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Adult locomotory activity mediates intralocus sexual conflict in a laboratory-adapted population of *Drosophila melanogaster*

Tristan A. F. Long* **and William R. Rice**

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

The strongest form of intralocus sexual conflict occurs when two conditions are met: (i) there is a positive intersexual genetic correlation for a trait and (ii) the selection gradients on the trait in the two sexes are in opposite directions. Intralocus sexual conflict can constrain the adaptive evolution of both sexes and thereby contribute to a species' 'gender load'. Previous studies of adult lifetime fitness of the same sets of genes expressed in both males and females have established that there is substantial intralocus conflict in the LH_M laboratory-adapted population of *Drosophila melanogaster*. Here, we investigated whether a highly dimorphic trait—adult locomotory activity—contributed substantially to the established intralocus sexual conflict. To measure the selection gradient on activity level, both this trait and adult lifetime fitness were measured under the same environmental conditions to which the flies were adapted. We found significant phenotypic variation in both sexes for adult locomotory activity, and that the selection gradients on this variation were large and in opposite directions in the two sexes. Using hemiclonal analysis to screen 99% of the entire genome, we found abundant genetic variation for adult locomotory activity and showed that this variation occurs on both the X and autosomes. We also established that there is a strong positive intersexual genetic correlation for locomotory activity. These assays revealed that, despite the strong, extant sexual dimorphism for the trait, locomotory activity continues to contribute strongly to intralocus sexual conflict in this population.

Keywords: intralocus sexual conflict; sexually antagonistic alleles; gender load; locomotory activity; sex chromosomes; fitness

1. INTRODUCTION

Darwin (1871) first recognized that the processes of natural and/or sexual selection may act differently on males and females—either as a consequence of their unique reproductive roles or due to competition between these two groups for limited resources—and that this divergent selection should ultimately produce adaptive sexually dimorphic phenotypes. The evolution of the sexes towards their respective adaptive peaks may be constrained, however, by the species' own genetic architecture. In males and females, most homologous traits are controlled by the same suite of genetic elements, and as such exhibit strong positive genetic correlations between the sexes (Lande 1980, 1987; Roff 1997). A strong positive intersexual genetic correlation (r_{MF}) will thus interfere with the processes of adaptation when alleles that enhance fitness when expressed in one sex also decrease fitness when expressed in the other sex, as the correlated responses to selection will interfere with members of the both sexes reaching their own fitness optima (Lande 1980; Rice 1984; Rice & Chippindale 2001).

Intralocus sexual conflict occurs when sexually antagonistic alleles (those favoured in one sex while disfavoured in the other sex) are present in the gene pool. When sexually antagonistic alleles are segregating, a negative correlation for fitness of the same genetic element (gene or group of genes) is diagnostic of intralocus sexual conflict (Rice 1992; Chippindale *et al.* 2001). In the currency of quantitative traits, the strongest, most unambiguous, form of intralocus sexual conflict occurs when there is (i) directional selection on the extant variation for a trait that is in opposite directions in the two sexes and (ii) a positive genetic correlation for the trait between the sexes. It is this form of intralocus sexual conflict that we consider in this study. However, we point out that intralocus sexual conflict can also occur when there is a negative intersexual genetic correlation for a trait when it interferes with each sex evolving towards a gender-specific optimum, and a weak form of sexual conflict occurs when genes are gender-limited in expression (zero intersexual genetic correlation) owing to the neutrality of alleles in the unselected sex and the consequent higher mutational load (Day & Bonduriansky 2004).

In principle, empirical testing for the presence of intralocus conflict should be relatively easy as it simply involves measuring (in the currency of lifetime fitness) heritable genetic variation for traits that are differentially selected between the sexes. In practice, however, logistical constraints usually prevent the accurate measurement of an individual's lifetime reproductive success, and fitness components must frequently be used as surrogates (e.g. Brommer *et al.* 2004). Unfortunately, potential negative correlations between fitness components (e.g. Rose & Charlesworth 1981*a*,*b*; Brooks 2000) can make this

* Author for correspondence (long@lifesci.ucsb.edu).

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approach unreliable. An alternative approach is to study divergent selection between the sexes in locally adapted laboratory populations in which complete measures of lifetime fitness are possible (laboratory island analysis, Rice *et al.* (2006)). Earlier studies (Chippindale *et al.* 2001; Gibson *et al.* 2002) using a laboratory-adapted population of *Drosophila melanogaster* (LH_M) found that much of the observed variation in adult fitness could be attributed to the expression of sexually antagonistic alleles. These studies, however, did not determine which phenotypic trait(s) contributed to the sexually antagonistic fitness variation. In this study, we expanded on the previous work on intralocus sexual conflict by identifying a phenotypic trait that is subject to divergent selection between the sexes, but is evolutionary constrained by its intersexual genetic correlation.

Besides identifying a trait that underlies intralocus sexual conflict, another goal of our study was to determine the location of genetic variation contributing to such a divergently selected trait. Theoretical work by Rice (1984) indicated that the sex chromosomes might be hot spots (i.e. highly enriched) for sexually antagonistic variation. Earlier work by Haldane (1962) and Kidwell *et al.* (1977) indicated that the parameter space permitting polymorphism for autosomal sexually antagonistic variation was limited unless selection coefficients were very large, or a fortuitous form of dominance occurred in which an allele is dominant in the sex where it was favoured and recessive in the sex where it was disfavoured. Later work by Rhen (2000) showed that autosomal polymorphism is facilitated in the special case where some mutations fortuitously exhibit gender-limited expression, but it is unclear how common such alleles are in nature. A previous study (Gibson *et al.* 2002) provided evidence that the X-chromosome of *D. melanogaster* was enriched with sexually antagonistic variation, but evidence for autosomal variation has also been reported (Rice 1992). In this study, we sought to make such a direct comparison in the context of a defined sexually antagonistic trait in order to ascertain the relative contribution of the X-chromosome and the autosomes.

Although there are numerous morphological, physiological and behavioural traits that could be excellent candidates for investigation, a review of the literature, combined with our own observations of males and females under typical culture conditions, led us to suspect a behavioural trait—adult locomotory activity—that was potentially subject to intralocus sexual conflict. First, the trait appeared to have a heritable basis, since others have successfully been able to artificially select for populations exhibiting high or low levels of activity (e.g. Ewing 1963; Connolly 1966; van Dijken & Scharloo 1979*a*). Equally important was the evidence that males and females may be selected to express different optimal levels of activity as adults, based on their respective fitness-enhancing 'strategies'. In *D. melanogaster*, male fitness is primarily limited by their ability to locate, court and mate with receptive females, at least under laboratory conditions (Bateman 1948). As such, upon eclosion, males engage in scramble competition for mates (Partridge *et al.* 1987), in which an important first step is locating females to court and ultimately copulate. Thus, even in the confines of laboratory culture, males may benefit from an increased activity rate, as sedentary males may obtain fewer reproductive opportunities. Consistent with this prediction are the observations that (i) females are more likely to mate with a male from a population that has been selected for high locomotory activity than from one from a population selected for low locomotory activity (van Dijken & Scharloo 1979*b*), (ii) the most reproductively successful males in a population show relatively high levels of movement when not courting (Partridge *et al.* 1987), (iii) artificial selection for low male mating activity resulted in flies of relatively low locomotory reactivity (Jordan *et al.* 2006), and (iv) males possessing 'general locomotor' mutations also show low reproductive success (Hall 1994). Cumulatively, these observations are suggestive of sexual selection favouring increased levels of activity in male *D. melanogaster*.

In contrast, females may be selected to behave in a markedly different manner as a result of their own sex-specific, fitness-maximizing 'strategy'. Numerous studies (e.g. Fowler & Partridge 1989; Chapman *et al.* 1995; Linder & Rice 2005; Kuijper *et al.* 2006) have demonstrated that, unlike males, females suffer a loss in fitness (measured as number of offspring produced) with each successive remating event that is not offset by the indirect benefits of good genes (Byrne & Rice 2005) or sexy sons (Orteiza *et al.* 2005). Since female movement is a stimulus for male courtship activity (Tompkins *et al.* 1982), excess activity by females is expected to attract harmful, persistent male courtship (Partridge & Fowler 1990). Additionally, in our LH_M population, adult females increase their reproductive success by consuming live yeast, which is the limiting resource for egg production (Linder & Rice 2005; Stewart *et al.* 2005). Since this yeast is located only on the medium surface (and takes up less than 2% of a vial's total internal surface area), females do not need to move around much to locate this valuable resource. Thus, an overly active female may needlessly expend energy that could otherwise be spent producing eggs.

Together these observations indicate that adult locomotory activity is a candidate phenotype for a trait that mediates intralocus sexual conflict. In this study, we set out to explicitly test whether this was the case, and if so, to compare the relative contribution of the X chromosome and autosomes with its heritable genetic variation.

2. MATERIAL AND METHODS

(a) *Study populations*

Each of the experiments described below used adult *D. melanogaster* obtained from either a large, outbred wild-type population (LH_M) or a replicate large, outbred population (LH_M-*bw*) into which a recessive, brown-eyed marker (*bw*) had been introgressed through repeated backcrossing. Briefly, populations are maintained on a two-week culture schedule that begins when flies are placed into 56 'oviposition vials' comprising 10 ml of standard cornmeal/molasses/killed-yeast medium (but no live yeast) for an 18 hour period. The eggs laid during this period are then manually culled to a density of 150–200 eggs per vial. Eggs hatch, develop and become sexually mature over the next 11 days in these vials, at which time flies are removed, mixed between vials, and 16 pairs of male and female flies are transferred to each of 56 'adult competition vials' containing new medium with 6.4 mg of live yeast applied to the surface. These vials are placed on their sides in the incubator for an additional 2 days. Eighteen hours before the end of the

two-week generation cycle, flies are transferred to a new set of 56 oviposition vials and the eggs laid during this period are used to found the next generation of the laboratory population. Further details on the history and maintenance of these populations can be found in Rice *et al.* (2005).

(b) *Assay of locomotory activity: general considerations*

Rather than placing adult flies into a novel test environment to measure locomotory activity (e.g. Connolly 1966; Angus 1974; van Dijken & Sharloo 1979*a*; Martin *et al.* 1999; Jordan *et al.* 2006), our experimental protocol was meant to mimic as closely as possible the conditions to which the flies had adapted for over 400 generations. Thus, our observations are hopefully free of potential confounds associated with novel genotype×environment effects and thus possess greater biological relevance with respect to fitness.

(c) *Individual analyses of locomotory activity and fitness*

In order to determine whether individual phenotypic variation in either of the two sexes for adult locomotory activity was related to variation in fitness, we collected eggs separately from both the LH_M-*bw* and the LH_M populations and cultured them at a density of 160 eggs per vial. After 11 days, standard adult competition vials (described above) were assembled containing 1 red-eyed male (bw^+bw^+), 1 red-eyed female (bw^+bw^+), 15 brown-eyed males (*bwbw*) and 15 brown-eyed females (*bwbw*). Vials were placed on their sides in a quiet, well-lit room and observations were made in 6 sessions, 4 on the 11th day post egg deposition and 2 on the following day. We chose to observe locomotory activity during this phase of the culture cycle, as females are known to compete for yeast, and males for mating opportunities (see Rice *et al.* 2006). In each session, the target (red-eyed) individuals were located in a vial and observed for 8 s. If during that 8 s period they made any movement that resulted in a physical displacement, they were scored as having been 'active'. 'Adult locomotory activity', defined as the fraction of times when activity was observed, across the six sessions, was then calculated for each individual. This measure is a composite trait that incorporates many more specific behaviours. Although it is possible to devise assays that measure some of these constituent elements more explicity (e.g. Connolly 1976; van Dijken & Sharloo 1979*a*; Martin 2004; Jordan *et al.* 2006), such attempts typically require placing flies in novel test environments for observation, the results of which may be context specific (e.g. Connolly 1967) and of little actual relevance to understanding the behaviour of individuals in their natural environment. By measuring our flies in a test environment that mimics their normal culture conditions, we can avoid this important confound.

Once activity observations were complete, vials were returned to the incubator until the end of the typical 2 days adult competition phase of the life cycle, at which time, all females were transferred to individual test tubes containing 5 ml of media, where they were allowed to oviposit for 18 hours before being discarded. A small cut was made on the surface of the medium in each test tube because previous studies in our laboratory (reviewed in Rice *et al.* (2005)) had shown that this procedure leads to normal levels of fecundity when the females oviposit in isolation. The number of eggs laid by each of the red-eyed LH_M females was counted and it represents their total adult fitness (as the LH_M base population is semelparous, the 18 hours oviposition phase represents total adult fitness for females). The eggs laid by the brown-eyed LH_M-*bw* females from each vial were incubated for an additional 11 days, at which time the number of eclosed red-eyed progeny in each clutch were counted and averaged among the number of females assayed. The average values of red offspring per vial were then multiplied by 16 to represent the total adult fitness of the single LH_M (red-eyed) male from each vial. Activity and fitness data were obtained during two replicate sessions, for a total of 99 individuals of each sex.

(d) *Hemiclonal analysis of locomotory activity*

In order to assess the genetic basis for phenotypic variation in locomotory activity, we measured the intersexual genetic correlation using hemiclonal analysis (see Rice *et al.* (2005) for review of the protocols). A hemiclone is a group of individuals that share a nearly genome-wide set of genes in common (all genes except the 0.5% of the genome located on the 'dot' fourth chromosome), each expressed in a different random genetic background. By analogy, if a single sperm genome were clonally amplified and used to fertilize a random collection of eggs from a population, the resulting offspring would represent a hemiclone. In our experiment, a random samples of 35 hemiclones from the LH_M population were used to measure genetic variation in adult locomotory activity.

In order to survey male and female hemiclones for activity level, eggs from each of the 35 hemiclones were placed into separate vials containing new media in sufficient numbers so that there were approximately 160 viable larvae per vial. Simultaneously, vials were set up with 160 eggs that had been collected from the LH_M-*bw* population. Both types of culture were then placed into incubators for 11 days until it was time to create adult competition vials, for observations. Each vial contained either 16 hemiclonal (red-eyed) males and 16 brown-eyed females or 16 hemiclonal (red-eyed) females and 16 brown-eyed males. Observation vials were constructed as described in §2*c*. Observations were made in 3 sessions, 1 on the 11th day post egg deposition and two on the following day. Observation sessions on the last day of the culture cycle were not performed as the accumulation of waste products on the sides of the vials obstructed clear viewing of the flies.

In each session, 10 out of the 16 target individuals in each vial (males or female from a prescribed hemiclone) were observed. To avoid potential observer bias in which flies to observe, the inside surface of each vial was divided into five regions, and each region was assigned a number. A random number generator was used to choose where the observer was to look for target (red-eyed) flies. If during a session, no flies were present in the randomly selected region, a new number was taken from a randomly generated list until a region containing target flies was selected. Once a target individual had been located in the predetermined region, it was observed for 8 s for the signs of locomotory activity. The adult locomotory activity score was calculated as the fraction (out of 10) of active individuals for each of the three observation sessions. The entire hemiclonal assay was replicated thrice. Each replicate provided one estimate of average adult locomotory activity for each sex for each of the 35-hemiclonal lines.

(e) *Chromosome-specific hemiclonal analysis of locomotory activity*

Once the above genome-wide hemiclonal assay had established a genetic component to the phenotypic variation in adult locomotory activity, we set out to determine the

chromosomal distribution of this genetic variation. To achieve this goal, it was necessary to decompose each hemiclone into its two constituent components: the X and the autosomes. Using the 35 genome-wide hemiclones as a starting point, a series of additional crosses were performed wherein either the X-chromosome or the autosomes were replaced with random chromosomes obtained from the base population (electronic supplementary material, figure S1). These new 35 X- and 35 autosomal hemiclones (X-hemiclones and A-hemiclones, respectively) were then assayed for locomotory activity as described above for the genome-wide hemiclones. In total, all 35 X- and 35 A-hemiclones were assayed for activity level three times for each sex.

(f) *Statistical analyses*

Analysis of covariance (ANCOVA) was used to test for an association between adult locomotory activity and fitness for individuals. For both male and female analyses, the dependent variable was adult fitness, and the independent variables were the individual adult locomotory activity score (the covariate), replicate and their interaction. When the interaction term was non-significant ($p > 0.05$), it was removed from the final model.

The data obtained from the X-hemiclonal, A-hemiclonal and whole genome-hemiclonal assays of adult locomotory activity were used to calculate the intersexual genetic correlations for this trait. This parameter was estimated by dividing the estimated additive genetic covariance between the sexes for adult locomotory activity by the square root of the product of the estimated additive variances of adult locomotory activity within each sex. The covariance was calculated by bivariate regressions (covariance = slope/var(X)). Each regression comprised 35 bivariate data points, and each data point was the average adult locomotory activity (across the three replicate assays) of the same hemiclone expressed in males or females. The additive genetic variance among hemiclones for each sex was estimated using a random effects one-way ANOVA with the average activity level of each hemiclone in each of the three replicates being the dependent variable and the random-effect, independent variable being hemiclone (1–35). In order to calculate the 95% lower bound for our estimates of the intersexual genetic correlations, the data were then bootstrapped by sampling (with replacement) the hemiclonal data 1000 times, recalculating the additive coefficient of variation between the sexes and the additive variance within each among hemiclones, and calculating the intersexual genetic correlation for each of these samples. The resulting values were then ranked in descending order, and the 95% lower bound was estimated from 950th value on that list.

The estimated additive genetic coefficient of variation (CV_{add}; Houle 1992) for locomotory activity (based on the X alone, the autosomes alone, or the X and autosomes together) was calculated separately for males and females. To obtain each estimated CV_{add}, we divided the square root of the estimated additive genetic variation among hemiclones (calculated as described in the previous paragraph) by the sample mean of the trait. To obtain an approximate 95% lower bounds for these estimates, we used bootstrap analysis. For each of 1000 bootstraps, we sampled 35 hemiclones randomly (with replacement) and then calculated the mean and additive genetic variance among hemiclones using a random effects ANOVA (as described in the above paragraph). The 95% lower bound of the CV_{add} was estimated as the 950th value on the ordered list of bootstrapped values.

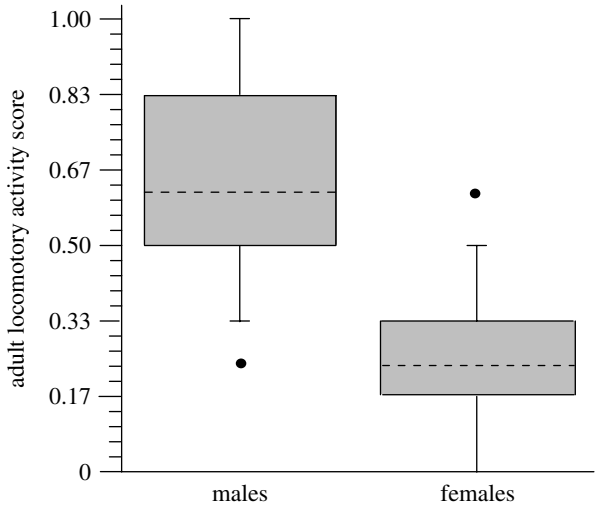

Figure 1. Activity levels (measured in the 'adult competition' phase of the life cycle as the fraction of sessions where movement was observed) for 99 individual male and female *D. melanogaster*s. Box plots show the 10th, 25th, 75th and 90th percentiles; dots show data outside that range; and dashed lines indicate the means of each group.

3. RESULTS

When we measured the activity of individual adult flies during the adult competition phase of their life cycle, we found that the mean ($\pm$s.e.) locomotory activity scores of the sexes differed, with males being nearly threefold more active (figure 1; males: 0.618 ± 0.018; females: 0.234 ± 0.022; generalized linear model with binomial error terms, $\chi^2_1 = 184.2$, $p < 0.0001$). Similarly, males were more active, on average (although to a lesser degree), in all other assays reported below (e.g. for whole-genome hemiclones (electronic supplementary material, figure S2); males: 0.479 ± 0.019; females 0.336 ± 0.013; matched-pairs t-test, $t = 9.72$, d.f. $= 34$, $p < 0.0001$).

In both males and females, individual locomotory activity was a significant predictor of adult fitness (figure 2). Females exhibited a negative association between locomotory activity and fitness, while this association in males was positive. More specifically, for males, ANCOVA analysis indicated a significant and positive slope ($p = 0.003$) with no heterogeneity of slopes among replicates ($p = 0.180$) or heterogeneity of intercepts among replicates ($p = 0.124$), so a single regression was plotted in figure 2*b*. For females, ANCOVA analysis indicated a significant and negative slope ($p < 0.0001$), with no heterogeneity of slopes among replicates ($p = 0.400$), but a significant replicate effect on average fecundity (heterogeneity among intercepts, $p < 0.0001$), so separate, parallel regression lines are shown for females in figure 2*a*.

Overall, we observed strong, positive, intersexual genetic correlations (r_{MF}) for locomotory activity between the sexes (figure 3 and electronic supplementary material, figure S3). Among nearly genome-wide hemiclones, we estimated the r_{MF} (and its bootstrapped 95% lower bound) to be 1.25 (0.69). Among hemiclones sharing only the X-chromosome, the estimate of r_{MF} (and 95% LB)

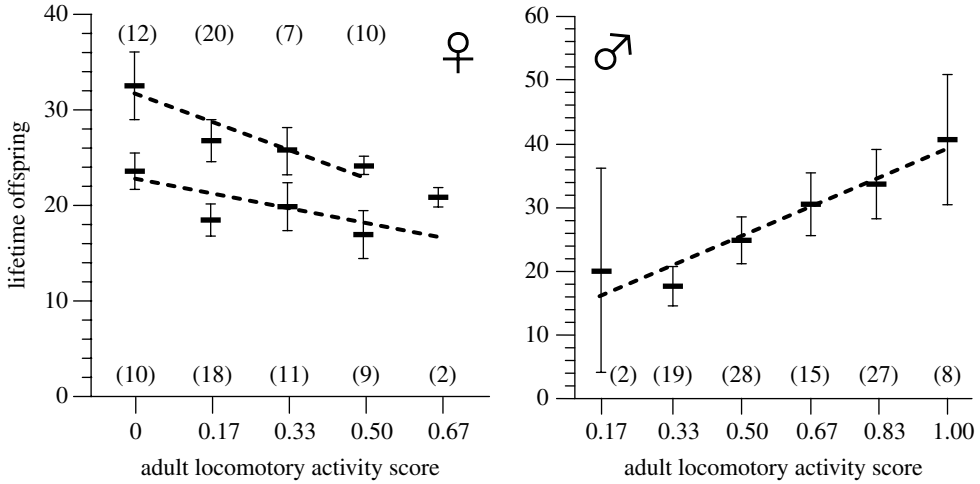

Figure 2. A plot of adult fitness (measured by mean (±s.e.) lifetime offspring production) versus locomotory activity (as measured in figure 1). Data from the two replicates are plotted separately for females, and together for males. In males, the correlation between the two variables is positive (ANCOVA: $F_{2,96}=4.797$; $p=0.0103$; $r^2=0.091$), while in females the correlation is negative ($F_{2,96}=14.467$; $p<0.0001$; $r^2=0.232$). The reduced major axis regression (McArdle 1988) though means are shown. The sample sizes are shown in parentheses.

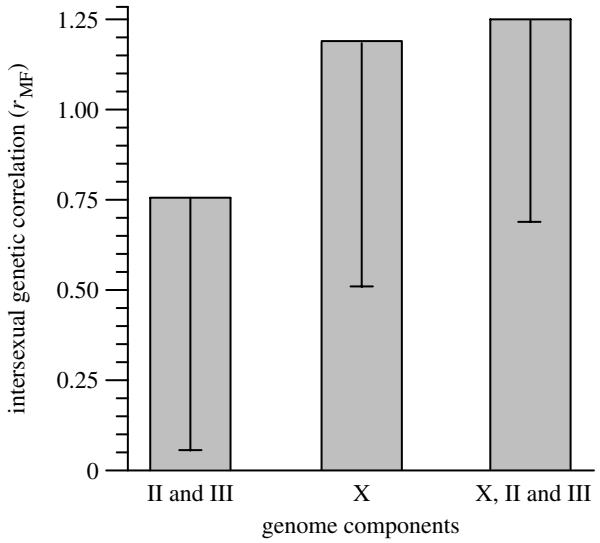

Figure 3. Estimates of intersexual genetic correlations (r_{MF}) of adult locomotory activity obtained from each of the three hemiclonal assays (major autosomes alone, X alone, X chromosome and major autosomes together, respectively). The error bars indicate the bootstrapped 95% lower bound estimate.

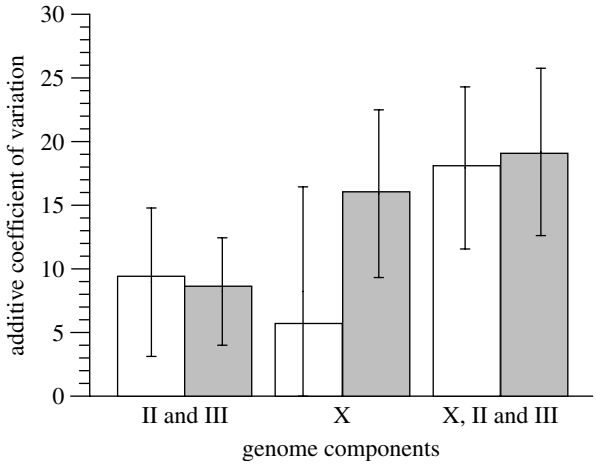

Figure 4. Estimates of additive coefficient of variation of locomotory activity in males (shaded boxes) and females (open boxes) obtained from each of the three hemiclonal assays (major autosomes alone, X alone, X chromosome and major autosomes together, respectively). The error bars indicate the 95% bootstrapped confidence limits for the estimates.

was 1.19 (0.51) and for those sharing only the autosomes, the estimated r_{MF} (and 95% LB) was 0.76 (0.07) (figure 3).

The capacity for adult locomotory activity to respond to selection, as measured by the additive coefficient of variation among hemiclones, was affected by the type of hemiclonal cross that was analysed and by the sex of the individuals that were assayed (figure 4). Among genome-wide hemiclones, the estimates of CV_{add} for males and females were 19.16 and 18.17%, respectively. When hemiclones shared only the autosomes, the CV_{add} estimates were roughly half the size of those obtained from the genome-wide assays (male $CV_{add}=8.86\%$, female $CV_{add}=9.48\%$). When hemiclones shared only the X-chromosome, the estimate of male CV_{add} was 16.15%, while that of the female CV_{add} was 5.92% (which was not significantly different from the value of 0).

4. DISCUSSION

Collectively, these results are consistent with the prediction that adult locomotory activity, a complex behavioural trait, is subject to intralocus sexual conflict. First, we found that the selection gradients on locomotory activity were in opposite directions for the two sexes (figure 2). Specifically, we observed that an increased level of activity in males was associated with a greater number of offspring sired, while female fecundity declined with increasing activity. We also observed that males were generally much more active than females during the adult competition phase of their life cycle (figure 1 and electronic supplementary material, figure S2). As discussed in §1, these results were consistent with our *a priori* predictions about the sex-specific costs and benefits associated with movement during the adult competition phase of their life cycle. Although our results on adult locomotory activity behaviour were obtained from a laboratory-reared

population of *D. melanogaster*, it is possible that similar selective pressures are also present in nature. In the wild, fruitflies congregate around decomposing plant matter where they both feed and mate. At these sites, females feed steadily on the yeasts and bacteria present, while males forgo most feeding in lieu of mate searching (reviewed in Spieth 1974). In this environment, females compete intrasexually for limited protein resources, while males must search for receptive mates, which, given the high rate of mated females found in the wild (Imhoff *et al.* 1998), may be a time-intensive task. Thus, it is conceivable that the selective pressures experienced by flies in the LH_M population are not completely unique to our laboratory environment. However, it should be noted that females in the wild may be selected for higher levels of activity than in the laboratory due to the increased need to search for feeding sites and to avoid predators. Our results of a significant negative correlation between female fitness and locomotory activity (figure 2*a*) contrast with those of Le Bourg *et al.* (1984) who found no correlation between individual activity (measured over an 8 hour period) and fecundity (measured as eggs laid over a 3-days period). Although we cannot state for certain why our results differed, we suggest that this earlier study may have been confounded by the use of a novel test chamber to score locomotory activity and/or by the use of a fitness correlate that may not have been a good predictor of lifetime adult fitness. Additionally, the method used to measure locomotory activity primarily measured spontaneous movement in isolated individuals, whereas we measured flies in a more 'reactive' environment (see Connolly 1966), and thus we were probably measuring the different facets of *D. melanogaster* locomotory behaviour.

A highly positive intersexual genetic correlation was also detected in our analyses. When observed in combination with directional selection in opposing directions between the sexes, a strong positive r_{MF} is a diagnostic element of intralocus sexual conflict. Males and females that shared identical, nearly genome-wide haplotypes showed similar relative levels of locomotory activity, indicating that this trait is under common genetic control in both sexes. This strong positive correlation between the sexes for locomotory activity was also observed in the X- and autosomal hemiclones (figure 3 and electronic supplementary material, figure S3). This is not a surprising result since the majority of genes in *Drosophila* are expressed to some extent in both sexes (e.g. Lindsley & Grell 1968; Jin *et al.* 2001; Arbeitman *et al.* 2002), and positive genetic correlations between the sexes for phenotypic traits are commonly reported in many species (see Roff 1997). Assuming that at least some of the extensive genetic variation that we have uncovered is additive, strong positive genetic correlations coupled with the presence of sexually antagonistic selection gradients within the range of the extant phenotypic variation should interfere with the adaptive evolution of sexual dimorphism (Lande 1980). Taken as a whole, we conclude that adult locomotory activity (or some important component thereof) is involved in an evolutionary tug of war between the sexes of the LH_M base population.

Although our assay is not capable of revealing how long this tug of war has been going on, we suspect that since selection pressures similar to those experienced by the LH_M population are likely to be present in nature; this case of intralocus sexual conflict had been a persistent component of this population's 'gender load' before it was brought to the laboratory. The large, positive intersexual genetic correlations observed in our assay (figure 3 and electronic supplementary material, figure S3) suggest that this intralocus conflict may be fairly recalcitrant to resolution, be it through the potential pathways of sex-limited gene expression (Rhen 2000; Rice & Chippindale 2001) or genomic imprinting (Day & Bonduriansky 2004). It is interesting to note that despite the fact that adult locomotory activity is an established, highly dimorphic trait (with male activity averaging nearly triple the level of females; figure 1), a strong intersexual genetic correlation persists. This observation indicates that a marked sexual dimorphism has evolved for adult locomotory activity without substantially reducing the intersexual genetic correlation, and hence the potential for further intralocus sexual conflict.

By assaying genome-wide hemiclones, as well as the X and autosomal components separately, we were able to compare the relative contribution of the X-chromosome and the autosomes with phenotypic variation in adult locomotory activity in both sexes. We found that a substantial amount of the additive genetic variation for this trait is autosomal in origin (figure 4). The contribution of the autosomes to variation in adult locomotory activity is supported by the recent quantitative trait loci (QTL) assays of Jordan *et al.* (2006) who uncovered four genomic regions that significantly affected locomotory reactivity in *D. melanogaster*, of which only one was located on the X-chromosome (and was only detected in males). The lack of a disproportionately large contribution of the X-chromosome to the genome-wide CV_{add} is in contrast with the earlier empirical study by Gibson *et al.* (2002), who found the X-chromosome to be a 'hot spot' for sexually antagonistic fitness variation (based on point estimates of additive variation among hemiclones).

Our observation of substantial autosomal sexually antagonistic variation suggests that the current theory for its maintenance may be incomplete. In this study, we found no significant correlation between the locomotory activity of genome-wide hemiclones and either their X- or their autosomal components in either sex (all $p > 0.05$; results not shown). In the absence of epistasis, one would have expected a positive correlation between the whole hemiclone and its constituent parts. These findings are consistent with strong epistatic interactions among genes influencing locomotory activity on the X-chromosome and the autosomes. Epistasis among QTLs influencing locomotory activity has been previously reported by Jordan *et al.* (2006) and it may be acting in our *D. melanogaster* population's genome. With the exception of Rhen (2000, 2007), sexual conflict theory has not considered the capacity of epistasis to promote polymorphism of sexually antagonistic variation. Our empirical results suggest that this may prove to be a productive area for future theoretical investigations.

This study of adult locomotory activity also revealed an imbalance of the CV_{add} on the X-chromosome, with males exhibiting, on average, a substantially higher CV_{add} than females. Although such a pattern may be due to sex-specific expression of some X-linked alleles, or to the expression of rare, recessive alleles that strongly influence activity levels, a more parsimonious explanation is that this

difference is the result of the dosage compensation of additive alleles on the X-chromosome in male *D. melanogaster*. Such dosage compensation can magnify the additive variation of the X-chromosome in males by as much as a factor of 2 (Cowley & Atchley 1988).

In summary, we describe how a complex behavioural trait, adult locomotory activity, meets the criteria of a character that mediates intralocus sexual conflict. We found opposing selection gradients between the sexes on the extant phenotypic variation in this trait. The phenotypic variation had a substantial genetic basis, and there was a large, positive genetic correlation between the sexes for adult locomotory activity. These findings add to the small but growing body of empirical evidence (reviews in Rice & Chippindale 2001, Arnqvist & Rowe 2005 and Prasad & Bedhomme 2006) that collectively suggest that resolution of intralocus sexual conflict is a slow process, leading to the persistence of a substantial gender load within species.

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