

ASSESSING THE POTENTIAL FOR AN ONGOING ARMS RACE WITHIN AND BETWEEN THE SEXES: SELECTION AND HERITABLE VARIATION

URBAN FRIBERG,^{1,2,3} TIMOTHY A. LEW,² PHILLIP G. BYRNE,^{2,4,5} AND WILLIAM R. RICE^{2,6}

¹Department of Ecology and Environmental Science, Umeå University, 901 87 Umeå, Sweden

²Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106-9610

⁴School of Botany and Zoology, Australian National University, Canberra, Australian Capital Territory 0200, Australia

⁵E-mail: phillip.byrne@anu.edu.au

⁶E-mail: rice@lifesci.ucsb.edu

Abstract.—In promiscuous species, sexual selection generates two opposing male traits: offense (acquiring new mates and supplanting stored sperm) and defense (enforcing fidelity on one's mates and preventing sperm displacement when this fails). Coevolution between these traits requires both additive genetic variation and associated natural selection. Previous work with *Drosophila melanogaster* found autosomal genetic variation for these traits among inbred lines from a mixture of populations, but only nonheritable genetic variation was found within a single outbred population. These results do not support ongoing antagonistic coevolution between offense and defense, nor between either of these male traits and female reproductive characters. Here we use a new method (hemiclinal analysis) to study genomewide genetic variation in a large outbred laboratory population of *D. melanogaster*. Hemiclinal analysis estimates the additive genetic variation among random, genomewide haplotypes taken from a large, outbred, locally adapted laboratory population and determines the direction of the selection gradient on this variation. In contrast to earlier studies, we found low but biologically significant heritable variation for defensive and offensive offspring production as well as all their components (P_1 , fidelity, P_2 , and remating). Genetic correlations between these traits were substantially different from those reported for inbred lines. A positive genetic correlation was found between defense and offense, demonstrating that some shared genes influence both traits. In addition to this common variation, evidence for unique genetic variation for each trait was also found, supporting an ongoing coevolutionary arms race between defense and offense. Reproductive conflict between males can strongly influence female fitness. Correspondingly, we found genetic variation in both defense and offense that affected female fitness. No evidence was found for intersexual conflict in the context of male defense, but we found substantial intersexual conflict in the context of male offensive sperm competitive ability. These results indicate that conflict between competing males also promotes an associated arms race between the sexes.

Key words.—Coevolution, *Drosophila melanogaster*, interlocus intersexual conflict, interlocus intrasexual conflict, sexual selection, sperm competition.

Received February 4, 2005. Accepted April 20, 2005.

It is common in many species for females to mate with multiple partners within a reproductive cycle (e.g., Parker 1970; Andersson 1994; Arnqvist and Nilsson 2000). To achieve high fertilization success under such conditions a male should (1) induce fidelity in his mates and prevent sperm displacement when remating occurs (defense) and (2) acquire new mates and supplant stored sperm from other males (offense). When expressed in different individuals, defense and offense become two opposing male phenotypes. When genes coding for offense and defense are at least partially non-overlapping, a coevolutionary arms race can ensue, because evolution of more efficient defense selects for improved offense and vice versa (Parker 1979; Rice and Holland 1997). In species where males rarely engage in agonistic interactions, male offense and defense phenotypes are mediated predominantly by male-induced changes of female behavior and reproductive physiology. As a consequence, adaptations by males to increase offense or defense may commonly influence the fitness of females via pleiotropy (Parker 1979; Wolfner 1997; Fowler and Partridge 1989; Rice 1996; Holland and Rice 1999; Civetta and Clark 2000; Prout and Clark 2000; Sawby and Hughes 2001; Chapman 2001; Chapman et al. 2003).

The pioneering work on the genetic control of male offense and defense was done with isogenic lines of *D. melanogaster* (Clark et al. 1995). These experiments found that genotype influences the ability of a male to: (1) prevent his mate from remating with another male (fidelity), (2) prevent displacement of his sperm when his mate remates (P_1), (3) remate previously mated females (remating), and (4) displace resident sperm in a previously mated female (P_2). However, this study pooled chromosomes that were extracted from widely separated populations, and the flies measured were inbred (completely homozygous) for the part of the genome (40%) that was screened for variation. Also, the females and the competitor males were from an unrelated inbred line. As a consequence, it is not clear that the observed genetic variation represent heritable, within-population variation. Instead, it is likely that inbreeding depression strongly contributed to the observed genetic variation.

Within-population variation in defensive and offensive sperm displacement (P_1 and P_2) in *D. melanogaster* was previously estimated by Hughes (1997). Her study of a large outbred laboratory population found no additive genetic variation among heterozygous third chromosomes. Consistent with earlier work on isogenic lines, substantial genetic variation was found among homozygous chromosomes that was associated with inbreeding depression. Thus, these studies collectively show that there exists genetic variation for offense and defense, but not necessarily additive genetic var-

³ Present address: Animal Ecology, Department of Ecology and Evolution, Evolutionary Biology Centre, Uppsala University, 752 36 Uppsala, Sweden; E-mail: urban.friberg@ebc.uu.se.

iation within populations. The lack of within-population additive genetic variation leaves open the question of whether antagonistic coevolution is currently occurring in the laboratory *D. melanogaster* model system. Positive results from selection experiments, however, provide indirect evidence for additive genetic variation in these traits, at least in novel environments (Service and Fales 1993; Service and Vossbrink 1996; Rice 1996; Holland and Rice 1999; Wigby and Chapman 2004).

Many studies indicate that males can influence components of female fitness. In promiscuous mating systems, the direct effect of males on females is primarily through courtship and substances transferred to females when mating. Only negative effects have been recorded for courtship (e.g., Partridge and Fowler 1990; Watson et al. 1998), while the influence of mating on females seems to depend on the particular system (for a review see Arnqvist and Nilsson 2000). In *D. melanogaster*, no material benefits from mating, apart from receiving sperm, have been recorded (Chapman et al. 1994; Pitnick et al. 1997). However, males transfer about 80 seminal fluid proteins to females (Swanson et al. 2001). Function has only been confirmed for a few of these, but in many cases it seems that their positive influence on male fitness has negative pleiotropic effects on females (reviewed in Chapman 2001). Accordingly, mating, as well as courtship, has been shown to have a negative effect on females in this species (Fowler and Partridge 1989; Partridge and Fowler 1990; Chapman et al. 1995; Rice 1996; Holland and Rice 1999; Pitnick and García-González 2002; Friberg and Arnqvist 2003). Although there is clear evidence that males can harm females, no study has quantified additive genetic variation among males in the degree to which they influence female lifetime fitness.

Here we use a new procedure, hemiclinal analysis, which makes it possible to study genetic variation over 99% of the genome instead of single chromosomes. With this method we (1) quantify the amount of standing genomewide genetic variation for male offense, defense, and all their components; (2) test for selection on these traits and for genetic correlations between them; and (3) estimate the extent to which female fitness is affected by variation in male defense and offense in a single outbred population of *D. melanogaster* that has adapted to a competitive, moderate-density laboratory environment, at large size, for over 300 generations.

Rationale for Testing for an Interlocus Arms Race

Interlocus antagonistic coevolution can be intra- or intergenomic. Intragenomic conflict occurs between nonallelic genes that are expressed in the same individual (such as occurs between meiotic drive elements and their suppressors). Intergenomic conflict occurs between nonallelic genes that are expressed in different individuals when their gene products interact and influence the outcome of conflicts of interest between individuals (Rice and Holland 1997). In this study we were concerned with intergenomic interlocus conflict between genes that code for male defense and male offense that interact when they are expressed in sexually competing males.

We will not explain the rationale of our testing procedure

directly with the traits of interest here, male offense and defense, because we know so little about the underlying mechanistic biology. Instead, we develop the logic of our approach with a simpler, more tractable example in which the biological interactions are more intuitively apparent. Consider an interlocus arms race between two groups of genes that influence different traits that mediate male-male competitive interactions: for example suppose that trait 1 is a toxin and trait 2 is an antidote, both used during intraspecific combat while establishing harems. Antagonistic coevolution is possible between the traits because evolutionary advance in one trait necessarily selects for compensating change in the other. Ideally, one would like to identify and study the genes controlling the two traits to test the hypothesis of an interlocus arms race. However, a quantitative genetics approach precludes the need to do so. To test for this antagonistic coevolution, three conditions need to be established: (1) evolutionary advance in trait 1 (the toxin) creates a lag-load (i.e., causes directional selection for a compensating adjustment) in trait 2 (the antidote), and vice versa; (2) there is unique genetic variation for trait 1 and trait 2 (i.e., there is genetic variation for both traits and the genetic correlation between them is <1); and (3) the traits have opposing selection gradients (i.e., selection for increased toxicity of the toxin and increased antitoxicity of the antidote).

If these three conditions are met, then the two traits would be shown to be evolving in opposing directions and provide strong empirical support for an arms race between them. There is the concern, however, that a "false arms race" might be detected if both traits were evolving in the prescribed directions for reasons other than antagonistic coevolution between them. But if the traits were shown to interact in nature as prescribed by the arms race (condition 1 above) then the evidence for an arms race would be strong.

It is important to point out that the rate of counteradaptation by a trait involved in a two-way antagonistic interaction, such as between a toxin and its antidote in the above example of male-male combat, increases with its deviation from its optimum value. To see why, suppose that the effect size of the toxin phenotype is presently at a prescribed value, say 10% mortality. The strength of selection on the antidote phenotype will influence the strength of selection on the spectrum of possible mutations that are recruited as counteradaptations by determining the maximum possible selection coefficient. When the toxin's mortality rate is 10% then the largest possible selective advantage of a counter-mutation at an antidote locus would be 10%, and any mutations with a pleiotropic harmful effect of more than 10% could not be favored by selection. However, if the rate of mortality from the toxin were 50%, then the maximal selection coefficient of a mutation would be raised to 50%, so that some counter-mutations at the antidote loci would accumulate faster owing to a larger selection coefficient, and a wider spectrum of counter-mutations would be favored by natural selection because the larger possible beneficial effect could compensate for larger harmful pleiotropic effects. In sum, although there is always selection for reduced mortality from the toxin, the rate of counteradaptation by the antidote loci is expected to increase as the effect size of the toxin increases because the average selection coefficient of potential counter-mutations is

larger and because a larger spectrum of possible counter-mutations is available. By the same logic, an elevated level of offensive P_2 increases the rate of counteradaptation for improved defensive P_1 , and vice versa.

One complication with this logic occurs when some of the genetic variation for the two traits is shared, that is, the traits are genetically correlated. An interlocus arms race cannot occur when the same gene controls both traits. When genetic variation for the two traits overlaps, then it must be shown that there is at least some nonoverlapping genetic variation for each of the two traits, and that there is a positive selection gradient on the trait-specific genetic variation.

Here we apply this logic to the two opposing traits that mediate competition between males to fertilize the eggs of females: male offense and defense. Condition 1 is necessarily met between these traits because, like toxin and antidote, evolutionary advance in offense must come at the expense of defense and visa versa, so long as at least some of the segregating genetic variation is unique to each trait (i.e., condition 2 is met). In this study we evaluate conditions 2–3 for male offense and defense in the *D. melanogaster* laboratory model system, to test the hypothesis that an intergenomic interlocus arms race is in progress between these traits.

Hemiclonal Analysis

The experimental paradigm that we employ is called hemiclonal analysis, and it has four stages. The first stage is the establishment of a large outbred laboratory population that has adapted to an experimentally controlled environment for hundreds of generations. Such a population emulates an island population in nature with the advantage that fitness can be suitably quantified in the same environment to which it has adapted (Service and Rose 1985; Fowler et al. 1997). We did not begin with a standard laboratory stock, such as Oregon-R and Canton-S, because they are highly inbred and because they have been reared under conditions of extreme crowding that interfere with meaningful behavioral interactions within and between the sexes. Instead we (with the help of L. Harshman) began with a large sample of flies (400 mated females) from a natural population in central California and founded a large outbred population (LH_M). To propagate the population, flies were transferred to three sequential sets of vials during their 14-day discrete generation cycle. Eggs were laid in 56 *juvenile competition* vials and trimmed to 150–200 per 10-dram vial containing 10 ml of cornmeal-molasses-killed-yeast medium (>8,400 eggs per total population)—a moderate density under laboratory conditions. Egg-to-adult viability at this density was approximately 90% in the LH_M base population (Chippindale et al. 2001). Larval, pupal, and early adult stages resided in these vials for 11.25 days, at which point they were mixed, and a sample of 1792 adults was transferred to *adult competition* vials (16 pairs per vial; the vials were kept on their sides to provide additional horizontal space for behavioral interactions). Here females competed for a limiting resource (10 mg of live yeast that is needed for egg production), and males competed to fertilize the females. Eighteen hours before the end of the 14-day generation cycle, the flies were transferred to 56 *oviposition* vials (which will become the juvenile competition vials of

the next generation). Only eggs laid at this time were used to propagate the next generation, so fecundity at this time represented lifetime fecundity in this population. At the start of these experiments the laboratory population (LH_M) had adapted to this laboratory environment for over 300 generations.

The second stage of hemiclonal analysis is the construction of a random set of hemiclones. A hemiclone is equivalent to a group of relatives produced by randomly selecting a sample of eggs from the base population and fertilizing them all with cloned copies of the same randomly selected sperm. To accomplish this, cytogenetic techniques are used to clone and amplify a random sample of nearly complete haploid genomes, which are equivalent to 99% of the genome of a gamete. The cytogenetic details of this process are described in Chippindale et al. (2001) and a schematic outline of the process is shown in Figure 1. The logical foundation for the procedure is to use cytogenetic constructs (compound X and an autosomal translocation) to cause a randomly sampled haploid genome (including the X and the two major autosomes, but not the “dot” fourth chromosome) to be transmitted father to son as if it were a concatenated, giant Y chromosome. Because male *D. melanogaster* lack meiotic crossing over, the technique allows genomic haplotypes to be clonally amplified just as a human male’s Y chromosome (nonrecombining region) is clonally amplified from a father to his sons. Lastly, males carrying the target genomic haplotype are crossed to specially constructed females (that have a genetic background derived from the LH_M base population) to produce a hemiclone (i.e., a group of individuals sharing one nearly complete genomic haplotype in common, while the other haplotype of their diploid genomes is randomly drawn from the base population, and thus half of each genome varies between individuals). These hemiclonal males express a completely wild-type genome free from any of the cytogenetic constructs used in the cloning process. Lastly, the whole process of constructing a hemiclone is repeated to produce a collection of random hemiclones, among which genetic variation and selection can be measured.

The third part of hemiclonal analysis is the measurement of the standing genetic variation among hemiclones for a trait of interest (such as male mating success, P_1 or P_2), under the same, or nearly the same, environmental conditions to which the laboratory population has adapted. The measured genetic variation is additive genetic variation among hemiclones. This measure is devoid of the influence of maternal effects and dominance (since each hemiclone is expressed in many different random genetic backgrounds inherited from many different mothers). The measure is also devoid of most forms of epistatic fitness variation. Epistatic interactions can occur between nonallelic genes that reside in the genomic haplotype inherited from: (1) the father (the haplotype shared in common among members of a single hemiclone), (2) the mother (the genetic background of each copy of the hemiclonal haplotype), or (3) between genes that reside in the two different haplotypes. Epistatic variation due to interactions of category (1) contributes to additive genetic variation among hemiclones but variation due to interactions of categories (2) and (3) does not. Traditional measures of heritable genetic variation (paternal half-sib designs and father-to-son

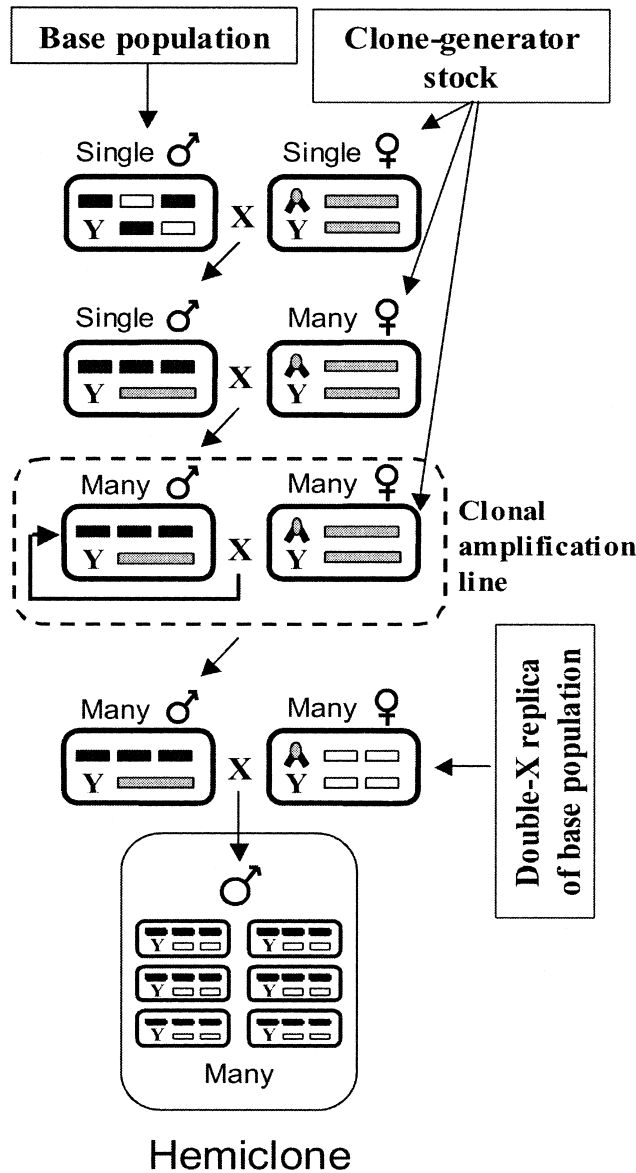


FIG. 1. A schematic of the cytogenetic cloning protocol. Progeny not shown were identified by genetic markers and discarded. Ellipses and rectangles depict individuals and chromosomes, respectively. The left, middle, and right positions of rectangles correspond to the X/Y, autosome-II, and autosome-III, respectively. The chevron depicts the compound X (C[1]DX, y,f) and the elongated rectangle represents a reciprocal translocation of the two major autosomes (T[2;3, rdg^C, st, p^p, bw^D]). The autosomes and the Y of the double-X replica of the base population carry autosomes and Y chromosomes that are derived from the LH_M base population through continuous backcrossing.

regressions) also include category (1) epistasis, since male *Drosophila* lack recombination between homologous chromosomes and chromosomes are inherited intact from father to son. However, past experiments and some theory indicate that epistatic variation will generally be small compared to the genomewide additive genetic variation for traits that are closely correlated with fitness (see for discussion Tachida and Cockerham 1988). Nonetheless we emphasize that although hemiclonal analysis is a powerful technique for mea-

suring genomewide genetic variation, the measured genetic variation may include some forms of epistatic variation. A straightforward way to demonstrate that some of the variation is heritable would be to show that the trait responds to directional selection.

The fourth and final part of hemiclonal analysis is the measurement of variation among hemiclones for lifetime net fitness (measured in the same environment to which the population is adapted). Once both net fitness and a phenotypic trait of interest (e.g., P_2) have been measured for a set of hemiclones, the bivariate data of average fitness and average trait value for each hemiclone can be regressed to obtain a selection gradient on the trait.

Hemiclonal analysis is an improvement to the traditional balancer chromosome technique often employed in *Drosophila* research to measuring genetic variation. The balancer technique only works well for single chromosomes because the multiple inversions in balancer chromosomes fail to fully suppress recombination when more than one is present in the genome simultaneously. Because hemiclonal analysis relies on the natural absence of meiotic crossing over in male *Drosophila*, and because the cytogenetic constructs (compound X and autosomal translocation) used in the procedure are highly stable, suppressed recombination across the full genome can be reliably achieved (Rice 1996; Chippindale et al. 2001). The compound X does break down at low rate ($<10^{-3}$ /generation), but offspring carrying the breakdown products are readily identified in the first generation and discarded. Whereas single chromosome studies cover 20–40% of the genome, hemiclonal analysis screens nearly the entire genome simultaneously in the native environment and genetic background to which the population has adapted.

MATERIALS AND METHODS

Offense and Defense Assays

In all assays, the densities and ages of flies; food composition; amounts of live yeast; and timing of events such as mating, egg production, and so on were matched to the conditions experienced by the base population. As a result, the flies were assayed in essentially the same environment to which they had adapted for over 300 generations.

To begin the assays, 35 hemiclone lines were constructed as described in Chippindale et al. (2001) and shown schematically in the upper portion of Figure 1. The hemiclones were assayed in two separate experiments. One experiment assayed male defense and the other male offense. Each experiment had five independent blocks, and each block measured all of the 35 hemiclones. In each block of each experiment, the hemiclonal males were constructed by crossing 25 males (carrying a target genomic haplotype) to 35 double-X females (see Fig. 1). From this cross, 27 males were collected to form each hemiclone/block. These hemiclones were next assayed for performance in offense or defense. Each male in a hemiclone expressed the same target genomic haplotype, but each copy differed with respect to the wild-type, random genetic background that it was expressed within.

When measuring defense and offense ability of the hemiclones we had them compete with males that were homozygous for a recessive brown-eye marker (*bw*). The brown-

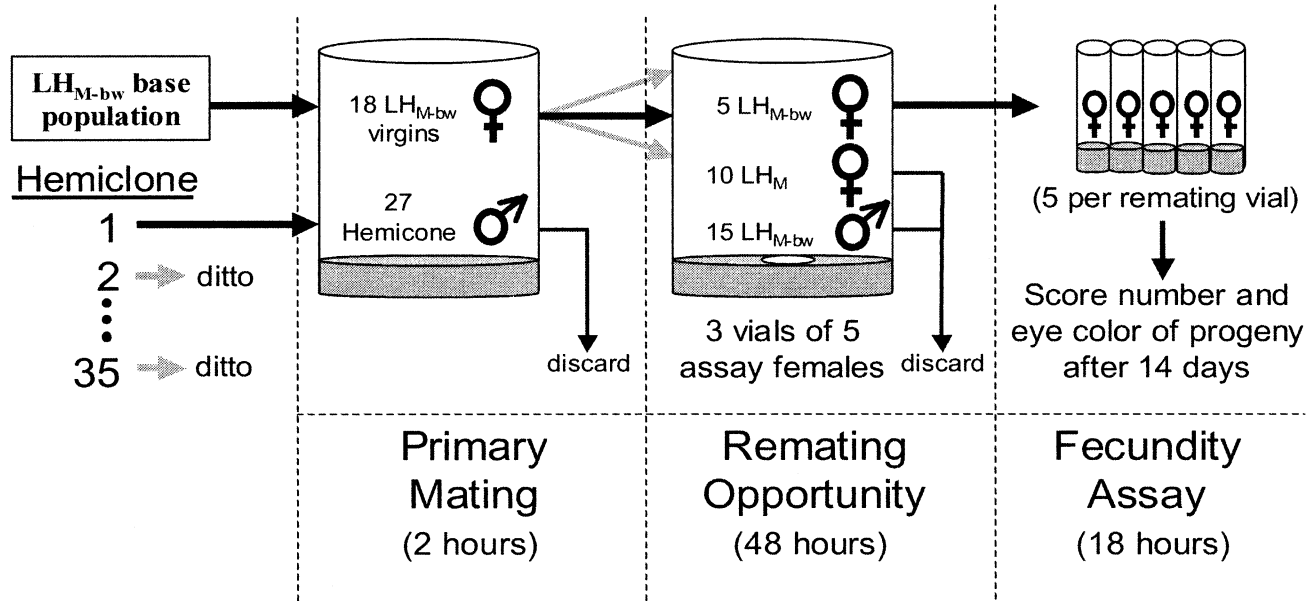


FIG. 2. The protocol used to measure male defense. The procedure was repeated five times to produce five replicated independent measures of mean defense for each of the 35 hemiclones. The competitor males and females in the remating vials were taken from the LH_{M-bw} and LH_M base populations, respectively. In the assay of male offense, the order of the hemiclonal and LH_{M-bw} competitor males was reversed.

eye marker had been introgressed into a replica (LH_{M-bw}) of the LH_M population by 13 rounds of backcrossing. The females that were used to assay sperm competition and remating (target females) were also homozygous for the brown-eye marker. Offspring fathered by a red-eyed hemiclonal male were red eyed (bw/bw^+), whereas those fathered by a brown-eyed competitor were brown-eyed (bw/bw). The brown-eye marker was used because it has only a small effect on the fitness of both males and females (Chippindale et al. 2001).

In the defense assay, virgin females (brown-eyed target females) were first mated to males from a specific hemiclone (red-eyed; see Fig. 2). Next the males were removed and then the mated females competed for live yeast for two days with red-eyed competitor females, while being courted and potentially remated by competitor males (brown-eyed). This two-day period corresponded to the adult competition stage of the laboratory culture of the LH_M base population. More specifically, to begin the assay, 27 males (aged three days posteclosion) from a hemiclone were put together with 18 virgin females (aged two days posteclosion) for 2 h to mate the females to a prescribed hemiclone of males. During the 2-h mating period virtually all females mate once and only once (Rice 1996; Holland and Rice 1999). The hemiclonal males were removed and the 18 mated females were divided into three groups of five newly mated females (the remaining three ‘‘spare’’ mated females, of the original 18, were discarded if not needed as a replacement). These three groups of five mated females were next assayed for their remating rate, sperm displacement when remated, and their fecundity while competing for limiting resources (live yeast). This was done by adding each group of five mated females to an adult competition vial containing 10 red-eyed competitor females (bw^+/bw^+ from the LH_M base population; aged three days

posteclosion) and 15 brown-eyed competitor males (bw/bw from the LH_{M-bw} population; aged three days posteclosion). In these adult competition vials, the females that had mated with the hemiclonal males competed for two days with the competitor females for a prescribed amount of live yeast (10 mg dry weight), while given the opportunity to remate with the competitor males. Females were then put into individual egg-laying vials for 18 h. Offspring emerging from these vials were counted and scored for eye color 14–16 days after eggs were laid. In this way the defense fitness of the hemiclones, the offense fitness of their competitors, and the fitness of their mates could be estimated. Male defense (proportion of offspring sired by primary males) could also be partitioned into its two causative components: fidelity (the fraction of females that had first mated to hemiclonal males that did not remate with competitor males) and P_1 (the fraction of the offspring sired by the hemiclonal male when his mate remated). To not confound P_1 with fidelity, only females that produced at least one offspring from the secondary males were included in our estimates of P_1 . Since sperm displacement is very high in *D. melanogaster* (e.g., Clark et al. 1995), the risk that we excluded females that did remate but only produced offspring from the first mating is very low.

In the offense assay, the protocol was identical except that females were first mated to competitor males (LH_{M-bw}) and then housed for two days with males from a hemiclone. From this assay, offense fitness for the 35 hemiclones was estimated as well as the defense fitness of their competitors and the fitness of their mates. Furthermore, offense (the proportion of offspring derived from secondary sires) could be decomposed into its causative components: remating (the fraction of females that remated) and P_2 (the fraction of offspring sired when females remated). Again, only females that produced at least one offspring from the secondary males were

used for measures of sperm competitiveness. To measure variation in body size among hemiclones, males from each hemiclone used during the offense assay were dried and weighed immediately after the assay.

Both of these assays were repeated in five independent blocks, and hence each of the 35 hemiclones was measured in 75 different genetic backgrounds. However, this sample size was reduced to 57, on average, because females that produced no or only a single offspring were removed from the analyses. Subsequent experiments in our laboratory (J. E. Linder and W. R. Rice) have shown that females in the LH_M population typically need an egg-laying stimulus in the form of an irregularity in the surface of the food medium (or eggs laid by other females), which was not uniformly provided in the vials containing the singly housed, experimental females. Most food vials have such irregularities by happenstance, but unusually uniform vials do not effectively induce egg laying by females. This resulted in a bimodal distribution of eggs, with one mode at about 25 eggs and another disjunct mode at 0–1 egg. When a small cut is placed in the food with a scalpel, the 0–1 egg mode is completely eliminated.

Long-Term Cost to Female Survival

To assess the long term cost to female survival due to interactions with males from a specified hemiclone, 25 hemiclone males (three days old) were mated to 25 tester females ($C[1]DX/Y;T[2;3]rdg^{C,st,p^p,bw^D}$, two to three days old) and then continuously housed together. The flies were transferred to freshly yeasted vials every second day, and the number of surviving females was scored. This continued for six days, and the number of dying females was recorded. Tester females are weakened by the expression of multiple visible mutations and are substantially more susceptible to male-induced harm than wild-type females. They nonetheless provide a useful measure of male-induced harm that would occur in wild-type females over longer periods of time. In our laboratory environment, wild-type flies live as adults at most six days, and because of the low density of only 16 pairs per vial in the adult competition phase of their life cycle, we observed virtually no adult mortality of wild-type females over this period of time. As a consequence, our measure of male harm to the survival of tester females is a measure of long-term harm that would be manifest in wild populations, or laboratory populations with overlapping generations, but not in our laboratory environment with a 14-day nonoverlapping generation cycle.

Juvenile Fitness

Juvenile fitness was measured for the 35 hemiclones as part of an unrelated, ongoing experiment. Briefly, newly laid eggs were collected and counted out into groups of 50 viable eggs per vial. Also added to the vials were 100 competitor eggs from the LH_M-bw population. On day 12 after egg deposition, the number of adult, target offspring was counted. This protocol measures competitive egg-to-adult viability under the same environmental conditions to which the population has adapted.

Statistical Analysis

We used ANOVA to test for genetic variation among hemiclones. In both the offense and defense assays, we had five blocks of data and all 35 hemiclones were assayed in each block. To improve normality of the dependent variables (Y), we took the average value from each hemiclone for each block and analyzed these average $(Y)_{ij}$ values, where i is the hemiclone (1–35) and j is the block (1–5). P_1 , P_2 , remate, and fidelity values were arcsine-transformed prior to analysis. The statistical model was $Y_{ij} = \text{hemiclone}_i + \text{block}_j + e_{ij}$, where Y_{ij} was the j th block average value for the i th hemiclone, hemiclone_i denotes the i th hemiclone, block_j denotes the j th block, and e_{ij} is the error term assumed to be normally distributed with equal variance. All analyses are based on weighted least squares, where weights are the number of observations contributing to each mean value. Hemiclone and block were modeled as random effects. Variance components were calculated using maximum likelihood methods as implemented by the REML option in the JMP statistical software package (SAS Institute, Cary, NC). Normality of the error terms, which was expected for the mean values due to the central limit theorem, was analyzed by distributional analysis of residual error terms. Correlations were done on grand means (across all five blocks). Because these grand means were averages of over more than 250 observations, they were expected to have approximate normal distributions. As a consequence, parametric correlations were calculated using the Pearson's product moment correlation. All correlation analysis was done with the JMP statistical software.

Estimating the Direction of Selection Gradients

An independent measure of the lifetime fitness of the 35 hemiclones was not available for the calculation of selection gradients. However, we were able to estimate the direction of the selection gradients, for defense, offense, and their components by using the lifetime reproductive success of the males in the offense and defense assays as a surrogate to an independent measure of lifetime fitness. This was possible because, in addition to male defense and offense, we also measured egg-to-adult viability for the hemiclones. These three traits together constitute most, if not all, of the components of male net fitness. Since we found no negative correlations between offense, defense, and egg-to-adult viability, as described in the results section, lifetime progeny production (measured in the offense and defense assays) was a useful index of net fitness for the purpose of estimating the direction of the selection gradient.

Second Set of Hemiclones

Because we found a genetic correlation between offense and defense, we needed to test for selection on the unique genetic variation for each trait. This required an independent measure of the net fitness of the 35 hemiclones, which we did not have. Data were available, however, from another, smaller set of 17 hemiclones taken from the LH_M base population (described in Lew and Rice 2005). Protocols to measure offense and defense, and survival of tester females were identical to those described here, except that a modified tech-

TABLE 1. Assay results for genetic variation among hemiclones.

Trait ¹	Additive CV ²	Heritability ³	Variance parameters ⁴
Offense assay			
P_2^5	5.92%	2.2%	(0.171, 0.015, 0.006)
Remate ⁵	8.74%	1.4%	(0.293, 0.045, 0.021)
Male adult net fitness	13.11%	1.6%	(42.956, 2.863, 0.969)
among remated females	5.18%	0.6%	(45.131, 0.939, 0.140)
Female adult net fitness	6.36%	1.7%	(37.126, 3.144, 1.003)
among remated females	10.77%	2.3%	(52.358, 4.060, 1.313)
among not remated females	6.42%	1.6%	(88.338, 2.990, 0.657)
Defense assay			
P_1^5	13.13%	1.88%	(0.092, 0.007, 0.003)
Fidelity ⁵	2.92%	0.05%	(0.199, 0.001, 0.0002)
Male adult net fitness	14.85%	1.5%	(25.176, 2.272, 0.789)
among remated females	14.56%	1.1%	(6.581, 0.280, 0.079)
among not remated females	3.65%	0.7%	(58.855, 0.841, 0.103)
Female adult net fitness	1.00%	0.1%	(19.203, 0.051, 0.010)
among remated females	NS	NS	
among not remated females	3.65%	0.7%	(58.855, 2.060, 0.103)

¹ Additive CVs and heritabilities are only shown when the 95% confidence interval for additive genetic variance did not overlap zero.

² Estimated additive CV = square root of the estimated additive variance (based on the variation of the block means)/estimated mean value.

³ Estimated heritability = estimated additive variance (based on block means)/estimated variance of individual observations within each block.

⁴ Total phenotypic variance among replicate means, estimated additive genetic variance among hemiclones, and 95% lower bound of the additive genetic variance among hemiclones.

⁵ Variance for P_1 , P_2 , remating, and fidelity are based on arcsin-transformed data.

nique was used to generate hemiclone males in the offense and defense assays that did not use double-X mothers (T. A. Lew and W. R. Rice, unpubl. data). These 17 hemiclones were hyperdispersed for male fitness (seven from the lower 10% of the distribution of male lifetime fitness, four from the middle 10%, and seven from the top 10%, taken from a screen of 119 hemiclones, as described in Lew and Rice 2005). Because the hemiclones were not a random sample from the base population, they could not be used to estimate standing genetic variation. However, we were able to use this smaller dataset to test for selection gradients on unique genetic variation for offense and defense. This was done by residual analysis. For example, to test for a positive selection gradient on offense that was independent of any covariation

with defense, we regressed the estimated offense values, of the 17 hemiclones, on their independently estimated defense values, and then calculated the residuals. We then tested for a correlation between the residuals and the independently estimated lifetime fitness of the 17 hemiclones.

RESULTS

Genetic Variation in Offense and Defense

Additive genetic variation among hemiclones was observed for adult male fitness in the context of both defense and offense and all of their components (fidelity, P_1 , remating, and P_2 ; see Table 1, Fig. 3). The additive coefficients of genetic variation for all of the male traits and their influ-

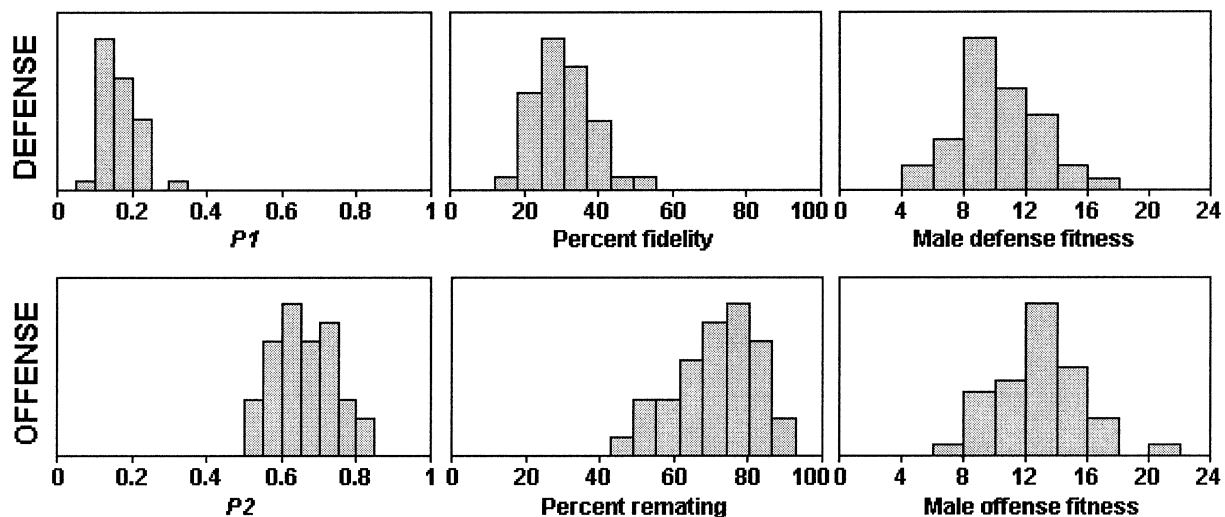


FIG. 3. Histograms of hemiclone means for male fitness (offspring production) in the context of defense and offense and their components (defense: P_1 and fidelity; offense: P_2 and remating).

TABLE 2. Correlations of components of offense and defense.

	Offense trait	Defense trait	Correlation	<i>P</i>
Seminal fluid traits	P_2	P_1	0.51*	0.002
	P_2	fidelity	0.46*	0.005
Male behavior/seminal fluid traits	remating	P_1	0.16	0.354
	remating	fidelity	0.28	0.107
All traits combined	net fitness	net fitness	0.41*	0.014

* Significant ($P < 0.05$) after sequential Bonferroni correction. All significant correlations remain significant after adjustment for male body size.

ence on female fitness were low, as were the heritability estimates, as would be expected for traits under strong directional selection.

Potentially Confounding Factors

Because females interacted with males for a period of two days, our measures of P_2 may be biased upward and those of P_1 downward, by multiple rematings. Measurements were made over two days in order to measure offense in the same environment to which the flies were adapted (i.e., the two days that the flies spent at low adult density in adult competition vials). If multiple rematings were common during this two-day period, they would increase the estimated value of sperm displacement, and genetic variation for remating rate would be confounded with genetic variation for P_1 and P_2 .

To check for this possibility we used residual analysis. This did not change our conclusion, since the additive genetic variation for P_1 and P_2 remains, when they are statistically adjusted for the level of remating that occurred in each vial. More specifically, we regressed our measures of the average values P_1 and P_2 for each hemiclone from each block on the remating rate that occurred in the remating vial during their measurement, and then analyzed the residuals of P_1 and P_2 that had been adjusted for remating rate with the same ANOVA design as the unadjusted values of P_1 and P_2 . The estimated standing additive genetic variances were not changed appreciably when the potentially confounding effect of remating rate was statistically removed (offense: estimated variance unadjusted was 0.0111 and adjusted was 0.0078; defense: estimated variance unadjusted was 0.0071 and adjusted was 0.0064).

Measures of P_1 and P_2 could also potentially be confounded by variation in egg-to-adult viability of different genotypes (Gilchrist and Partridge 1997). Because the hemiclones were measured in the heterozygous state, genetic variation in viability is small and uncorrelated with adult male fitness (Chippindale et al. 2001). Measures of viability of the 35 hemiclones were available from a different study in our laboratory. To test the idea that our observed variation in P_1 and P_2 was associated with variation in viability, we correlated average P_1 and P_2 of the 35 hemiclones versus their egg-to-adult survival. No significant correlation was observed for P_1 ($r = 0.03$, $P = 0.86$), nor for P_2 ($r = 0.09$, $P = 0.61$), indicating that variation in egg-to-adult viability cannot explain the observed variation in P_1 and P_2 .

We also tested whether variation in male offensive and defensive fitness was due to variation in the egg-to-adult viabilities of the 35 hemiclones. No significant correlation

with egg-to-adult viability was observed for defense fitness ($r = 0.06$, $P = 0.71$) nor offense fitness ($r = -0.15$, $P = 0.38$) indicating that variation in egg-to-adult survival cannot account for the observed variation in male offensive and defensive fitness.

Genetic Covariation in Offense and Defense

Male defense and offense fitness were positively correlated (see Table 2), but none of them was correlated with juvenile fitness (defense: $r = 0.06$, $P = 0.71$; offense: $r = -0.15$, $P = 0.37$). Because the offense and defense measures were carried out independently (different times, random genetic backgrounds, maternal effects, female mates, and competitors) there was no environmental covariance between them, and the significant phenotypic correlation between offense and defense fitness demonstrates a significant genetic correlation. This implies that some of the segregating alleles for offense and defense simultaneously influence both traits. By the same logic, we also found positive genetic correlations between offensive P_2 and both defensive P_1 and fidelity (see Table 2). No correlation was found between the behavioral offensive trait remate and the two defensive seminal fluid traits P_1 and fidelity (see Table 2). The positive correlations across the offense and defense assays provides strong corroborating evidence to the ANOVA analyses that there is additive genetic variation among hemiclones for defense and offense phenotypes.

To test for genetic variation among hemiclones that contributes exclusively to male offense and defense, we correlated the offense and defense traits with a third, independently measured phenotype of the hemiclones; that is, survival of tester females when constantly confined with males. Previous experiments with our base population have demonstrated that remating reduces female survival (Rice 1996; Holland and Rice 1999), so we expected a priori that the offensive remating rate would correlate negatively with the survival of the tester females. We found a significant partial correlation of offensive remating rate with survival of tester females ($r = -0.39$, $P = 0.02$, directed test), after adjustment for defensive P_1 and fidelity, indicating genetic variation for offensive male mating behavior that is nonoverlapping with genetic variation for defense. Defensive fidelity also was significantly correlated with the survival of tester females ($r = -0.36$, $P = 0.03$). The statistical significance of this correlation was slightly reduced ($P = 0.06$) after taking out the effects of offensive P_2 and remate.

As an independent check on the nearly significant negative association between defensive fidelity and survival of tester females, data were available from the additional set of 17

hemiclones that were hyperdispersed for male lifetime fitness (Lew and Rice 2005). When we calculated the partial correlation between defensive fidelity and survival of tester females, adjusting for P_2 and offensive remate, a significant partial correlation was found ($P = 0.04$), corroborating the existence of genetic variation (in the LH_M base population) for defense (fidelity) that is independent of offense. Collectively, these partial correlations indicate that although offense and defense are genetically correlated there is also evidence for at least some independent genetic variation for each trait.

Selection on Components of Male Offense and Defense

Higher values of P_1 , P_2 , remating rate, and fidelity are not necessarily favored by natural selection because high values may come at the expense of reduced female fecundity. Unfortunately, the intuitive test of correlating the averages of these traits with average progeny production is statistically invalid because the two averages are intrinsically positively correlated when both measures are taken from the same data. To circumvent this problem, we correlated the offense and defense components from each block to the male offense and defense offspring production values, respectively, taken from the remaining four blocks. This produces five tests for a correlation of the trait with male fitness. We verified that the tests are stochastically independent by a computer simulation. Each test has low statistical power due to reduced sample size associated with using only a single block to estimate P_1 , P_2 , remating rate, and fidelity. However, the five tests, each with low statistical power, can be combined with a combined probability test to recoup statistical power (Rice 1990). The combined P -values for the correlations between adult male offensive or defensive offspring production and P_1 , defensive fidelity, P_2 , and offensive remating rate are 0.04, 0.02, 0.06, and 0.01, respectively. Although we expected each trait to be favored by selection, we have reported directed rather than one-sided P -values (Rice and Gaines 1994). Because the P -value for selection on P_2 was only close to significant ($P = 0.06$), we used the smaller set of 17 hyperdispersed haplotypes to check this result. In this dataset there was a significant correlation between P_2 and an independent measure of lifetime fitness ($P = 0.02$), corroborating the result from the larger dataset.

Because we found a genetic correlation between offense and defense, we needed to establish whether there was a positive selection gradient for the nonoverlapping genetic variation for offense and defense. This was not possible for the main dataset because we do not have independent measures of net fitness for the 35 hemiclones. A test was possible for the smaller set of 17 hemiclones for which we had an independent measure of offense, defense, and total fitness. We found a significant correlation between the residuals of offense adjusted for defense and net fitness ($P = 0.005$), indicating a positive selection gradient of offense variation that was nonoverlapping with defense variation. However, we did not find a corresponding significant selection gradient on defense variation that was nonoverlapping with offense variation ($P = 0.82$), which indicates that any such selection, if present, was too weak to be detected with this smaller dataset.

TABLE 3. Influence of male offense and defense traits on female fitness.

Male trait	Female trait	Correlation	P
Defense			
P_1	lifetime fecundity	0.04	0.806
	long-term survival	0.07	0.678
Fidelity	lifetime fecundity	0.58	0.0002*
	long-term survival	-0.36	0.033**
Offense			
P_2	lifetime fecundity	-0.38	0.024*
	long-term survival	0.17	0.320
Remating rate	lifetime fecundity	-0.07	0.172
	long-term survival	-0.44	0.009*

* Significant ($P < 0.05$) after sequential Bonferroni correction; ** $0.05 < P < 0.1$ after Bonferroni correction.

Effects of Male Defense and Offense on Female Fitness

Hemiclones differed in their influence on the fecundity of their mates in both the offense and defense assays (see Table 1). Table 3 displays the correlations between male offense and defense traits and the fitness of females that interacted with the males. Fidelity to the first male to mate a female in the defense assay was positively correlated with the fecundity of their mates ($r = 0.58$, $P = 0.0002$). Part of this effect was because, irrespective of the genotype of their mates, females that remated experienced reduced fecundity in the defense assay (paired Student's t -test; $t = -4.76$, $P < 0.0001$). However, the positive correlation between defensive fidelity and the fecundity of their mates is also manifest among females that did not remate ($r = 0.41$, $P = 0.02$), indicating that the positive effect on female fecundity of males that induce high fidelity is not solely due to protection from remating.

The beneficial effect of defensive fidelity was reversed in the context of survival of tester females (higher fidelity was associated with reduced female survival, $r = -0.36$, $P = 0.03$), but this harmful effect on survival would not be realized in our laboratory environment due to the short lifespan of the females. The parameter P_1 did not influence female fecundity ($r = 0.07$, $P = 0.68$) or survival of tester females ($r = 0.04$, $P = 0.81$). P_2 was negatively associated with female fecundity ($r = -0.38$, $P = 0.02$) but did not affect the survival of tester females ($r = 0.17$, $P = 0.32$). To reaffirm the negative correlation between P_2 and female fecundity, we calculated the correlation for each of the five blocks separately and then combined inference by using a consensus-combined P -value. This procedure was used because it allows the negative relationship between P_2 and female fecundity to vary between blocks, which makes it more powerful than the previous analysis. This more detailed analysis confirmed the negative association that was based on mean values ($P = 0.0003$).

Remating, in the offense assay, was not significantly associated with reduced female fecundity ($r = -0.06$, $P = 0.71$) but it was associated with reduced survival of tester females ($r = -0.44$, $P = 0.009$). In a related study (Linder and Rice 2005; T. A. Lew, E. H. Morrow, and W. R. Rice, unpubl. ms.), using a protocol similar to the offense assay—but with random males genotypes and female hemiclones—found that increased remating was significantly associated

with reduced lifetime fecundity of females. As a consequence, the nonsignificance of the correlation between remating in the offense assay and female lifetime fecundity may reflect lack of statistical power rather than absence of male-induced harm.

DISCUSSION

The main finding from this study was that male defense and offense and all their subcomponents (P_1 , fidelity, P_2 , and remating rate) show low but statistically significant additive genetic variation among hemiclones. This pattern is expected for traits subject to strong directional selection, and the positive correlation of all of the offense and defense traits with progeny production (in their respective assays) supports the conclusion that this form of selection is operating in the LH_M base population. We also found evidence that male offense (specifically P_2) is harmful to females but defense is not (although fidelity was associated with reduced long-term survival of tester females, harm that is not manifest in our base population due to its short generation cycle). Lastly, we found evidence that the male offense and defense traits are jointly influenced by some segregating alleles in the LH_M base population, but that there was also some independent genetic variation for each trait.

Male defense and offense are two major components of male adult fitness. Empirical data suggest that such traits have substantial evolvability but low heritabilities, due to biologically significant levels of additive genetic variation but high relative levels of environmental variation (Houle 1992). Prior to our experiments, earlier studies demonstrated that genetic variation for male defense and offense phenotypes existed among inbred genotypes (homozygous for 40% of their genomes; Clark et al. 1995, 1999; Hughes 1997). However, the study by Hughes (1997) found that the effect of genotype only was due to dominance variation and not to additive genetic variation when outbred genomes were studied, indicating that many of the genotypic effects from past studies may have been due to inbreeding depression rather than the additive effects of alleles. Unlike past studies, our genome-wide assays of outbred individuals found significant additive genetic variation among hemiclones for offense and defense. However, heritabilities for offense and defense fitness are low (CV_A : offense 13.11%, defense 14.85%; h^2 : offense 1.6%, defense 1.5%). These results are in line with previous estimates of other major components of fitness (CV_A : fecundity 11.90%, lifespan, 9.89%; h^2 : fecundity 6%, lifespan 11%; see review by Houle 1992).

Additive genetic variation among hemiclones represents additive genetic variation among gamete genomes, and approximately half the additive genetic variation among diploid individuals, except for variation that is due to epistatic interactions among nonallelic genes contained in the same hemiclone haplotype (thus, as an approximation to convert our measures to those for diploid individuals, the above CV and h^2 values should be multiplied by $\sqrt{2}$ and 2, respectively). Two lines of evidence indicate that much of the additive genetic variation among hemiclones represents additive genetic variation among individuals. First, theoretical work concerning standing genetic variation for traits closely

associated with fitness indicates that epistatic variation will be a minor component of total standing genetic variation for fitness (see Materials and Methods). Second, and most important, two previous studies with our LH_M base population found a rapid evolutionary change in either male defense (Rice 1996) or a major component of male offense (courtship rate; Holland and Rice 1999) in response to experimental manipulation of the environment, which is consistent with substantial standing additive genetic variation. Overall, we conclude that our base population has substantial additive genetic variation for both offense (P_2 and remating rate) and defense (P_1 and fidelity), but that the levels of additive variation are much lower than one might conclude based on previous studies of inbred lines (Clark et al. 1995).

To provide evidence for an ongoing arms race between offense and defense we needed to verify the three conditions outlined in the introduction were met: (1) adaptive evolution by each trait creates a lag load at the other, (2) there is at least some unique genetic variation for each trait, and (3) the selection gradients on each trait are opposing. Because offense and defense are intrinsically opposing traits, like a toxin and antitoxin, condition (1) is fulfilled. We also demonstrated that there is at least some unique genetic variation for both offense and defense, so condition (2) is met. Although we found evidence for mutually opposing selection gradients on the two traits (based on the positive correlations between total offense [combined remating rate and P_2] and defense [combined fidelity and P_1] and progeny production in their respective assays), the positive genetic correlation between offense and defense requires that we further demonstrate a positive selection gradient on the variation for both traits that was nonoverlapping. We were unable to carry out this test with our random sample of 35 hemiclones because we do not have independent measures of their net fitness. However, data from the smaller sample of 17 nonrandom hemiclones provided evidence that there was a positive selection gradient on the genetic variation for offense that was not shared with defense, but not vice versa. In sum, we have strong evidence that at least half of the arms race (the offense side) is currently in progress between male offense and defense. The other half (defense) may lack selected variation, or we may have had too little power to detect it with our smaller sample of 17 hemiclones. The fact that even half of the arms race could be detected at an arbitrary single point in time is consistent with the conclusion that antagonistic coevolution between offense and defense is a perpetual process that can foster rapid evolution and contribute strongly to genetic divergence among isolated populations.

Additionally, we have evidence that variation in offense, and to a lesser degree in defense, has implications for female fitness. Variation in male offense was, not unexpectedly, associated with variation in male fitness. But it also caused substantial variation in female fitness; in the context of male offense the CV_A in the influence of males on female fitness was nearly half as large as that for male fitness itself (6.36% vs. 13.11%), and even higher when only considering females that remated (10.77%). Our results further show that advance in male offense (specifically P_2), but not defense, leads to reduced fecundity of a male's mates, causing sexual conflict over the expression of this trait. Even if higher P_2 was some-

how associated with increased quality of a female's offspring, females would nonetheless be selected to eliminate any costly side effects associated with elevated P_2 . If such counteradaptations caused the optimal P_2 to differ between the sexes, antagonistic coevolution between male offense and defense would fuel a parallel antagonistic coevolution between the sexes.

Our study corroborates previous studies by showing a negative correlation between fidelity and female lifespan (Civetta and Clark 2000) and no correlation between P_2 and female lifespan (Civetta and Clark 2000; Sawby and Hughes 2001). Because our study used completely outbred flies, and the earlier studies used lines homozygous for 40% of their genome, the congruence of results suggests that inbreeding depression alone cannot account for genetic variation in male-induced harm to their mates' survival. However, because of the short generation time of our laboratory population, this survival cost to females is not manifest and therefore cannot contribute to antagonistic coevolution between the sexes in our laboratory population. Nonetheless, it would potentially contribute to intersexual conflict over fidelity in wild populations or laboratory populations with overlapping generations.

Genetic variation in male reproductive traits (e.g., testis size, ejaculate size, sperm length) has been found in several studies (e.g., Pitnick and Miller 2000; Morrow and Gage 2001; Simmons and Kotiaho 2002). In general, the measured heritabilities have been considerably higher than the values reported here for heritabilities of P_1 and P_2 . The fact that these heritabilities are so high compared to our estimates of P_1 and P_2 , and other traits closely related to fitness, indicate that some male reproductive traits are probably under only weak selection and therefore have low predictive value for a male's sperm competitiveness. However, the only other study that has reported significant levels of additive genetic variation in sperm competition itself among outbred individuals found a much higher level of heritability for this trait in bulb mites than we did in *D. melanogaster* (Radwan 1998).

Concerning the pattern of genetic correlations among components of offense and defense, our results are different from earlier studies. Although we found a positive correlation between defensive P_1 and offensive P_2 , Clark et al. (1995) found a close-to-significant negative genetic correlation, and Hughes (1997) found none for homozygous genomes and a nonsignificant trend toward positive correlation in heterozygous genomes. In our study, fidelity and P_2 were positively correlated, whereas a nonsignificant trend in the opposite direction was found by Clark et al. (1995). Furthermore, we found no correlation between fidelity and remating ability, whereas these were found to be strongly negatively correlated by Clark et al. (1995). The one case where our correlations coincide with those of Clark et al. (1995) is the lack of a significant correlation between P_1 and remating. Although there is no reason to expect to find identical correlations between traits among different studies, we were nonetheless surprised to see so little concordance among studies. The expression of inbred homozygous versus outbred heterozygous chromosomes may explain some of the lack of concordance among studies (Rose 1984; Phillips et al. 2001),

but clearly additional surveys will be needed to determine whether there are any general patterns.

In conclusion, this study demonstrates that our large outbred base population (LH_M) contains additive genetic variation for male reproductive performance, both in the context of offense and defense. A positive genetic correlation was found between offense and defense, indicating some shared genetic variation, but we also found evidence for nonoverlapping genetic variation for these male traits. Offense was found to be under positive directional selection, and indirect evidence indicates that defense also has a positive selection gradient (based on a positive correlation between defense ability [combined fidelity and P_1] and progeny production in the assays). We found strong evidence that increased offense ability is currently evolving in the base population and creating a lag-load in defense. However, we were unable to statistically demonstrate ongoing counterevolution by defense, so only half of the arms race between offense and defense could be established to be ongoing in our base population at this time. Lastly, we found evidence for harm to a male's mates in the context of male offense (P_2) but not in defense. This harm to females is expected to create a lag-load in females and fuel a parallel arms race between male offense and female resistance.

ACKNOWLEDGMENTS

We thank J. Linder and G. Rice for technical assistance and G. Arnqvist, A. G. Clark, and A. Maklakov for providing comments on a previous version of the manuscript. This study was financially supported by two grants from the National Sciences Foundation to WRR (DEB-0128780 and DEB-0410112), scholarships from the Sweden-America Foundation and Stiftelsen för internationalisering av högre utbildning och forskning (STINT) to UF, and a grant from the Swedish research council to G. Arnqvist.

LITERATURE CITED

- Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.
- Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* 60: 145–164.
- Chapman, T. 2001. Seminal-fluid mediated fitness traits in *Drosophila*. *Heredity* 87:511–521.
- Chapman, T., S. Trevitt, and L. Partridge. 1994. Remating and male-derived nutrients in *Drosophila melanogaster*. *J. Evol. Biol.* 7: 51–69.
- Chapman, T., L. F. Liddle, J. M. Kalib, 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.
- Chapman, T., G. Arnqvist, J. Bangham, and L. Rowe. 2003. Sexual conflict. *Trends Ecol. Evol.* 18:41–47.
- Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 98: 1671–1675.
- Civetta, A., and A. G. Clark. 2000. Correlated effects of sperm competition and postmating female mortality. *Proc. Natl. Acad. Sci. USA* 97:13162–13165.
- Clark, A. G., M. Aguade, T. Prout, L. Harshman, and C. H. Langley. 1995. Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. *Genetics* 139:189–201.

- Clark, A. G., D. J. Begun, and T. Prout. 1999. Female \times male interactions in *Drosophila* sperm competition. *Science* 283: 217–220.
- Fowler, K., and L. Partridge. 1989. A cost of mating in female fruit flies. *Nature* 338:760–761.
- Fowler, K., C. Semple, N. H. Barton, and L. Partridge. 1997. Genetic variation for total fitness in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* 264:191–199.
- Friberg, U., and G. Arnqvist. 2003. Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. *J. Evol. Biol.* 16:797–811.
- Gilchrist, A. S., and L. Partridge. 1997. Heritability of pre-adult viability differences can explain apparent heritability of sperm displacement ability in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* 264:1271–1275.
- 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. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130:195–204.
- Hughes, K. A. 1997. Quantitative genetics of sperm precedence in *Drosophila melanogaster*. *Genetics* 145:139–151.
- Lew, T. A., and W. R. Rice. 2005. Natural selection favors harmful male *Drosophila melanogaster* that reduce the survival of females. *Evol. Ecol. Res.* 7:633–641.
- Linder, J. E., and W. R. Rice. 2005. Natural selection and genetic variation for female resistance to harm from males. *J. Evol. Biol.* 18:568–575.
- Morrow, E. H., and M. J. Gage. 2001. Artificial selection and heritability of sperm length in *Gryllus bimaculatus*. *Heredity* 87: 356–362.
- Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* 45:525–567.
- . 1979. Sexual selection and sexual conflict. Pp. 123–165 in M. S. Blum, and N. A. Blum, eds. *Sexual selection and reproductive competition in insects*. Academic Press, London.
- Partridge, L., and K. Fowler. 1990. Non-mating costs of exposure to males in female *Drosophila melanogaster*. *J. Insect Physiol.* 36:419–425.
- Phillips, P. C., M. C. Whitlock, and K. Fowler. 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. *Genetics* 158:1137–1145.
- Pitnick, S., and F. García-González. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* 269:1821–1828.
- Pitnick, S., and G. T. Miller. 2000. Correlated response in reproductive and life history traits to selection on testis length in *Drosophila hydei*. *Heredity* 84:416–426.
- Pitnick, S., G. S. Spicer, and T. A. Markow. 1997. Phylogenetic examination of female incorporation of ejaculates in *Drosophila*. *Evolution* 51:833–845.
- Prout, T., and A. G. Clark. 2000. Seminal fluid causes temporarily reduced egg hatch in previously mated females. *Proc. R. Soc. Lond. B* 267:201–203.
- Radwan, J. 1998. Heritability of sperm competition success in the bulb mite, *Rhizoglyphus robini*. *J. Evol. Biol.* 11:321–327.
- Rice, W. R. 1990. A consensus combined *P*-value test and the family-wide significance of component tests. *Biometrics* 46: 303–308.
- . 1996. Sexually antagonistic male adaptations triggered by experimental arrest of female evolution. *Nature* 381:232–234.
- Rice, W. R., and S. D. Gaines. 1994. “Heads I win, tails you lose:” testing directional alternative hypotheses in ecology and evolution. *Trends Ecol. Evol.* 9:235–237.
- Rice, W. R., and B. Holland. 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intra-specific Red Queen. *Behav. Ecol. Sociobiol.* 41:1–10.
- Rose, M. R. 1984. Genetic covariation in *Drosophila* life history: untangling the data. *Am. Nat.* 123:565–569.
- Sawby, K., and K. A. Hughes. 2001. Male genotype affects female longevity in *Drosophila melanogaster*. *Evolution* 55:834–839.
- Service, P. M., and A. J. Fales. 1993. Evolution of delayed reproductive senescence in male fruit flies: sperm competition. *Genetica* 91:111–125.
- Service, P. M., and M. R. Rose. 1985. Genetic covariation among life-history components: the effect of novel environments. *Evolution* 39:943–945.
- Service, P. M., and R. E. Vossbrink. 1996. Genetic variation in “first” male effects on egg-laying and remating by female *Drosophila melanogaster*. *Behav. Genet.* 26:39–48.
- Simmons, L. W., and J. S. Kotiaho. 2002. Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competitive traits. *Evolution* 56: 1622–1631.
- Swanson, W. J., A. G. Clark, H. M. Waldrip-Dail, M. F. Wolfner, and C. F. Aquadro. 2001. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 98:7375–7379.
- Tachida, H., and C. C. Cockerham. 1988. Variance components of fitness under stabilizing selection. *Genet. Res.* 51:47–53.
- Watson, P. J., G. Arnqvist, and R. R. Stallmann. 1998. Sexual conflict and the energetic costs of mating and mate choice in water striders. *Am. Nat.* 151:46–58.
- Wigby, S., and T. Chapman. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution* 58:1028–1037.
- 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: T. Tregenza