

# Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*

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Because of their distinctive roles in reproduction, females and males are selected toward different optimal phenotypes. Ontogenetic conflict between the sexes arises when homologous traits are selected in different directions. The evolution of sexual dimorphism by sex-limited gene expression alleviates this problem. However, because the majority of genes are not sex-limited, the potential for substantial conflict may remain. Here we assess the degree of ontogenetic conflict in the fruit-fly, *Drosophila melanogaster*, by cloning 40 haploid genomes and measuring their Darwinian fitness in both sexes. The intersexual genetic correlations for juvenile viability, adult reproductive success, and total fitness were used to gauge potential conflict during development. First, as juveniles, where the fitness objectives of the two sexes appear to be similar, survival was strongly positively correlated across sexes. Second, after adult maturation, where gender roles diverge, a significant negative correlation for reproductive success was found. Finally, because of counterbalancing correlations in the juvenile and adult components, no intersexual correlation for total fitness was found. Highly significant genotype-by-gender interaction variance was measured for both adult and total fitness. These results demonstrate strong intersexual discord during development because of the expression of sexually antagonistic variation.

Selection may operate in different directions on each gender, frequently leading to the evolution of sex-specific gene expression and sexual dimorphism (e.g., dimorphism for body size). Yet most genes in the genome are expressed in both sexes, setting the stage for intersexual conflict during ontogeny through the effects of sexually antagonistic alleles (i.e., those beneficial to one sex but harmful to the other; ref. 1). The extent of this ontogenetic conflict can be explored by treating the sexes as distinct environments interrelated by their genetic correlation (2). Genes that are identically expressed in both sexes will produce a strong, positive, intersexual genetic correlation, whereas gender-limited gene expression will reduce the correlation toward a value of zero (3).

When the quantitative trait measured is Darwinian fitness, the intersexual genetic correlation can be used to assess the degree of ontogenetic conflict between the sexes. Alleles with sexually antagonistic effects will contribute to a negative intersexual correlation for fitness. Measured over a representative portion of the genome, the intersexual genetic correlation for fitness provides a weighted average of genetic variation expressed concordantly, independently, and discordantly between the sexes. In the few cases where it has been calculated, the intersexual genetic correlation for morphological traits has been high and positive (i.e.,  $>0.8$ ; refs. 3 and 4). It is notable that, for several traits ostensibly linked to reproductive success, lower correlations have been found (5–7). Trade-offs involving floral traits in dioecious plants (e.g., refs. 5 and 8) present a particularly attractive system for investigating ontogenetic conflict between the sexes. However, to date, the intersexual genetic correlation for total fitness itself has not been reported.

To estimate the degree to which ontogenetic conflict is manifest in a model organism, we measured the intersexual

genetic correlation for fitness across most of the genome of *Drosophila melanogaster*. Haploid genomes, functionally equivalent to nearly complete sperm genotypes, were randomly sampled from a base population, clonally amplified, and used to fertilize many different eggs from the same base population. By manipulating the maternal karyotype, we were able to produce sons and daughters expressing the same haploid genome in its normal outbred state. Fitness was measured over a complete generation, allowing measurement of total (or net) fitness and its two sequential components: egg-to-adult viability (juvenile fitness) and adult reproductive success (adult fitness).

## Materials and Methods

**Sampling and Cytogenetic Cloning of Genomes.** A total of 40 haploid genomes were sampled from the base population ( $LH_M$ ), cloned, expressed in both males and females, and then fitness was compared between the sexes. Each of the sampled haploid genomes (designated “target genomes”) constituted 99% of a full set of genes and contained chromosomes I (X), II, and III, but excluded the minute chromosome IV. The  $LH_M$  population was large (adult  $n > 1,600$  per generation) and had adapted to the laboratory for over 200 generations.

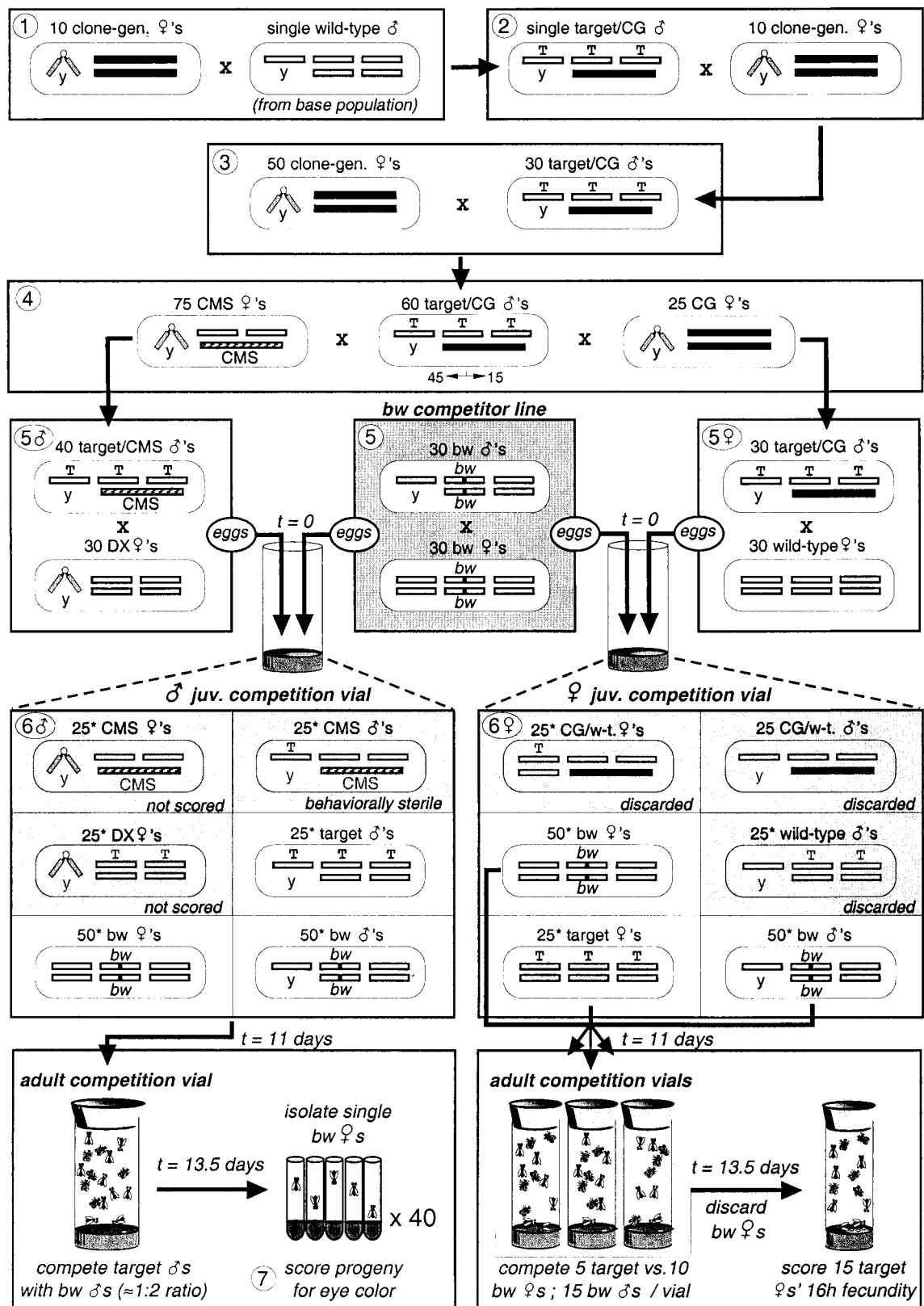
The cytogenetic cloning of genomic haplotypes is based on two factors. First, male *D. melanogaster* do not have intrachromosomal recombination, so paternal chromosomes are transmitted intact to progeny. Second, specially constructed clone-generator females (9) were used to make an entire genomic haplotype cosegregate from father to son each generation. The genotype of clone-generator females included a compound X chromosome [C(1)DX, y, f], a Y chromosome (from the  $LH_M$  base population), and a homozygous-viable translocation of the two major autosomes [T(2;3) *rdgC st in ri p<sup>P</sup> bw*; depicted in Fig. 1 by elongated rectangles spanning chromosomal positions 2–3]. The Y and compound X chromosome cause the X chromosome to be transmitted from father to son. The autosomal translocation causes the two major autosomes to cosegregate each generation. A haploid genome was captured by mating a single male to clone-generator females (Fig. 1, step 1) and then extracting one set of chromosomes by repeating this cross by using a single son (Fig. 1, step 2). By subsequently retaining only those sons that received both the wild-type X and autosomes from their father, each genomic haplotype was cloned and amplified into 60 identical copies (Fig. 1, step 3). After the target genomes were amplified, their gender-specific juvenile, adult, and total fitness was measured by placing them into many different random genetic backgrounds from the base population (see below).

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**Fig. 1.** Protocol for sampling and cloning a single haploid genome (denoted T for “target”; steps 1–4), returning it to its original (wild-type) genetic background (step 5), and then measuring its fitness in males and females over a full generation (steps 5–7). Numbered steps indicate generations. Chromosomes I (X), II, and III are depicted as rectangles arranged *Left to Right*, respectively. Excepting the compound X (depicted as a chevron symbol) and autosomal translocations (shown as elongated rectangles spanning chromosomal positions 2 and 3), all chromosomes were derived from the base population; wild-type chromosomes are shown as open rectangles and marked ones with fill or crosshatching. For clarity, only offspring that were viable and used in a subsequent cross are shown in steps 1–5. By using this protocol, 40 haploid genomes were cloned and measured for juvenile, adult, and total fitness. Steps 4–7 were independently repeated three times for each genome. Asterisks indicate average numbers of viable eggs used to begin the fitness assay. See *Materials and Methods* for additional details.

Fitness variation among the target haploid genomes is equivalent to fitness variation among the nuclear genomes of sperm or eggs in a random-mating population.

**Fitness Assay.** Both female and male fitness assays (Fig. 1, steps 5–7) were designed to closely replicate the culture conditions of the base population. To place a cloned target genome back into the original genetic background of the base population, two steps were taken. First, to begin the assay generation, males carrying a target genome were crossed to females whose chromosomes [except the C(1)DX in the male assay] were randomly derived from the base population (Fig. 1, step 5♂ and 5♀). These females were allowed to oviposit onto small plates of standard food medium over a period of 12–14 h. Eggs were counted by using fine brushes lubricated with an isotonic saline solution and then transferred on a small layer of food to the larval competition vial. Because some eggs received inviable karyotypes, the number of eggs counted into each fitness assay vial was adjusted upward to produce an expected number of 25 zygotes expressing the target genome in the appropriate sex. Second, by using the same techniques described above, eggs from a vigorous competitor line [LH<sub>M</sub>-*bw*; a replica of the base population carrying a brown-eyed (*bw*) recessive marker that had been introgressed through 12–13 back-cross generations into the LH<sub>M</sub> background] were counted into the fitness assay vial to obtain a ratio of 2:1, competitor eggs to eggs carrying the target genomes (i.e., 50 brown-eye/25 wild type; Fig. 1, step 5). The overall density of 200 larvae per 28.5 × 95-mm shell vial and all other general features of the fitness assays (temperature, light, timing of handling events or counts) were standard rearing conditions for the base and competitor populations. The protocol (Fig. 1, steps 3–7) was independently repeated three times for each genome. Thus each genome's fitness was assayed in an average of 75 different genetic backgrounds.

**Conditional Male Sterility (CMS) Construct.** In the cross shown in step 5♂ of Fig. 1, two types of males were produced: (i) those expressing the complete target genome in a heterozygous background randomly derived from the base population, and (ii) those expressing only the target X chromosome along with a translocation of chromosomes II and III. To remove the latter from sexual competition, a special translocation that produced CMS was used, replacing the original clone-generator translocation (Fig. 1, step 4). The CMS translocation [T(2;3) *Ubx<sup>Cbx-Hm</sup> Dr<sup>1</sup> Pr<sup>1</sup>*] carries three dominant mutations producing a phenotype with virtually no eyes, no wings, and no bristles. As adults, males expressing the CMS phenotype are crippled to the point of behavioral sterility in competition with normal males but are fully fertile in the absence of mating competition. Because the males expressing the CMS translocation were competitively debilitated (here gaining <0.8% of matings), the CMS construct enabled us to restrict competition for mates to only two functional male types (wild-type target males and marked competitor males; Fig. 1, step 6) without invasive manipulation of the flies during the fitness assay.

**Fitness Components.** Juvenile fitness was measured as the proportion of target adults, censused 11 days from oviposition, relative to the expected number of target eggs (i.e., 25) founding each juvenile competition vial. Adult fitness of males was measured by the per capita reproductive success of target adults at the end of the 14-day population cycle. In the male experiment, 40 *bw/bw* females were sampled from each adult competition vial after 13.5 days (of the 14-day cycle) and individually housed in small culture tubes overnight to lay eggs. The recessive eye-color marker of these females allowed us to score paternity (Fig. 1, step 6♂); brown-eyed offspring were sired by competitor males and red-eyed offspring by target males.

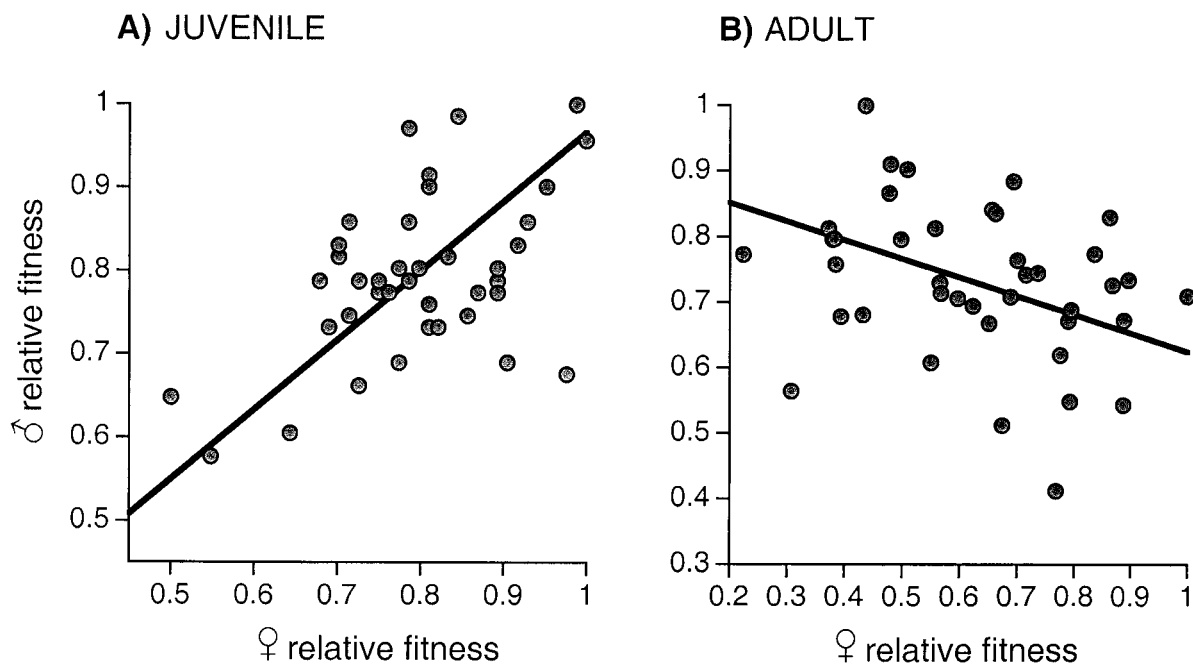
For each of the 4,495 female broods assayed, paternity was assigned by a binning procedure involving five categories: all target genome (100% red-eyed), majority target (more than 2/3 red eyed), tie (approximately equal numbers of wild-type and brown-eyed), majority *bw*-competitor (more than 2/3 brown eyed), and all *bw* competitor (100% brown-eyed). Broods that were difficult to resolve within culture tubes were anesthetized and counted. “All” and “majority” categories were then pooled for target or competitor males, respectively, creating a dichotomous score on the basis of which type of male sired most (67% or more) of the offspring. Closely mixed broods (34–66% of either type of sire) were considered uninformative ties and excluded from the data set. Because of strong sperm precedence, ties comprised only 3.9% of females scored. This binning procedure has two important advantages over counting exactly each brood: first, it increased efficiency and hence substantially increased the number of females that could be surveyed for paternity. Second, it does not confound the viability of the target male with the viability of his progeny, and therefore juvenile survivorship is not counted twice in an experiment designed to measure fitness over one complete generation. Also, because the majority (69%) of broods fell into the “all target” or “all brown-eyed competitor” categories, and because the “majority” categories were typically highly biased to one or the other eye color, the binning procedure will closely match a distribution based on individual counts of each female's brood. Male reproductive success was calculated as the deviation (observed – expected) of percent females mated to target males minus the percent target males in the pool of available fertile males (target and *bw* competitor).

Adult fitness of females was measured by the per capita fecundity at the end of the 14-day population cycle, the period in the base population's propagation protocol when eggs were collected to produce each subsequent generation. In the base population, female fecundity was limited by the amount of live yeast added to the culture medium before the beginning of each generation (days 11–13.5 of the 14-day cycle). To create these conditions during the female fitness assay, target females competed for 2.5 days with *bw*-competitor females at a ratio of 1:2 (5 target/10 *bw* competitor) for 16 mg of live yeast (the base medium, containing killed yeast, was not limiting). Competitor females were then discarded, and per capita egg production was measured between days 13.5 and 14.0 in vials containing 15 target females and 15 males. Apart from brief (<60 s) anesthesia to sort females on day 13.5, this protocol matched the environment to which the flies had adapted.

**Total Fitness.** Total fitness combines juvenile survival and adult reproductive success. In males, total fitness was measured as a deviation (observed – expected) the proportion *bw*-competitor females fertilized by target males minus 0.333 (i.e., the proportion of original target vs. competitor males beginning the assay as eggs). Female total fitness was per capita egg production multiplied by egg-to-adult survival.

## Results and Discussion

Juvenile fitness, as measured by egg-to-adult survival under competitive conditions, was strongly positively correlated between the sexes (Fig 2A). The intersexual symmetry of genotypic effects is illustrated by the close conformation of the slope to a value of +1 when juvenile fitness of one sex is regressed on the other [Fig. 2A; principal axis (10) slope = 0.83; intersexual genetic correlation = 0.49; *P* = 0.001]. Juvenile survival was high in both sexes, with mean viability of 75.4 and 89.1% for males and females, respectively. This measure of survival included a development time component, because adult flies were moved from the juvenile competition vial to the adult competition vial after 11 days, and any flies that had not yet eclosed were



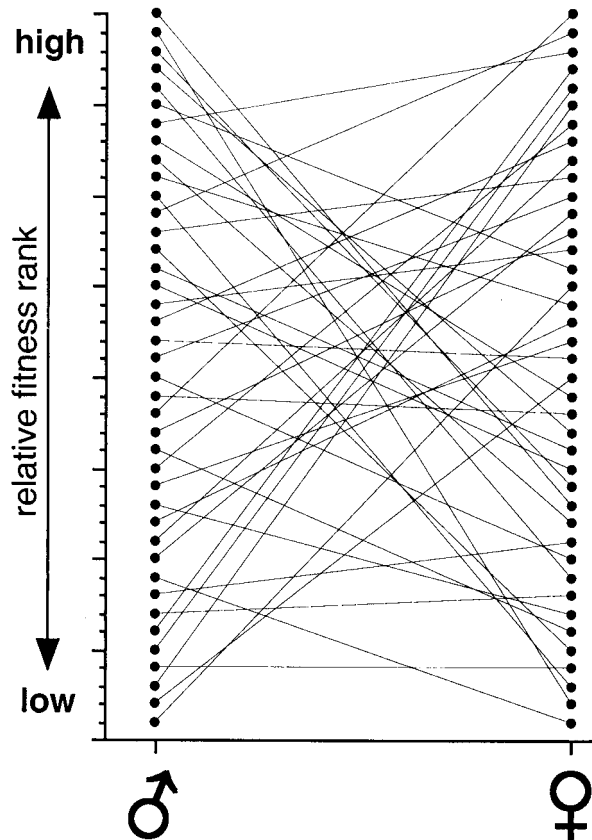
**Fig. 2.** Intersexual regression for juvenile and adult fitness components: (A) egg-to-adult viability and (B) adult reproductive success (male fertilization success or female fecundity), calculated as the principal axis regression. Data are expressed as relative fitness values by dividing, in each sex separately, by the value of the fittest genotype. The null expectation is that each genome will have identical effects on fitness in both sexes, therefore creating a strong positive association. The intersexual genetic correlations were +0.49 and  $-0.30$ , for juvenile and adult fitness, respectively.

considered dead. The slower development time of males compared with females may have contributed to their lower average survival.

Developmental conflict between the sexes was evident only after sexual maturation. Reproductive success of adults was significantly negatively correlated (Fig. 2B; principal axis slope =  $-0.28$ ; intersexual genetic correlation =  $-0.30$ ;  $P = 0.03$ ), demonstrating that, on average, genomes that promoted greater egg production in females reduced fertilization success in males, as well as the reverse. The observed concordance between the sexes for the juvenile component of fitness probably reflects a lower opportunity for sexually antagonistic selection during the larval stages, where sexual dimorphism is less evident and gender roles appear to be more similar. In contrast, the discordance between the sexes observed for the adult component of fitness indicates substantial ontogenetic tension accompanying the differentiation of genders at maturity.

Total fitness combines the sequential effects of juvenile survival and adult reproduction. The genetic correlation between the sexes for total fitness did not differ significantly from zero (principal axis slope =  $-0.14$ ; intersexual genetic correlation =  $-0.16$ ,  $P = 0.37$ ), indicating a net canceling between the positive correlation for juvenile survival and the negative correlation for adult reproduction. This net zero correlation between the sexes occurs despite the fact that genotype was a powerful predictor of total fitness when each sex is analyzed separately (ANOVA,  $r^2_{\delta} = 0.45$ ,  $P = 0.04$ ;  $r^2_{\varnothing} = 0.69$ ,  $P < 0.0001$ ). ANOVA of the combined data for the total fitness of males and females demonstrated a strong interaction between genome and gender ( $P < 0.001$ ). This genetic interaction was associated with many reversals in relative fitness between genders: the genomes that produced high fitness in males frequently produced low fitness in females and *vice versa* (Fig. 3).

Our findings reveal the existence of substantial ontogenetic conflict between the sexes in *Drosophila*. Because many genes are expressed throughout the entire life cycle, much of the strong



**Fig. 3.** Interaction plot of the rank of total fitness of a genome in males compared with females. The strong genome  $\times$  gender interaction for total fitness (ANOVA,  $P < 0.001$ ; see text) is indicated by the reversal in fitness rank of many genomes when tested in males vs. females.



positive intersexual correlation observed for juvenile fitness would be expected to carry over to the adult stage as well. However, for the adult component of fitness, the correlation was reversed, not merely diminished, indicating extensive genome-wide polymorphism for sexually antagonistic alleles. The present data cannot resolve whether this pattern results from many loci with alleles of small effect or from a small number of loci with strong-effect alleles. But, given the potential for divergent selection between the sexes on a multitude of traits, our view is that many loci are likely to be involved.

If these results from *Drosophila* can be extended to other species, then several important conclusions result. First, the expression of male-benefit sexually antagonistic alleles in females (“masculinizing”) and corresponding female-benefit alleles in males (“feminizing”) blurs the phenotypic dichotomy between female and male. Substantial sexually antagonistic fitness variation, as demonstrated here, will cause the average phenotype of each sex to deviate from its gender-specific optimum, so the adaptive evolution of each sex is impeded by counterselection in the other sex. Gender-specific selection on

loci expressed in both sexes may therefore be an important factor contributing to the maintenance of high levels of genetic variance for fitness within each sex (e.g., see ref. 1). Second, our finding of substantial ontogenetic conflict between the sexes indicates a strong potential advantage to asexual reproduction, because all-female (clonal) lineages will be released from counterselection in males, allowing a closer approach to the optimal phenotype for female reproduction. Third, models of sexual selection must accommodate the fact that “good genes” may be highly gender-specific. As a consequence, our data suggest that females mating with the fittest males would be expected to produce, at best, only average-fitness daughters. Last, our experiments indicate that intersexual developmental conflict may have broad application to the more general phenomenon of intragenomic conflict (e.g., refs. 11 and 12).

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