An Evolutionary Cost of Separate Genders Revealed by Male-Limited Evolution

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abstract: Theory predicts that intralocus sexual conflict can constrain the evolution of sexual dimorphism, preventing each sex from independently maximizing its fitness. To test this idea, we limited genome-wide gene expression to males in four replicate *Drosophila melanogaster* populations, removing female-specific selection. Over 25 generations, male fitness increased markedly, as sexually dimorphic traits evolved in the male direction. When male-evolved genomes were expressed in females, their fitness displayed a nearly symmetrical decrease. These results suggest that intralocus conflict strongly limits sex-specific adaptation, promoting the maintenance of genetic variation for fitness. Populations may carry a heavy genetic load as a result of selection for separate genders.

Keywords: intralocus conflict, sexual dimorphism, *Drosophila melanogaster*, experimental evolution, genetic constraints.

The two sexes often have divergent roles in reproduction and are therefore expected to evolve toward different optima for a number of the fitness related traits. This pattern of selection has the potential to create two distinct forms of conflict—interlocus and intralocus sexual conflict—with very different evolutionary consequences. Interlocus conflict has been topical because it can cause open-ended cycles of adaptation and counteradaptation between the sexes (Parker 1979; Rice and Holland 1997), potentially driving speciation (Rice 1996; Parker and Partridge 1998; Gavrilets and Hayashi 2005). Intralocus conflict arises because the sexes share the same gene pool, with expression of the same alleles having opposite effects on relative fitness in each sex. Rather than an arms race, this form of sexual conflict evolution resembles a tug-of-war over gene expression in which each sex is prevented from freely evolving toward its optimal phenotype by the genetic correlation with the other sex (Rice and Chippindale 2001). The ultimate effect of sexual conflict is a reduction in the average fitness of the sexes (and therefore that of the population), a phenomenon that we refer to as "gender load" (Rice and Chippindale 2002; Long et al. 2006). Specifically, gender load can result from direct antagonistic interactions between the sexes (i.e., the interlocus component) and the accumulation of alleles favored in one sex despite counterselection in the other sex (i.e., the intralocus component).

Although the existence of sexually antagonistic (SA) alleles producing intralocus conflict has long been implied (Fisher 1931; Lande 1980) or explicitly theorized (Rice 1984), interest in the magnitude of the conflict and the resulting gender load has been recent. A number of mechanisms, including genomic imprinting (Day and Bonduriansky 2004) and sex-limited expression of SA alleles (producing sexual dimorphism; Rice 1984), can potentially ameliorate the gender load resulting from intralocus conflict. This form of sexual conflict may therefore only be important as a precursor to, or short-lived transient state in, the evolution of sexual dimorphism. Arnqvist and Rowe (2005, p. 8) concluded that although intralocus sexual conflict is "potentially common and consequential, its evolutionary importance is debated." Here we contribute to this outstanding debate by briefly summarizing recent evidence for intralocus sexual conflict from the literature as well as describing the first results from a new experiment of our own.

Recent evidence for the importance of SA genes causing intralocus sexual conflict includes the production of low-fitness daughters by high-fitness fathers (Calsbeek and Sinervo 2004; Fedorka and Mousseau 2004; Pischedda and Chippindale, forthcoming) and the reverse (Pischedda and Chippindale, forthcoming) in animals. Furthermore, the existence and reduced fitness of heritable intersex phe-

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notypes in plants (Delph et al. 2004) is again suggestive of intralocus sexual conflict. Perhaps the most compelling evidence for the maintenance of a large pool of sexually antagonistic variation comes from the studies of Chippindale et al. (2001) and Gibson et al. (2002) in which cytogenetic cloning of haploid sets of chromosomes in *Drosophila melanogaster* was used to measure the intersexual genetic correlation for fitness. These studies reported a negative genetic correlation between the sexes for adult fitness that can only be explained by sexually antagonistic gene expression. While appropriate for diagnosing a pattern of sexual antagonism, these studies, being snapshots in time, had some limitations from a quantitative perspective. Specifically, the measurement of relative fitness will be sensitive to the distribution of genetic variation sampled, and segregating gene combinations were "frozen" in the clones. With this approach, an admixture of positive and negative genetic correlations between the sexes could belie the expression of SA alleles, producing no correlation between the sexes, as shown in Chippindale et al. (2001) for total fitness. Additionally, these studies did not identify any specific traits underlying intralocus conflict, simplifying the life history into juvenile viability and adult fertility.

Experimental evolution is a powerful approach with the potential to overcome some of the problems associated with correlative studies. Rice (1996, 1998) restricted the expression of near complete haploid genomes of *D. melanogaster* to males for 41 generations. This approach should (1) release males from any cost associated with selection for female function (i.e., the intralocus component of the gender load) and (2), because of the special constructs employed, allow male specialization for fitness with the females used to create male-limited transmission. Consistent with these predictions, Rice demonstrated that males expressing male-limited evolved genomes had increased in several key fitness-related traits (sperm offense, remating rate, mating speed). However, emphasis was placed on the second potential avenue of adaptation: direct adaptation of the males to the female population used to cultivate the lines rather than on the benefits of removing gene expression in females. Measurements of the fitness of females expressing these genomes were very limited and did not reveal the predicted reduction in female fitness, although slower development was documented (Rice 1998).

In another ingenious experiment directly aimed at testing for intralocus sexual conflict, Rice (1992) forced a pair of eye-color markers to segregate as a female-determining factor for 29 generations. Regions proximal to these new sex-determining genes were predicted to accumulate SA alleles that were female-benefiting but male-harming. While males expressing these female-determining regions were found to have reduced fitness (Rice 1992), there was no evidence for the increased fitness of females expressing the same regions (Rice and Chippindale 2001). One potential explanation for the lack of female effect, however, is that 29 generations of evolution is simply not long enough. Theory and recent empirical evidence suggest that little-standing sexually antagonistic variation should be maintained on autosomes (Rice 1984; Gibson et al. 2002). Because the experimental sex-determining factors used in this study were normally autosomal, most SA alleles would first have to arise by mutation and then increase in frequency during the 29 generations of experimental evolution. Additionally, this kind of experiment may predispose an asymmetric outcome, as SA genes with very large male-harming effects are expected to accumulate, unchecked by selection. In this case the predicted increase in female fitness may have been small and difficult to detect. In both of the aforementioned selection experiments, relatively low levels of replication (two selected and two control populations) and adaptation of the lines to different culture conditions (the base population [LH] was kept under unregulated population densities) may also have reduced the potential to detect intralocus conflict.

Thus, while these earlier laboratory evolution experiments developed an excellent strategy for assessing the evolutionary impact of intralocus sexual conflict, each had technical limitations. Ideally, one would start with a population that is at evolutionary equilibrium, manipulate the strength of selection on one sex, specifically, and then measure the evolutionary response of both sexes. This would allow selection to act on genome-wide levels of standing sexually antagonistic variation and should therefore yield an immediate evolutionary response if biologically relevant frequencies of SA alleles are segregating. For example, if a population of chromosomes was restricted to being expressed exclusively in one sex, say, males, eliminating any countervailing selection pressures on female fitness, then this population should evolve higher fitness as it moves closer to the male sex-specific optimal phenotype (as shown by Rice [1996, 1998]). We would expect to see sexually dimorphic traits become more male-like, and thus female fitness would suffer if these males were allowed to produce daughters again.

We set out to conduct such an experiment with laboratory-adapted populations of *D. melanogaster* by using sex-limited experimental evolution. The basic rationale was to conduct the above "ideal" experiment by eliminating female-specific selection on over 99% of the haploid genome of *D. melanogaster*. We created eight replicate populations of which four were subjected to male-limited (ML) evolution and the other four were matched controls (C). In the ML populations, nearly complete sets of chromosomes (cI(X), cII, and cIII, but excluding cIV [a tiny

dot chromosome comprising less than 0.5% of the genome]) were forced to cosegregate as haplotypes, being transmitted from father to son, like Y-bearing sperm. In the C populations, the genomes were not sex limited and thus submitted to both male and female selection pressures.

After 25 generations of selection, we expressed ML and C genomes as males and females and characterized intralocus sexual conflict in terms of both total Darwinian fitness and three key preadult fitness components. If intralocus conflict results from incomplete sex-limitation when traits are under selection to be sexually dimorphic, then obvious candidate traits would be those that are dimorphic and yet known to be genetically variable. We chose to investigate adult body size and preadult traits related to growth that are known to readily respond to selection and to be sexually dimorphic, with fruit fly males showing longer developmental time, lower weight at eclosion, and lower overall growth rates compared to females.

Methods

Experimental Evolution Protocol

We derived four replicate base populations from a single long-term, laboratory-adapted population (LH_M; a controlled-density derivative of the LH population, described in Chippindale and Rice 2001) and maintained them in parallel for multiple generations. After nine generations, we derived four replicate selected populations labeled $ML_{1–4}$ (male-limited genomes) and four control populations labeled $C_{1–4}$ (control genomes). Populations bearing the same numerical subscript were more closely related to each other, through both common ancestry and subsequent handling, than to other selected or control populations.

To establish the selected populations, we initially sampled ~1,000 haploid genomes from each replicate base population. This was done by crossing ~1,000 males from the replicate base population to "clone-generator" (CG) females that carried a compound X(C(1)DX, *y*, *f*), a Y chromosome (from LH_M base population) and a homozygous-viable translocation of the two major autosomes (T(2 : 3)*rdgc st in ri* p^p *bw*). The compound X (DX) consists of two X chromosomes fused together by a common centromere. The two X's are therefore inherited together and passed from mother to daughter each generation, whereas the CG female's Y is transmitted to her sons. This unusual arrangement means that sons inherit their father's X chromosome and a Y chromosome from their mother (note: the Y has no known effect on female phenotype or fitness). The translocation between chromosomes II and III means that both chromosomes must be inherited together to form a complete haploid complement of genes. These chromosomal constructs mediate the transmission of cI (X), cII, and cIII chromosomes (99.5% of the haploid genome) from father to son because (1) there is no molecular recombination in male *Drosophila melanogaster*, (2) zygotes carrying three X chromosomes, or no X, are inviable, (3) individuals carrying two X chromosomes and a Y chromosome are female, and (4) the zygotes that do not inherit the two translocated autosomes together are inviable. Thus, because of the genetic markers used and the mortality of aneuploid genotypes, all wild-type males derived from such a cross carry the unrecombined X and major autosomes of their father.

After initially capturing the haploid genomes, 1,040 males were randomly selected each generation to establish each ML population 11 days postoviposition (fig. 1). These males were distributed into 52 vials at a density of 20 males/vial. On day 12 postoviposition, the males in each vial were combined with 15 virgin CG females and allowed to interact for 18 h. Later, the females were separated from the males under light CO_2 anesthesia and allowed to oviposit for 20 h. The establishment of the control populations was identical to that of the ML populations. Thereafter, we set up 15 vials/population with 20 males and 15 females/vial. The females were isolated as virgins from about seven to eight vials over the entire eclosion profile and were combined and resampled on day 11 post oviposition. The males were harvested from the other seven to eight vials on day 11 post oviposition. The females received a limiting quantity of yeast supplement before and during their interaction with the males. The ability of the females to obtain this yeast greatly affected their fitness (Stewart et al. 2005), while for the males, interaction with the females during the last 18 h of their life determined their fitness.

Larval densities were maintained at ~150 per 8-dram vial. The larvae and the adults were maintained at 25°C ($\pm$0.5°C) temperature, 50% relative humidity, and a 12L : 12D cycle on standard cornmeal-molasses food. These were identical conditions to the ancestral populations.

Allowing Recombination

The absence of molecular recombination in male *Drosophila*, combined with the chromosomal constructs used to make the genome male-limited, completely prevents recombination in the ML populations. This could slow the rate of adaptation by reducing the rate at which beneficial alleles accumulate through genetic hitchhiking of deleterious variation or through clonal interference. However, previous work (Rice 1996) has shown that a small rate of recombination can ameliorate these problems. Thus, each

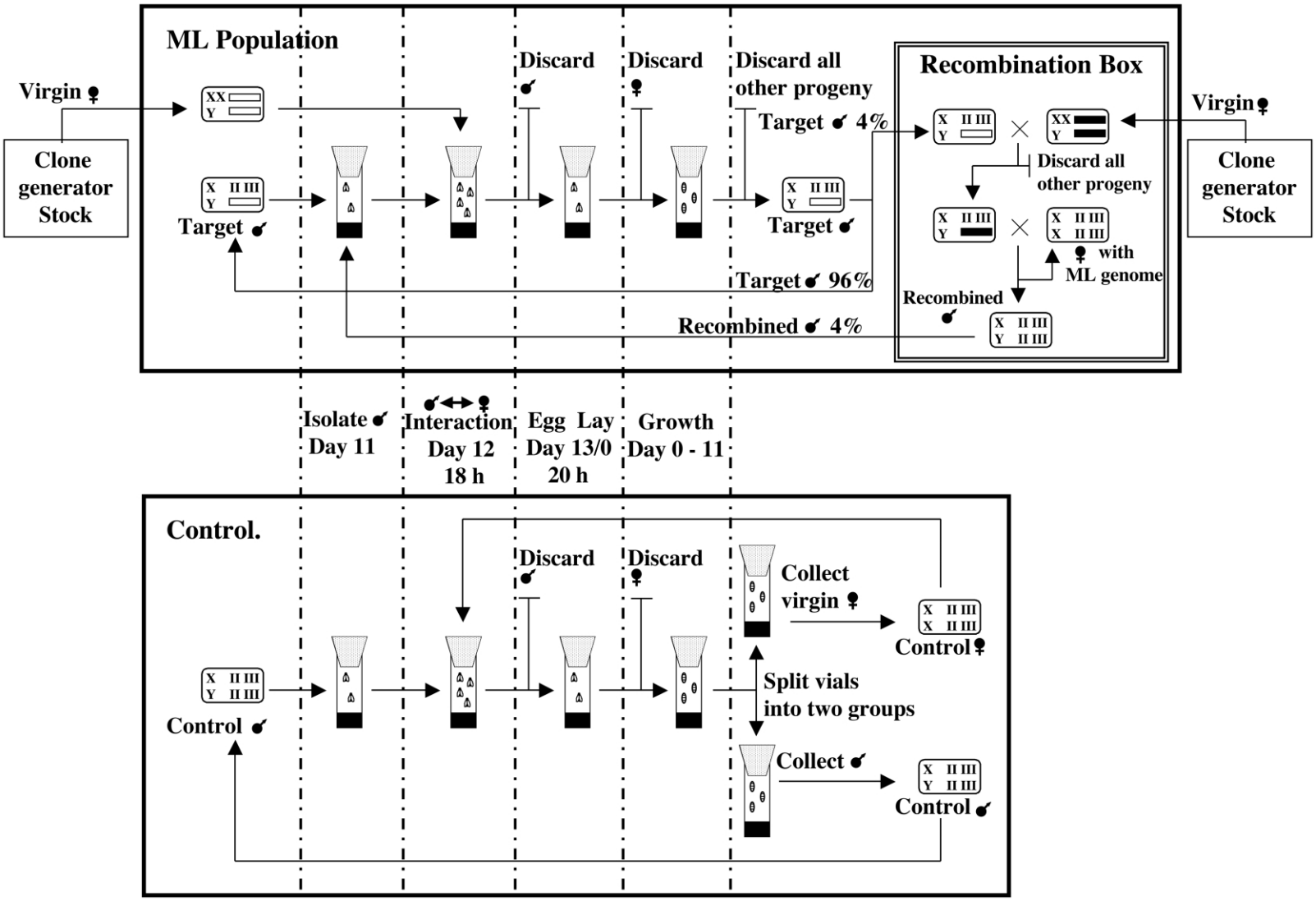

Figure 1: Schematic representation of the experimental evolution protocol. Bars represent the translocation between chromosomes 2 and 3 (see "Experimental Evolution Protocol" for details). Open bars represent translocation with recessive markers. Closed bars represent translocation with dominant markers. X, Y, II, and III represent the respective chromosomes. XX represents the compound X chromosome (see "Methods" for details).

generation, we allowed 4% of the male-limited evolving genomes to recombine by passing them through a "recombination box," where they were expressed in females for a single generation. Recombined genomes were then returned to the evolving populations as sons, where their reduced genetic linkage was expected to aid in the response to selection. The recombination box consisted of a pair of crosses designed to extract the haploid genome from ML males and recombine them by expressing them as females for a single generation. Each generation, recombined genomes constituted about 4% of the total ML genomes. The crosses were carried out as follows (fig. 1). For cross 1, from each of the ML populations, 21 males (2% of the population of haploid males) were extracted and crossed with an excess of virgin clone generator females (see above) that carried a dominant eye color marker (*bwD*). For cross 2, from the progeny of cross 1, 21 males heterozygous for the ML genome were isolated and crossed with 15 wild-type virgin females. Wild-type female progeny from cross 2 contain the ML genome and are hence responsible for the recombination of these genomes. These females were isolated as virgins and reused in cross 2 of the next cycle. The wild-type male progeny from cross 2 contain the recombined ML genome, and 21 of these males (4% of the population) were introduced back into the ML population each generation.

Overview of Experimental Assays

After 25 generations of selection, we sampled 300 haploid genomes from each of the ML and C populations and passed them through a series of crosses to express them in an appropriate genetic background. Prior theoretical and empirical work suggests that the X chromosome may be especially rich in SA alleles (Rice 1984; Gibson et al. 2002). Furthermore, theory predicts that male-benefit SA alleles on the X are expected to be recessive. These would therefore be masked when in the heterozygous condition in females if matched with unevolved chromosomes (e.g., control chromosomes). Therefore, we ensured that when the sampled genomes were expressed as females, both of the X chromosomes were derived from the same population, though from different individuals. During all of the assays reported here, both males and females remained heterozygous for the autosomal translocation used during selection to match the normal culture state of the male-limited genomes. This translocation carried only recessive markers and had been backcrossed to the same base population from which we derived our experimental lines. Although we expected it to be nearly neutral in our experiments, we were mindful of the potential for adaptation to be specific to this genetic construct.

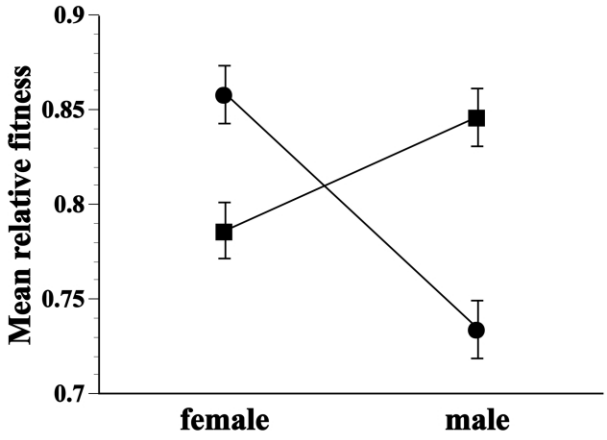

Figure 2: Mean ($\pm$SE) relative fitness (averaged across the four replicate populations) of male-limited (*squares*) and control (*circles*) genomes when expressed as males and females.

Expressing the Genomes as Males and Females

For each experimental assay testing for a relationship between the sexes, it was necessary to express ML-evolved and control chromosomes in both sexes. To accomplish this, we followed three steps. (1) Three hundred haploid genomes were captured per population by crossing males with clone generator females. (2) The progeny F1 males were then mated to females that were homozygous for a balancer X chromosome (FM7) and translocation (T (2 : 3)*rdgc st in ri p^p bw*). (3) Progeny females that were heterozygous for balancer X but homozygous for the translocation were again crossed to the F1 males. The progeny of this cross yielded males and females expressing the genome of interest.

Female Fitness Assay

Females were isolated as virgins and housed in groups of 10 along with five competitor females from a replica of the base stock (LH) homozygous for the relatively benign recessive scarlet eye marker (called LH_{st}) and were provided with 10 mg of yeast/vial. On day 12 post egg lay, females were combined with 20 males from LH_{st} for 18 h after which they were separated from the males and allowed to oviposit for 20 h. The progeny eclosing from these vials were counted 12 days later. Fifteen such vials were set up per population.

Male Fitness Assay

Males were harvested 11 days post oviposition. Ten males from ML (or C) populations were combined with 10 males

from LH_{st} population. Fifteen such vials were set up per population. On day 12 post egg lay, males were combined with 15 virgin clone-generator females and allowed to interact for 18 h after which the females were separated from the males and allowed to oviposit for 18 h. The progeny from the two types of males can be distinguished because of their eye color. Twelve days later, a fraction of the progeny sired by each type of male within each vial was scored.

Development Time, Dry Weight, and Growth Rate Assay

The assay began with the cross described in step (3) of the section titled "Expressing the Genomes as Males and Females." Twenty vials were established per population, and the larval density was regulated at ~150/vial. Development time checks were done three times a day, and at each time point, the number of eclosing flies of each sex that expressed the genome of interest was noted. The flies were then immediately frozen. The checks were done until no more flies eclosed from the vials. The frozen flies from each vial were grouped by sex, dried for 36 h at 70°C, and weighed. The mean growth rate was calculated as (mean dry weight)/(mean development time) for each sex and vial combination.

Statistical Analyses

In the male and female competitive fitness assay, we calculated relative fitness by setting the value of the vial with highest fitness within each sex × replicate combination to 1 and scaling the fitness of all other vials relative to the highest fitness vial. Population means were the units of analyses. All the data were subjected to two-way ANOVA treating selection and sex as the fixed factors. All analyses were implemented using JMP statistical software (SAS Institute 2002). In "Results," values of developmental traits are presented as means ± SE.

Results

Males expressing the ML-evolved genomes (ML males) had 15% higher fitness than males expressing control genomes (C males), while females expressing ML genomes (ML females) had 10% lower fitness than females expressing control genomes (fig. 2), leading to a strong selection-by-sex interaction (table 1). Separate two-sample *t*-tests on raw fitness values indicated that the differences in fitness were significant in both males ($t = 4.187$, df = 6, $P = .006$) and females ($t = -4.387$, df = 6, $P = .005$).

Flies expressing the ML genomes had a significantly longer development time (male = 229.8 ± 0.54 h; female = 227.0 ± 0.57 h) than flies expressing the C genomes (males = 225.9 ± 0.59 h; females = 224.8 ± 0.61 h). The ML populations were also significantly lighter at eclosion (males = 212.5 ± 1.43 μg; females = 267.4 ± 1.82 μg) than the C populations (males = 217.9 ± 1.25 μg; females = 275.9 ± 1.45 μg). These differences resulted in substantially reduced preadult growth rates in ML populations (fig. 3; table 1). Therefore, compared to the C populations, both males and females experimentally expressing ML genomes displayed phenotypes that were shifted toward the male end of the sexual dimorphism continuum.

Discussion

If sharing the genome with females imposes a load on male fitness, restricting gene expression to males should lead to an increase in male fitness at the expense of female fitness. Here we confirm the male half of this result, seen earlier in the studies of Rice (1996, 1998), by showing a rapid increase in male fitness. Since such a pattern could also result from the specialization of males on "target" females that are incapable of counterevolving to male adaptations, finding reduced female fitness from the expression of masculinized genomes would constitute critical evidence for the involvement of intralocus conflict. Rice's work on the female half of the intralocus sexual conflict problem was experimentally underpowered and produced equivocal results for fitness. Using a larger selection experiment and more extensive assays of the evolved populations, we demonstrate a strong cost to females resulting from male-limited evolution. We consider three possible alternative explanations for the observed pattern of differentiation in fitness and developmental traits: (1) differential genetic drift and inbreeding, (2) female-specific

Table 1: Summary of results from two-way ANOVA treating selection and sex as fixed factors crossed among themselves

		Relative fitness		Development time		Dry weight		Growth rate	
Effect	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Selection	1	1.027	.3309	10.0341	.0081	10.8601	.0064	34.2283	.0001
Sex	1	2.5771	.1344	4.0087	.0684	743.7235	.0001	1161.621	.0001
Selection × sex	1	21.2473	.0006	.7776	.3952	.564	.4671	.4411	.5192

mutation accumulation, and (3) adaptation to the genetic constructs employed before concluding that intralocus sexual conflict is the most likely explanation for the evolutionary patterns observed.

The first alternative explanation, genetic drift and inbreeding, is unlikely because the number of haploid genomes transmitted each generation was identical in the ML and C populations, the populations were fairly large, and the response occurred consistently in all four replicated experimental versus control comparisons. In this context, it is interesting to note that we found no significant effect of selection on population fitness (i.e., the average fitness of ML and C populations averaged over the sexes was similar) because gains in male fitness were approximately balanced by losses in female fitness.

The second alternative explanation, female-specific mutation accumulation, posits that disuse and the subsequent accumulation of mutations in genes with female-limited expression accounts for the reduced fitness of females. This stochastic process is statistically unlikely because (1) all four replicate populations traced approximately the same pathway, (2) male fitness improved dramatically with female loss of function, and (3) if these mutations were mainly recessive, they would not be expressed when all experiments were conducted with animals in the heterozygous state.

The third alternative explanation, adaptation to the genetic constructs employed, is also unlikely given that males and females carrying the same genetic construct showed opposite patterns of fitness change. Moreover, experiments conducted several generations before the work reported here failed to detect significant adaptation to the translocation and other genetic constructs that could explain improvements in the fitness of the selected line males. The controlled, identical maintenance of ML and C populations with respect to growth conditions (medium, demography, larval density, etc.) allows us to rule out obvious inadvertent direct selection in these populations.

Given the above considerations, we conclude that the differentiation of developmental traits and changes in fitness in the ML line males and females is most likely to be an adaptive response to male-limited selection. While we continue to investigate all of the potential avenues of evolutionary change, we suggest that the most tenable explanation for our finding of reduced female fitness during male-limited evolutionary gains is a fundamental conflict over gene function in the two sexes, that is, intralocus sexual conflict.

The discrepancy between the results of this study and those of Rice (1998) with respect to female fitness could be due to the handling of the X chromosome or simply to differences in experimental power. Rice (1998) combined X chromosomes from different male-limited evolved

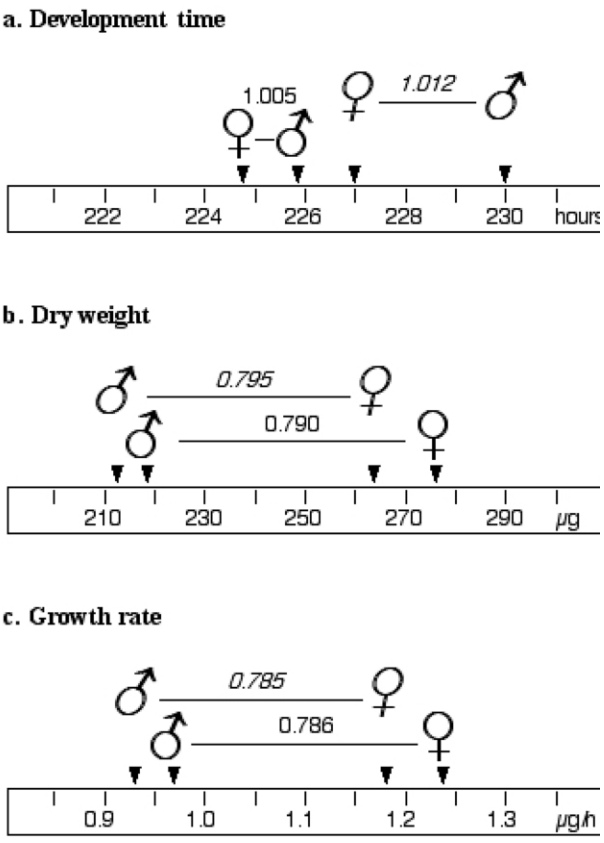

Figure 3: Direct and correlated responses to male-limited (ML) selection in (*a*) development time, (*b*) dry body mass, and (*c*) net growth rate. In each character, trait values moved in the male direction in ML lines (*italic* and *upper symbols*) compared to controls (*roman* and *lower symbols*), leading to a correlated change in females in the same direction. No statistically significant change in the level of sexual dimorphism (M/F; shown by the number above the line connecting the female and male symbols) was detected. Values and errors are reported in the text.

populations to measure female fitness. In the present study, X chromosomes from the same male-limited population were combined to measure female fitness. Given that different male-benefit SA alleles may have accumulated in different replicate populations and that these alleles are predicted to be recessive (Rice 1984), our approach probably had greater power to detect X-linked effects. The comparison may be moot, however, because Rice's fitness assay was conducted on a very small scale (22 females/treatment; 44 females total) and, because he combined his two selected lines and controls, without population replication.

This study also suggests that effects of intralocus sexual conflict are manifested during the preadult stages in *Drosophila melanogaster*, as indicated by our results on preadult growth rates. This is in apparent contradiction to the

study of Chippindale et al. (2001), who found no evidence of intralocus conflict in the preadult stages. However, it should be pointed out that Chippindale et al. (2001) assayed preadult viability as the singular measure of juvenile fitness. While viability is a potentially strong component of overall fitness, it fails to capture a wide range of fitness consequences stemming from development. For example, an individual emerging late, small, or in poor condition may suffer reduced fitness after eclosion. Therefore, the existence of intralocus conflict over growth and development as documented by our study is expected (see below) and does not contradict the results of previous studies except insofar as it points out the inadequacy of characterizing juvenile fitness as survival alone.

Female-biased sexual size dimorphism as seen in *D. melanogaster* is often assumed to be related to fecundity selection (Reeve and Fairbairn 1999). In *D. melanogaster*, female fitness is positively correlated with size (Mueller 1985; Zwaan et al. 1995; Houle and Rowe 2003), which in turn is dependent on larval resource acquisition through rapid growth (Nunney 1996; Chippindale et al. 1997; Prasad et al. 2000). On the other hand, male fitness is most strongly correlated with mating success, which has no simple relationship with body size (Joshi et al. 1999; Da Silva and Valente 2001; Bangham et al. 2002). Perhaps this is not surprising, given that males are small relative to females in this species and cannot easily assert physical dominance. Rather, male fitness is likely to depend on a combination of mobility, persistence, territory defense, and attractiveness to females in sexual selection. If body size (or growth rate) trades off with agility or physical attractiveness (e.g., through reduced developmental stability), then selection on male mating success will dictate relatively diminutive size (Chippindale et al. 2003). Here, in response to male-limited evolution, developmental traits all moved toward the male end of the dimorphic spectrum, suggesting that males in normal populations are faster developing, faster growing, and larger than their sex-specific evolutionary optimum because of selection acting on females.

Patterns of heritable variation consistent with intralocus sexual conflict have recently been reported in a variety of organisms beyond *Drosophila*. For example, genotype-by-sex interactions for characters related to fitness have been found in snakes (Forsman 1995), field crickets (Fedorka and Mousseau 2004), lizards (Calsbeek and Sinervo 2004), dioecious plants (Delph et al. 2004), and perhaps even in the maintenance of male homosexuality in humans (Camperio-Ciani et al. 2004). The growing list of examples underscores the fact that this form of sexually antagonistic variation may not only be important in theory but also common and detectable in a wide range of sexually reproducing organisms.

By showing that female fitness declines rapidly under selection for male-specific fitness, we provide the clearest demonstration to date of unresolved conflict over the evolution of sexual dimorphism. This form of conflict appears to be a powerful force promoting the maintenance of genetic variation in characters related to fitness. Our *Drosophila* populations, kept in simplified fitness microcosms for many generations before male-limited evolution, responded immediately to release from counterselection in females with average gains of 0.6%/generation in total fitness. We suggest that chronic disruptive selection acting on adult size in the two sexes has led to the maintenance of a spectrum of larval growth patterns and the failure of either sex to achieve its optimal size, even under the defined and uniform evolutionary conditions of laboratory culture. Moreover, because sex-limited evolution is predicted to create genomes enriched for SA genes and thus define the unresolved conflicts within the genome, these lines are near-ideal material for further investigations into other life-history and reproductive traits that mediate intralocus conflict. The utility of *Drosophila* in genomic research should facilitate characterization of underlying genetic mechanisms, thus opening the way to an integrated understanding of the evolution of separate genders.

Our view of the sexes is, increasingly, that an individual's gender is merely a point on a masculine-feminine continuum maintained by a combination of intralocus sexual conflict and its genetical remedies (e.g., sex-limited gene-expression, genomic imprinting, hormonal regulation). This perspective helps to explain the common occurrence of intersex phenotypes in species that are sexually dimorphic, on average. Our data and the growing body of evidence we review suggest that substantial levels of sexual conflict remain unresolved in the genome of many species. The "gender load" carried by each sex represents a cost to sex, beyond the well-known twofold cost of males, which will be felt to varying degrees by sexual species in ecological competition. Intralocus sexual conflict may therefore be a powerful and pernicious source of genetic variation in microevolution and an important force in macroevolution.

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