as virgins following the protocol of the main experimental lines. The males were then pooled and combined with test females, using 1-min exposure to CO_2, into fresh, yeasted, large vials (10 males and 10 females/vial; $n = 20$ vials/population). Adults were transferred without anesthesia into fresh, yeasted, large vials, every 20 h thereafter until at least 90% of the females were dead in all populations (four days after combining the sexes). Dead females (and males) were scored at each transfer.

After each transfer of test females to fresh vials, the deposited eggs were transferred to 100-μm nylon filters using a fine brush and tap water. To quantify the total egg mass, the collected eggs were boiled in deionized water for 20 sec (to dissolve particles of media), rinsed in deionized water, and then dried overnight at 60°C before weighing. Fecundity was calculated as the total egg mass produced over the assay.

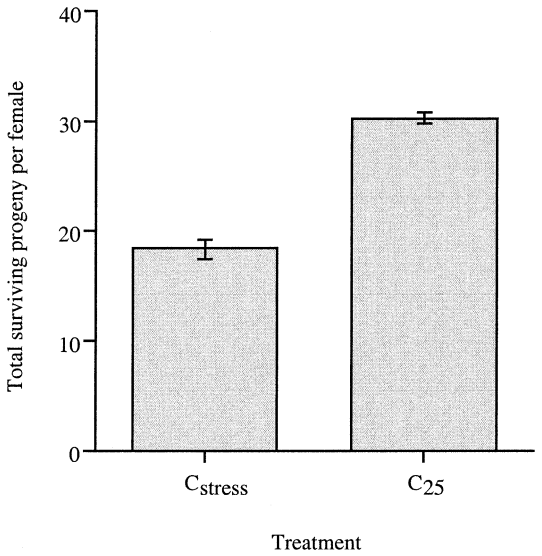

FIG. 2. The number of surviving progeny per female of the thermal control populations is greater when reared at 25°C (C_{25}) then when reared under the thermal stress treatment (C_{stress}). Error bars are ± one standard error (based on the variance between replicate populations).

Test female survival was measured as the fraction of females surviving each day multiplied by the number of days survived. One male died during the assay.

Statistical Analyses

Student's *t*-tests were used to assess statistical significance. Whenever the direction of a test could be unambiguously prescribed a priori, one-sided tests were employed. To avoid pseudoreplication, independent lines ($N = 2$ experimental + 2 control populations = 4) were used as the data for statistical analysis (i.e., Student's *t*-tests and measures of dispersion), rather than the individual flies or vials of flies that generated these population measures. A normal distribution of the data can be inferred because each measure is an average (or a total) over a large number of contributing individuals.

RESULTS

Thermal Stress

The number of surviving progeny was significantly greater in the thermal control populations maintained under the ancestral temperature (25°C) than in those same populations maintained under the thermal treatment (Fig. 2; $P = 0.004$, Student's one-tailed *t*-test, $N = 4$, df = 2). In this species, development rate increases with temperature until approximately 28°C and then it begins to decrease (reviewed in Ashburner 1989). The development rate within the thermal control populations reared at 28°C was significantly faster than in those same populations reared under the thermal treatment (Fig. 3; $P = 0.0001$, Student's one-tailed *t*-test, $N = 4$, df = 2). Lower progeny production and slower development rate while under the thermal treatment indicated that the thermal treatment induced stress within the experimental populations.

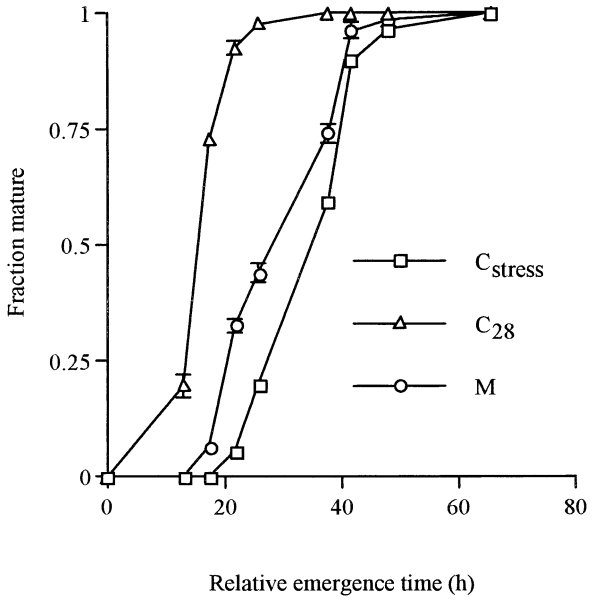

FIG. 3. The development rate of the thermal control populations is retarded when reared under the thermal treatment (C_{stress}) compared to 28°C (C_{28}). Also shown are data for the monogamy lines, after 47 generations of selection for tolerance to thermal stress, illustrating adaptation of these populations. Relative emergence time is the elapsed time since the first flies emerged from their pupal cases. Error bars are ± one standard error (based on the variance between replicate populations); some error bars are not visible.

Thermal Adaptation

The level of adaptation of the experimental lines was measured by comparing the performance of two thermally stressed (monogamy) lines to that of the two temperature control lines reared under identical, thermally stressful, conditions. Adaptation was assessed after 35 and 46 generations, respectively, of exposure to the thermal treatment. The monogamous populations exhibited a greater net reproductive rate (Fig. 4a; Table 1a) and produced a greater number of surviving progeny (Fig. 4b; Table 1b) than the thermal control populations. Development rate, measured after generation 47, in the monogamous populations also was faster than in the thermal controls (Fig. 3; $P = 0.003$, Student's one-tailed *t*-test, $N = 4$, df = 2). Collectively, these data indicate that the monogamous populations have adapted substantially to the thermal treatment and that development rate was a substantial component of that adaptation.

Sexual Selection and Thermal Adaptation

Thermal adaptation did not differ between the monogamous and polyandrous populations. Net reproductive rate did not differ significantly between monogamy and polyandry populations when both were reared under the monogamy protocol (Fig. 5a; generations 36, 37, and 38; $P = 0.5$, 0.6, and 0.4, respectively; Student's *t*-test, $N = 4$, df = 2, in each comparison) nor when all populations were reared under the polyandry protocol (Fig. 5b; generations 49, 50, and 51; $P = 0.4$, 0.9, and 0.6, respectively; Student's *t*-test, $N = 4$, df = 2, in each comparison). These data indicate that sexual selection did not increase the ability of the polyandrous populations to adapt to their new thermal environment.

Intersexual Mutualism

Monogamous and polyandrous males showed no difference in their harm to the test females' fecundity (Fig. 6a; $P = 0.7$, Student's one-tailed *t*-test, $N = 4$, df = 2) or survival (Fig. 6b; $P = 0.7$, Student's one-tailed *t*-test, $N = 4$, df = 2) after 55 generations of divergence.

DISCUSSION

Heritable Variation in Thermal Stress Tolerance

By generation 46 the net reproductive rate improved more than six standard deviations (the difference between the mean net reproductive rate of the monogamy replicates and the thermal controls divided by the standard deviation of the thermal controls). Therefore, heritable variation in male condition is not a limitation of this experiment.

The Relevance of the Thermal Treatment

The temperature treatment used in this experiment was clearly stressful and within the range encountered by *D. melanogaster* in nature (Feder et al. 1997). Natural clines of thermal adaptation have also been found in this species (James and Partridge 1995). Because low-grade thermal stress is a pervasive selective agent in this species' natural environment and because it must affect a wide variety of loci (White and Somero 1982), it would seem appropriate to view low-grade thermal stress as an excellent selective agent for maximizing indirect benefits of sexual selection. It should also be noted that any broadly acting stress can generally be expected to affect complex traits, including female preferences. For example, in *D. montana* the female preference function for pulse song varies between 15°C and 25°C (see Ritchie et al. 2001). Additional experiments that stressed only one sex would allow male and female effects to be distinguished.

Sexual selection could have favored the more thermally adapted males in several ways: active female selection for condition dependent courtship traits, for example, fluctuating asymmetry; passive female selection, for example, for males who are able to deliver more courtship; and intermale contests. Although intermale contests do occur in this species (Dow and von Schilcher 1975), during many hours of observation agonistic male interactions were not observed, while courtship was common.

Even if thermal stress was a poor choice for some unforeseen reason, it was not the only source of selection. Unconditionally deleterious mutations, in and of themselves, should have provided a sufficient source of heritable variation for sexual selection to act upon (Charlesworth 1987; Kondrashov 1988; Rice 1988) and apparently produced the difference in viability (1% over one generation) seen by Partridge (1980).

Mating Treatment

In the polyandrous populations each female was potentially free to mate with the best of four males, while in the mo-

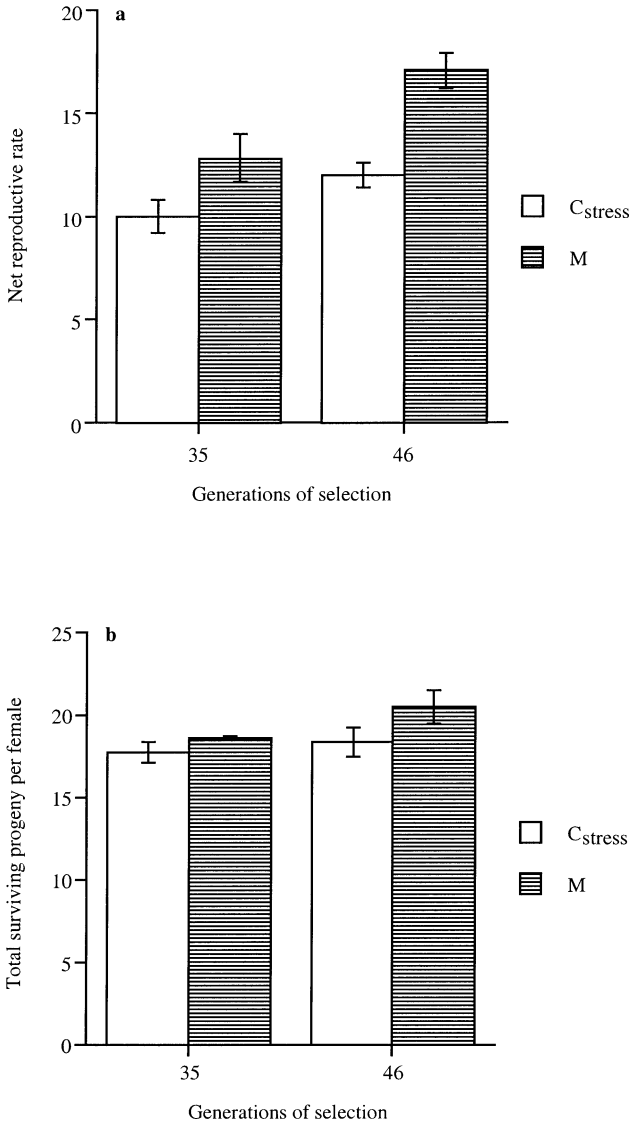

FIG. 4. (a) The net reproductive rate (the offspring produced prior to the standard termination of virgin collection); and (b) the total number of surviving progeny (the same as in [a] but including the slow-developing offspring of the monogamous [M] and thermal control populations exposed to thermal stress [C_{stress}]). These measurements taken after the M populations had been exposed to the thermal treatment for 35 and 46 generations, respectively, indicate adaptation. Error bars are ± one standard error (based on the variance between replicate populations).

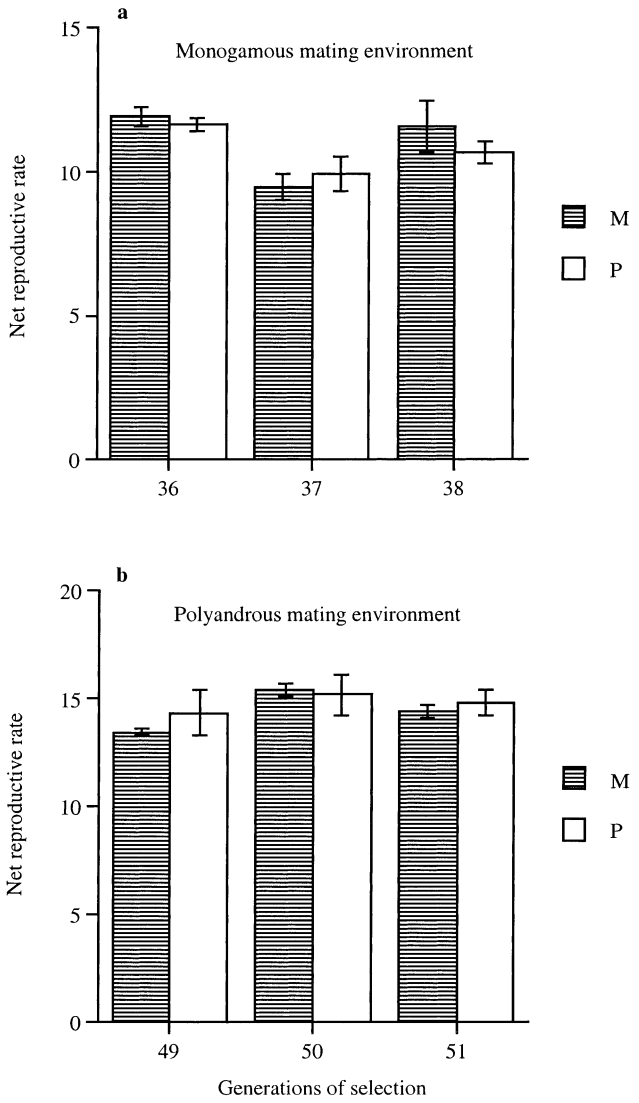

FIG. 5. Comparison of the net reproductive rate of the monogamous (M) and polyandrous (P) populations (when reared under identical environments; monogamous conditions in generations 36–38 and polyandrous conditions in generations 49–51) demonstrates no difference in their level of adaptation to thermal stress. Error bars are ± one standard error (based on the variance between replicate populations).

TABLE 1. Analysis of variance of adaptation to thermal stress.

Source	df	Sum of squares	Mean square	*F*-value	$P_{\text{one-tailed}}$
a. Dependent variable, net reproductive rate					
Treatment (monogamy vs. control)	1	30.77	30.77	18.48	0.008
Generations diverged (35 vs. 46)	1	19.74	19.74	11.86	0.02
Residual	5	8.32	1.66		
b. Dependent variable, total progeny					
Treatment (monogamy vs. control)	1	4.53	4.53	4.5066	0.02
Generations diverged (35 vs. 46)	1	3.29	3.29	3.2731	0.03
Residual	5	5.03	1.00		

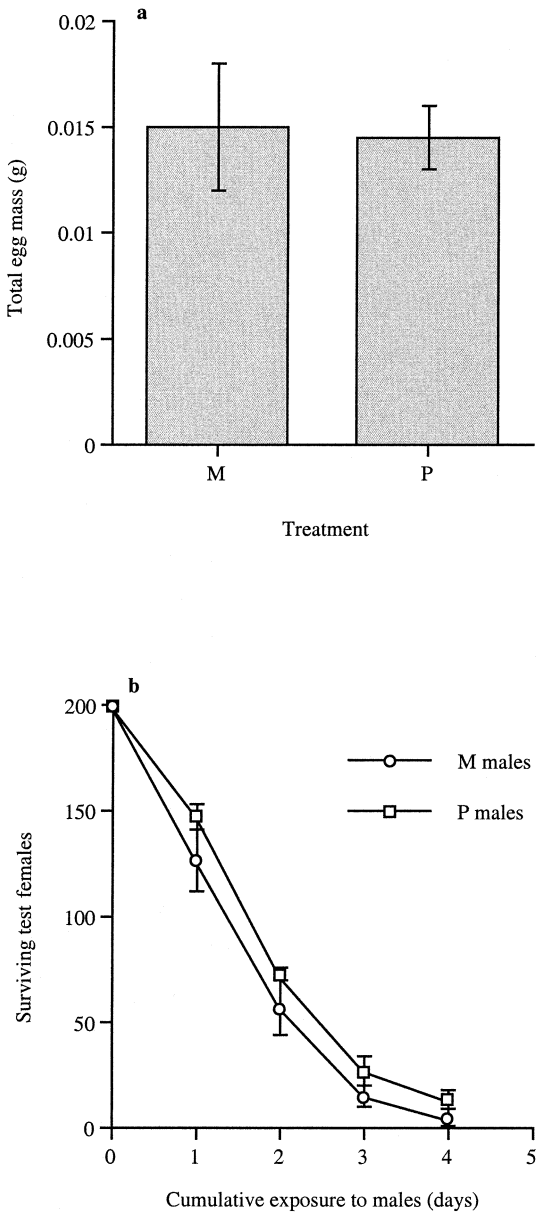

FIG. 6. Comparisons of the influence of monogamous (M) and polyandrous (P) males on the fecundity and survival of test females demonstrate no difference in harm inflicted on females: (a) total cohort fecundity; and (b) survival. Measures were taken after 55 generations of evolution. Error bars are ± one standard error (based on the variance between replicate populations).

nogamous populations each female was constrained, on average, to mate with an average male. A population cage could have been used to further increase the strength of sexual selection. However, preliminary experiments indicated that such a design would also have changed the microthermal environment (e.g., through differences in evaporative cooling of food) and would have reduced control over population size (see Materials and Methods, Standard Protocol). The sexual selection differential between the treatments is proportional to the product of the number of males competing for each female per generation and the number of generations over which divergence occurred. Therefore, after 36 generations, 144 male-generations of differential sexual selection separated the treatments. While experimental power may have limited some of the previous experiments that used fewer generations, or stabilizing conditions, it is unlikely to be a limitation here.

Intersexual Mutualism

Another explanation for the observation that sexual selection did not appear to augment nonsexual adaptation is that it actually did improve adaptation but that its benefit was exactly countered by mutualistic coevolution between the sexes that was induced by monogamy. Promiscuity necessarily maintains an opportunity for sexual conflict (Trivers 1972; Charnov 1979; Parker 1979, 1984; see reviews in Rowe et al. 1994; Stockley 1997; Partridge and Hurst 1998). Previous studies of *D. melanogaster* have shown strong intersexual conflict (Fowler and Partridge 1989; Chapman et al. 1995; Rice 1996, 1998; Holland and Rice 1999). The Holland and Rice (1999) study also removed sexual selection by enforced, life-long monogamous mating, specifically to test the effect of promiscuity in perpetuating intersexual conflict. Enforced monogamy make the reproductive success of mates identical so any conflicts between mates are necessarily reversed. Within 47 generations of enforced monogamy, there were reductions in both male harm to females and female resistance to male harm. Those changes apparently contributed to the 30% improvement in net reproductive rate that also evolved under pure monogamy. That experiment was performed at 25°C and a sexual interaction period of four days, prior to egg laying, to which the starting population had been adapting for more than 200 generations.

The current experiment used the same starting population but used strong directional selection for thermal tolerance that would interfere with selection for other traits due to negative pleiotropy. Perhaps more importantly, the current experiment also halved the period of interaction between the sexes relative to the ancestral laboratory period. Such a reduction should relax male-induced stress on females, which is known to be cumulative in nature (Fowler and Partridge 1989; Chapman et al. 1995; Rice 1996, 1998; Holland and Rice 1999). Therefore, females of both mating treatments within this experiment experienced a relaxation of male-induced harm relative to the ancestral population from which they were derived, thereby limiting selection for benign males under monogamy. Not surprisingly, no difference was seen in the harm to test females caused by monogamous and polyandrous males. These results indicate that the evolution of intersexual mutualism was trivial compared to the degree of thermal adaptation and cannot explain the equal rates of adaptation between the mating treatments.

Independence between Sexual and Nonsexual Selection

Finally, genetic variation for male sexual fitness (e.g., through courtship, promiscuity, sperm competition) may be largely unrelated to the genetic variation for the remainder of fitness (i.e., juvenile survival, development rate, and female fecundity). Alternatively, sexual selection may reinforce some components of nonsexual selection but oppose others, yielding, on average, no relationship. This would ex-

plain the inconsistent results obtained between previous studies that measured different fitness components, as well as the results obtained here that evaluate net nonsexual fitness. A major difficulty in evaluating the data relevant to the benefits of sexual selection is the potential for publication bias. Positive evidence is easy to interpret, stimulating, and frequently published in widely read journals.

Evaluating Sexual Selection

If sexual selection does not reinforce nonsexual selection, then what else might drive the evolution of the extravagant courtship? Numerous studies have demonstrated that courtship traits mediate male fitness (see Andersson 1994) and that most of the variation in male fitness is in the adult components (courtship, promiscuity, sperm competition; reviewed in Brittnacker 1981). Much of sexual selection is intersexual. If females are not benefiting, then what are they selecting? One seemingly inevitable possibility is that sexual selection is a self-reinforcing process in which females select males who will in turn produce sons who are sexually competitive (Fisher 1952). See Andersson (1994) for a review of this hypothesis.

Antagonistic coevolution between the sexes

Mating can be costly for a variety of reasons (e.g., sexually transmitted diseases and seminal fluid toxicity), and there can be conflict between the sexes in a variety of mating decisions (when, where, how often; see recent reviews in Rice and Holland 1997; Stockley 1997; Holland and Rice 1998; Partridge and Hurst 1998). Conflict is expected to be proportional to the difference in parental investment and the opportunity for the lower investing sex to divert resources in pursuit of promiscuity (Bateman 1948; Trivers 1972; Parker 1979). In general, in the absence of life-long monogamy, internal fertilization is an enormous opportunity for sexual conflict because female physiology is laid open to the products of hundreds of male loci, whose interests necessarily differ from the female-derived gene products they interact with. Male seminal fluid products are known to alter female physiology in ways that clearly benefit the inseminating male, sometimes at a direct cost to their mate (reviewed in Rice and Holland 1997; Stockely 1997; Wolfner 1997).

The observed mating patterns within a population should represent intermediates between sex-specific optima. But such intermediates cannot generally represent a stalemate; novel mutations that express a benefit within one sex, at a direct cost to the other sex, will inevitably arise. Such a cost should, in turn, counter-select a response at other loci expressed within the opposite sex. Therefore, antagonistic coevolution between opponent loci should be widespread and perpetual (Rice and Holland 1997). Evidence from water striders demonstrates a morphological arms race between male traits used to coerce prospective mates and female resistance to copulating maladaptively (Arnqvist and Rowe 1995). Such a process within a population is similar to the antagonistic coevolution that has been documented between species (Van Valen 1973; Vermeij 1983). There is a recent body of evidence indicating that such arms races also occur between noncoercive sexual traits as well.

Antagonistic seduction

Recent phylogenetic evidence indicates that in a variety of taxa male courtship traits have evolved in response to preexisting female preferences and that female preferences are the incidental result of viability selection on female sensory systems (West-Eberhard 1984; Kirkpatrick 1987; Endler and McLellan 1988; Basolo 1990; Ryan 1990; see reviews in Endler and Basolo 1998; Ryan 1998). Several studies also indicate that female preferences have, in turn, diminished (i.e., required more stimulation to elicit the same response) following the evolution of ornaments that exploit the preference. Collectively, the above data suggest that courtship traits can be the sensory equivalent of weapons, manipulating females through superstimulation rather than coercion. Spectacular ornaments and choosiness may simply reflect chronic coevolution between genes that mediate seduction and resistance to being maladaptively seduced, respectively (Holland and Rice 1998). The idea that advertisements that are very stimulating and successful may come at a cost to receivers is not new (Dawkins and Krebs 1978). That consumers should evolve to reduce their sensitivity to manipulation, and that such coevolution should be self-perpetuating, seems inevitable.

Conclusion

This study was intended to resolve the ambiguous evidence concerning the relationship between sexual selection and nonsexual fitness. It estimates an upper bound for the realized heritable benefit of sexual selection in its entirety, as first proposed by Darwin (1871). The design used here should have no deficiency in experimental power due to the substantial response to selection. Yet, the hypothesized positive association between sexual selection and adaptation was not found. This result, combined with recent theory and experiments, implies that sexual selection need not be driven by adaptive choice for mates with superior nonsexual fitness, but may occur simply through the benefits to those genes that themselves increase the mating and fertilization rates of the males expressing them.

Acknowledgments

I thank my dissertation advisor, W. R. Rice, for suggesting the use of thermal stress and for advising me throughout this project. I thank A. Chippindale, J. Gibson, N. Johnson, T. Leonardo, B. Lyon, M. Noor, S. Nuzhdin, G. Pogson, M. Servedio, P. Stockley, and an anonymous reviewer for many useful comments on earlier drafts. This work was supported by the National Science Foundation through grants DEB-9307735, DEB-9509191, and DEB-9623479.

Literature Cited

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.

Arnqvist, G., and L. Rowe. 1995. Sexual conflict and arms races between the sexes: a morphological adaptation for control of mating in a female insect. Proc. R. Soc. Lond. B 261:123–127.

Ashburner, M. 1989. *Drosophila*: a laboratory handbook. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Basolo, A. L. 1990. Female preference predates the evolution of the sword in swordtail fish. Science 250:808–180.
Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349–368.
Brittnacher, J. G. 1981. Genetic variation and genetic load due to the male reproductive component of fitness in *Drosophila*. Genetics 97:719–730.
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.
Charlesworth, B. 1987. The heritability of fitness. Pp. 21–40 *in* J. W. Bradbury and M. B. Andersson, eds. Sexual selection: testing the alternatives. Wiley, Chichester, U.K.
Charnov, E. 1979. Simultaneous hermaphroditism and sexual selection. Proc. Natl. Acad. Sci. 76:2480–2484.
Civetta, A., and A. G. Clark. 2000. Correlated effects of sperm competition and postmating female mortality. Proc. Natl. Acad. Sci. USA 97:13162–13165.
Darwin, C. 1871. The descent of man and selection in relation to sex. Murray, London.
David, J. R., R. Allemand, V. Herrwege, and Y. Cohet. 1983. Ecophysiology: abiotic factors. Pp. 105–170 *in* H. L. Carson, M. Ashburner, and J. J. N. Thompson, eds. The genetics and biology of *Drosophila*. Vol. 3. Academic Press, London.
Dawkins, R., and J. R. Krebs. 1978. Animal signals: information or manipulation? Pp. 282–309 *in* J. R. Krebs and N. R. B. Davies, eds. Behavioural ecology: an evolutionary approach. Blackwell, Oxford, U.K.
Dow, M. A., and F. von Schilcher. 1975. Aggression and mating success in *Drosophila melanogaster*. Nature 254:511–512.
Endler, J. A., and A. L. Basolo. 1998. Sensory ecology, receiver biases and sexual selection. Trends Ecol. Evol. 13:415–410.
Endler, J. A., and T. McLellan. 1988. The process of evolution: toward a newer synthesis. Annu. Rev. Ecol. Syst. 19:395–421.
Feder, M. E., N. Blair, and H. Figueras. 1997. Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. Funct. Ecol. 11:90–100.
Fisher, R. A. 1952. The genetical theory of natural selection. Dover, New York.
Fowler, K., and L. Partridge. 1989. A cost of mating in female fruitflies. Nature 338:760–761.
Hall, J. C. 1994. The mating of a fly. Science 264:1702–1714.
Holland, B., and W. R. Rice. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. Evolution 52:1–7.
———. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. Proc. Natl. Acad. Sci. USA 96:5083–5088.
James, A. C., and L. Partridge. 1995. Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. J. Evol. Biol. 8:315–330.
Kirkpatrick, M. 1987. Sexual selection by female choice in polygynous animals. Annu. Rev. Ecol. Syst. 18:43–70.
Kondrashov, A. S. 1988. Deleterious mutations as an evolutionary factor. III. Mating preference and some general remarks. J. Theor. Biol. 131:487–496.
Markow, T. A., and R. F. Ankney. 1984. *Drosophila* males contribute to oogenesis in a multiple mating species. Science 224: 302–303.
Markow, T. A., and S. Sawka. 1992. Dynamics of mating success in experimental groups of *Drosophila melanogaster* (Diptera: Drosophilidae). J. Insect Behav. 5:375–383.
Parker, G. A. 1979. Sexual selection and sexual conflict. Pp. 123–166 *in* M. S. Blum and N. B. Blum, eds. Sexual selection and reproductive competition in insects. Academic Press, New York.
———. 1984. Sperm competition and the evolution of animal mating strategies. Pp. 123–166 *in* R. L. Smith, ed. Sperm competition and the evolution of animal mating systems. Academic Press, New York.
Partridge, L. 1980. Mate choice increases a component of offspring fitness in fruitflies. Nature 283:290–291.
Partridge, L., and L. D. Hurst. 1998. Sex and conflict. Science 281: 2003–2008.
Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. Nature 371:598–599.
Promislow, D. 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.
Rice, W. R. 1988. Heritable variation in fitness as a prerequisite for adaptive female choice: the effect of mutation-selection balance. Evolution 42:817–820.
———. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature 361:232–234.
———. 1998. Male fitness increases when females are eliminated from gene pool: implications for the Y chromosome. Proc. Nat. Acad. Sci. USA 95:6217–6221.
Rice, W. R., and B. Holland. 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE) and the intraspecific red queen. Behav. Ecol. Sociobiol. 41:1–10.
Ritchie, M. G., M. Saarikettu, S. Livingstone, and A. Hoikkala. 2001. Characterization of female preference functions for *Drosophila montana* courtship song and a test of the temperature coupling hypothesis. Evolution 55:721–727.
Rowe, L., G. Arnqvist, A. Sih, and J. J. Krupa. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. Trends Ecol. Evol. 9:289–293.
Ryan, M. J. 1990. Sexual selection, sensory systems, and sensory exploitation. Oxf. Surv. Evol. Biol. 7:156–195.
———. 1998. Sexual selection, receiver biases, and the evolution of sex differences. Science 281:1999–2003.
Schaeffer, S. W., C. J. Brown, and W. W. Anderson. 1984. Does mate choice affect fitness? Genetics 107:S94.
Stockley, P. 1997. Sexual conflict resulting from adaptations to sperm competition. Trends Ecol. Evol. 12:154–159.
Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136–179 *in* B. Campbell, ed. Sexual selection and the descent of man. Heinemann, London.
Van Valen, L. 1973. A new evolutionary law. Evol. Theor. 1:1–30.
Vermeij, G. J. 1983. Intimate associations and coevolution in the sea. Pp. 311–327 *in* D. M. Futuyma and M. Slatkin, eds. Coevolution. Sinauer Associates, Sunderland, MA.
Welch, A., R. D. Semlitsch, H. C. Gerhardt. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. Science 280:1928–1930.
West-Eberhard, M. 1984. Sexual selection, competitive communication and species-specific signals in insects. Pp. 283–324 *in* T. Lewis, ed. Insect communication. Academic Press, Toronto.
White, F. N., and G. Somero. 1982. Temperature and the internal milieu. Physiol. Rev. 62:41–90.
Wolfner, M. F. 1997. Tokens of love: Functions and regulation of *Drosophila* male accessory gland products. Insect Biochem. Mol. Biol. 27:179–192.

Corresponding Editor: M. Noor