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Simpler mode of inheritance of transcriptional variation in male *Drosophila melanogaster*

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Sexual selection drives faster evolution in males. The X chromosome is potentially an important target for sexual selection, because hemizygosity in males permits accumulation of alleles, causing tradeoffs in fitness between sexes. Hemizygosity of the X could cause fundamentally different modes of inheritance between the sexes, with more additive variation in males and more nonadditive variation in females. Indeed, we find that genetic variation for the transcriptome is primarily additive in males but nonadditive in females. As expected, these differences are more pronounced on the X chromosome than the autosomes, but autosomal loci are also affected, possibly because of X-linked transcription factors. These differences may be of evolutionary significance because additive variation responds quickly to selection, whereas nonadditive genetic variation does not. Thus, hemizygosity of the X may underlie much of the faster male evolution of the transcriptome and potentially other phenotypes. Consistent with this prediction, genes that are additive in males and nonadditive in females are overrepresented among genes responding to selection for increased mating speed.

microarray | sexual antagonism | sexual conflict | sexual selection | transcription

Sex differences have been a focus of discussion in evolutionary biology ever since Darwin's *Origin of Species* (1). As Darwin pointed out, sexual selection likely drives much of sexual dimorphism. The mechanism underlying phenotypic sex differences, however, has remained a mystery. Heterogametic males and females have the same complement of genes, with the exception of a handful located on the Y chromosome; therefore, differences between the sexes must arise from differential function of the same genes. The most appealing mechanism for differential function is differential expression, although certainly differential function mediated by mechanisms at the posttranscriptional level (e.g., translational or posttranslational differences) are also possible. In adult flies, approximately half of all genes are differentially expressed in males and females (2). As many as 25% of genes may experience sex-specific splicing (3). Gene expression may mediate sexual dimorphism either by limiting expression to one sex only (sex-limited expression), or by changing expression for the same genes between sexes (differential expression). In addition, the mode of inheritance for gene expression could be sex-specific.

How evolution shapes gene expression depends on the mode of inheritance of standing transcriptome variation, particularly with respect to how much of this variation is additive, or heritable. Additive variation responds to selection more quickly than nonadditive variation, because the effect of additive alleles is independent of other alleles at both the locus in question and other loci in the genome. Genetic variation for expression is frequently nonadditive, involving both intra- and interlocus interactions (4–6). It remains unclear how much genetic variation for gene expression is additive and thus available to selection, and whether the mode of inheritance in males is similar to that in females. Sex-specific differences in the mode of transcriptome inheritance could explain Darwin's observation of different evolution in males and females.

Males evolve more quickly than females for many characters, likely because of sexual selection, which is stronger in males than females. Three examples of faster male evolution in flies follow. First, sexual selection causes male morphology to evolve more quickly than female morphology, including but not limited to morphology of external genitalia (7). Second, genes underlying sexually selected and reproductive traits in males evolve more quickly at the sequence level than randomly sampled genes and/or genes associated with female reproductive traits (8). Finally, interspecific divergence of expression is faster in genes with higher expression in males (i.e., male-biased genes) than in genes with higher expression in females (i.e., female-biased genes; (9), and male-biased genes are more likely than female-biased genes to show signatures of positive selection (10). In *Drosophila*, mutation rates are equal in females and males (11), so male-driven mutation cannot contribute to faster male evolution.

Genes located on the X, as well as autosomal genes with transcriptional modifiers on the X, might be expected to have sex-specific inheritance for expression because of hemizygosity in males. In species with heterogametic males, sexually selected traits are frequently located on the X (12, 13). Quantitative trait loci (QTL) controlling sexually selected traits in flies such as sex-comb tooth number and pigmentation have been mapped to the X chromosome, although QTL for these traits have also been mapped to autosomal loci (14, 15). Interestingly, multiple cis-regulatory targets of sexual selection have been mapped to the X-linked *yellow* locus (16–18). These data are suggestive of a special role for the hemizygous X chromosome in sexual selection in general, and expression variation in particular, although precisely mapped QTL are too few to make a quantitative conclusion.

We have conducted a comprehensive study of both differential gene expression between the sexes and the mode of inheritance of transcript abundance in both sexes using a full diallel analysis. The diallel is a classic crossing design, measuring the phenotype of interest. We measured transcript abundance in all possible heterozygous cross progeny, from nine wild-type homozygous

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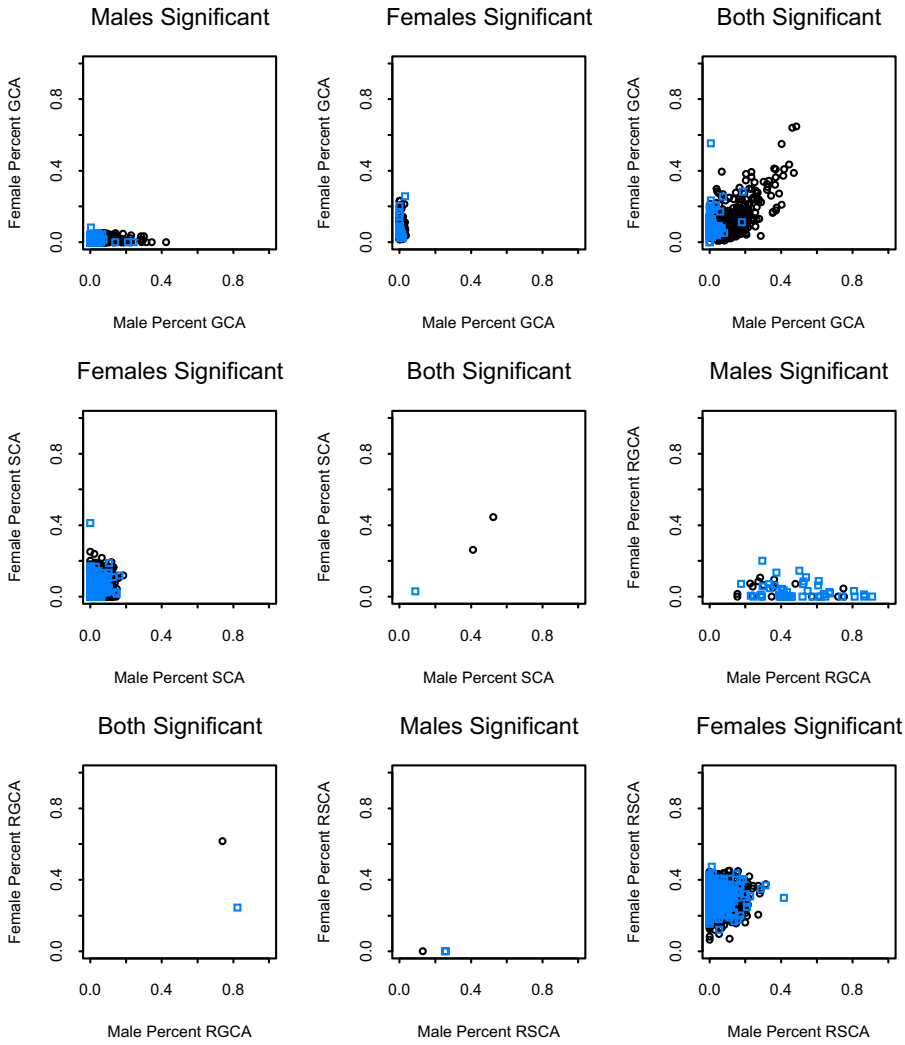

Fig. 1. Percent variance explained for different components of genetic variation, plotted by sex. Blue squares represent genes on the X; black circles represent genes on the autosomes. Genes could be either significant for the particular term in one sex (males or females) or significant in both sexes (both significant). Plots are not presented for genes significant in SCA for males, for RGCA for females, or for RSCA for both sexes, because there were no significant genes in any of these categories.

lines of *Drosophila melanogaster* extracted from natural populations using a custom Agilent oligonucleotide microarray platform (3) (Agilent Technologies, Palo Alto, CA). The diallel allows identification of relative contributions of additivity and dominance to total genetic variation by comparing effects of a particular parental genotype regardless of its mate's genotype (additivity) or in combination with specific mate genotypes (dominance; see *Materials and Methods*; ref. 19). By measuring transcript abundance in both sexes and focusing on comparisons between the X and autosomes, we are able to characterize and quantify extensive sex differences in the mode of inheritance for expression.

Results

We find large differences between the two sexes in transcript abundance, including sex-limited genes and sexually dimorphic genes. Only 8% of genes were sex-limited (expressed in only one sex; 467 male-limited and 238 female-limited, from a total of 9,312 genes examined). Sex-limited genes were not differentially represented on the X chromosome relative to the autosomes within sexes [supporting information (SI) Data Set 1]. Of the genes expressed in both sexes, 7,617 of the 8,607 genes have sexually dimorphic expression (i.e., sex bias). Of these, 4,070 are female biased, whereas 3,547 are male biased. The majority of the biased genes located on the X (764 of 1,243) are female biased ($P < 0.001$), whereas the biased genes on the autosomes are more evenly divided between males and females (3,306 genes are female biased and 3,068 genes are male biased).

We also find striking evidence for differential modes of inheritance of transcript abundance between the two sexes: of the 8,607 genes expressed in both sexes, 4,210 show evidence for genetic variation in either males or females, but only 889 show evidence for genetic variation in both sexes. Thus, the agreement between the sexes is quite low, although it is significantly different from zero [$\kappa = 0.12$; 95% confidence interval (CI) 0.10–0.15; Fig. 1].

However, if we ask to what extent does heritable genetic variation [general combining ability (GCA)] agree between the sexes, we see a better, although still low, correlation ($\kappa = 0.46$; 95% CI 0.42–0.48), which does not differ between the X and autosomes ($P = 0.76$). Thus, the major difference in genetic variation between the sexes must be due to differences in dominance variance: males have virtually no dominance variance, with only six genes showing significant dominance variation.

Of the 1,570 genes showing heritable genetic variation in males (exclusive of the 4th chromosome), 682 (43.44%) have no genetic variation for expression in females. For the remaining 57% that show significant variation in females (888 genes), 404 genes show evidence for nonadditive variation, and 349 of these (86.4%) are autosomal. Interestingly, of the 404 genes that show evidence for dominance variance in females, 281 do not show evidence of GCA or RGCA. In other words, 281 genes have additive variation in the males, but have only nonadditive variation in the females. 246 of these 281 genes are on the autosomes, implying that the mode of inheritance must differ between males and females because of sex-specific and/or X-linked trans effects, or cis by trans interactions.

For genes expressed in both sexes (SI Table 2 and SI Data Set 2), sex differences in mode of inheritance are greatest on the X chromosome. Although more genes vary in females than in males, both on the X and on the autosomes, sex differences in mode of inheritance are more pronounced on the X chromosome (43.89% vs. 14.19% in females, $P < 0.0019$; 40.37% vs. 19.10% in males, $P < 0.0001$). Females have relatively more genes with any type of genetic variation on the X than on the autosomes (43.89% vs. 40.37%; $\chi^2 = 6.08$; $P < 0.0137$), whereas males show the opposite pattern (fewer genes varying on the X than on the autosomes; 14.19% vs. 19.10%; $\chi^2 = 19.21$; $P < 0.0001$). Additionally, there is greater agreement between the sexes on the autosomes ($\kappa = 0.14$, 95% CI 0.12 - 0.16) than on the X ($\kappa = 0.06$, 95% CI 0.02–0.10), and the difference in correlation between the X and autosomes is significant ($\chi^2 = 12.13$; $P = 0.0005$). Only 109 X-linked genes of 1,424 varied in both sexes.

Differences between reciprocal crosses allow detection of effects attributable to the X, to cytoplasmic (including maternal and epigenetic) effects, and/or to nuclear–cytoplasmic interactions. We can infer which mechanism is most likely responsible by comparing differences between the sexes of the progeny within and between reciprocal crosses and considering whether the effects of a given line are independent of the second parent (RGCA) or depend on the specific combination of genotypes [reciprocal specific combining ability (RSCA)]. If the transcript abundance of a gene differs similarly between reciprocal crosses regardless of the specific combination of parental genotypes (RGCA), either the X chromosome or the cytoplasm is most likely responsible. The cytoplasm is the same in male and female cross progeny within each member of a pair of reciprocal crosses, but the nuclear (X) genotype is not: males are hemizygous haploids, whereas females are heterozygous diploids. Thus, if effects are seen in both sons and daughters, cytoplasmic effects are the most likely explanation; but if effects are predominantly limited to males, then cis or trans effects of the X are more likely to be responsible, because daughters of reciprocal crosses have identical nuclear X genotypes but sons do not (see Fig. 2). The overall reciprocal effect (RGCA) is significant for 69 genes in males but only 2 in females, indicating that the cytoplasm is unlikely to explain differences in males and females and thus indicating a substantial X effect (Table 1, Fig. 2), 46 genes on the X (cis or trans effects) and 23 on the autosomes (trans effects).

Considering the sexes of progeny across specific pairs of reciprocal crosses, females are again identical heterozygotes, and males from the two crosses have a single X from the female parent only. Cytoplasms are shared between sexes within a cross, but systematically differ between pairs of crosses and cosegregate with the X in sons. Thus, differences in reciprocal crosses in females for specific parental genotypic combinations (RSCA) must be due to interactions between the common nuclear genotype and the different cytoplasms or, possibly, cross-specific epigenetic effects, present in reciprocal cross-progeny. These effects are common in females and rare in males (2,222 vs. 3 genes, respectively) and are not distributed similarly between X and autosomes (females: 30% of X genes and 25% of autosomal genes).

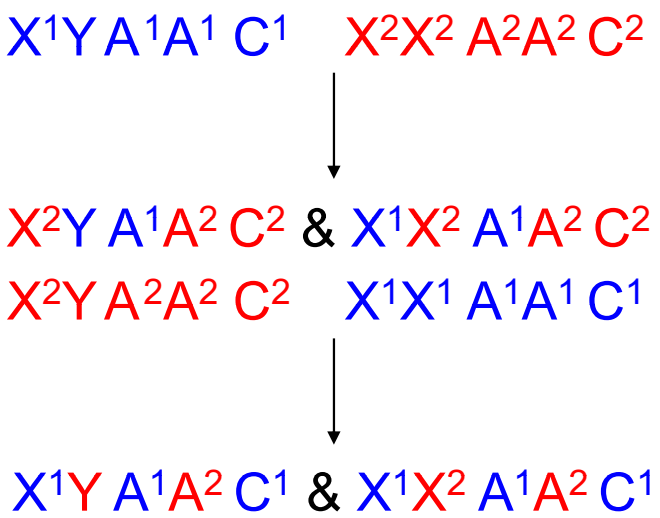

Fig. 2. A reciprocal cross: In the first cross, parent 1 (blue) is the sire, and parent 2 (red) is the dam; in the second, parent 2 (red) is the sire and parent 1 (blue) is the dam. Sons differ between pairs of reciprocal crosses for their single X chromosome, but daughters from both crosses are heterozygous for the X. Within each reciprocal cross, sons and daughters have identical cytoplasms but different X genotypes because sons are hemizygous, whereas daughters are heterozygous diploids. Reciprocal crosses also differ in both males and females for epigenetic and parent-of-origin effects, not illustrated here.

In principle, the sex-specificity of transcriptome variation might be due to differences between genetic networks associated with reproduction in the two sexes. To test this hypothesis, we analyzed variation in ovary-specific, testes-specific, and soma-specific genes (20). We did not see any obvious association between significance for different components of genetic variance and membership in these groups of genes (SI Data Set 3), indicating that the differences between males and females in the patterns of mode of inheritance for transcript abundance are not attributable to any specific sex-limited gene expression.

Discussion

A straightforward but seemingly underappreciated explanation for widespread sexual dimorphism in gene expression is that hemizygosity causes genes on the X, or controlled by X-linked trans-acting factors, to have a simpler mode of inheritance in males than in females. The presence of a single X chromosome in males eliminates intralocus interactions (dominance) and most interlocus interactions (epistasis) that are possible in females for X-linked genes and autosomal genes with X-linked trans-acting factors. Males pass their X chromosomes only to their daughters and inherit an X only from their mothers; thus, the heritability for X-linked genes changes from simple to complex each time an X chromosome is passed through a male (see Fig. 3). The conversion of nonadditive, epistatic variance to additive variance when the X moves from females to males is similar to the loss of nonadditive variation by genetic drift or inbreeding (19), which could confer a faster response to selection (21).

To further test the hypothesis that hemizygosity mediates faster evolution in response to selection, we compared genes with additive variation attributable to the X in males to a list of genes whose expression differed between lines selected for mating speed and controls in *D. melanogaster* (22). We found that selection response was more frequently attributable to genes with our predicted fast evolving genes than expected by chance ($P = 8.3 \times 10^{-11}$; SI Data Set 3).

Table 1. Mode of inheritance for transcription for genes in both sexes, with respect to chromosomal context [X or autosomes (A)]

		Males		Females	
		X	A	X	A
Any genetic variation	NS	1,222	5,811	799	4,283
	S	202	1,372***	625*	2,900
Additive genetic variation (GCA)	NS	1,229	5,812	1,328	6,516
	S	195	1,371***	96	667**
Nonadditive genetic variation (SCA)	NS	1,423	7,181	1,124	6,135
	S	1†	2	300***	1,048
Additive genetic variation associated with the X (RGCA)	NS	1,378	7,160	1,423	7,182
	S	46***	23	1†	1
Nonadditive genetic variation [nuclear–cytoplasmic interactions (RSCA)]	NS	1,422	7,182	999	5,386
	S	2*†	1	425***	1,797

Significant differences between numbers of genes on chromosomes within sexes are indicated by asterisks associated with the chromosome with proportionately more genes with genetic variation: *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.001$.
†Comparisons with small cell counts.

We predict that genes with purely additive variation in males and purely nonadditive genetic variation in females ("simple male genes") mediate faster evolution in males, for example, in response to sexual selection. One testable corollary of this prediction is that there should be less additive genetic variation in simple male genes than for other autosomal genes with more similar modes of inheritance between the sexes, because of erosion of additive variation by selection. Indeed, both heritability and CV_A are lower for simple male genes ($n = 246$) than other autosomal genes ($n = 1{,}125$) in males, consistent with the erosion of variation by selection (for h^2, median 0.08 compared with median 0.11; for CV_A, median 1.78 compared with median 2.35; see SI Table 3). Furthermore, some genes should have not merely reduced, but no detectable additive variation remaining. Therefore, there should be a group of genes noteworthy by their absence of significant additive variation. If simple male genes are caused by hemizygosity, they should reside predominantly on the X chromosome; thus, we should see relatively fewer genes with segregating additive variation on the X than the autosomes for both sexes, which is indeed the case (Table 1).

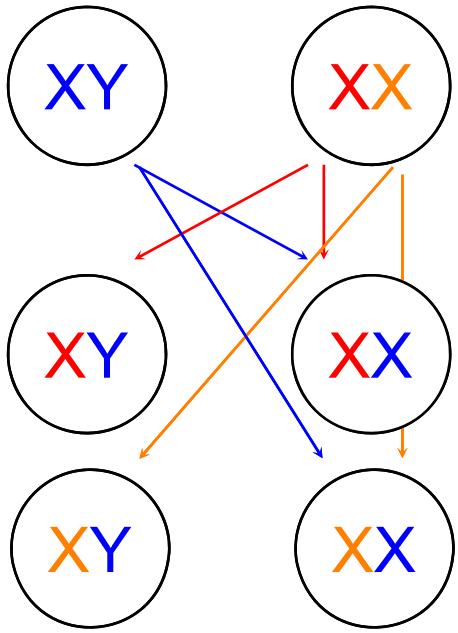

Fig. 3. Each X chromosome from a female has an equal probability of being transmitted to a son or a daughter [red and orange Xs; the X from males is always transmitted to daughters, never to sons (blue X and arrow)].

However, it is unclear whether low heritabilities are caused by depletion of additive variation by selection acting on the trait, or whether, in fact, low heritabilities are not indicators of past selection but are due solely to the vagaries of the underlying genetic architecture, irrespective of selection. A low heritability in this context would imply that these traits will be unable to respond to selection effectively. One possible nonselective explanation for low heritabilities in traits affected by hemizygosity is that, under a strictly additive model, heritability will be lower for hemizygous genes than for diploid genes, simply because there fewer cross progeny genotypic states. However, diploidy permits nonadditivity, and there is no clear theoretical prediction what the relationship between heritabilities should be for hemizygous and diploid genes where nonadditivity is permitted, because, if genetic variation is partitioned into a combination of additive and nonadditive in the diploid case, one might expect heritability to be lower than in the haploid case.

There are only 607 genes that have additive variation in both sexes, and these are primarily autosomal (534). In contrast to the simple male genes described above, neither the heritability nor the CV_A among these genes are significantly different between the two sexes. The genetic correlation for these genes is high (0.848), and in many cases, the CI includes one (SI Data Set 3).

Hemizygosity of the X and resulting increased additivity in males is also consistent with patterns of inheritance of sexually antagonistic (SA) alleles, which have sex-specific effects on fitness. SA alleles are expected to accumulate on the X if mutations that are deleterious to females and beneficial to males are partially recessive. Such alleles would be available to selection in hemizygous males but concealed from selection in diploid females. In other words, SA alleles would be additive in males and nonadditive in females. Indeed, SA alleles are preferentially located on the X in flies (23). However, males do not pass their X chromosomes to their sons but, rather, to their daughters, causing the sign of selection of a given SA to switch every generation (24). Both recessivity in females and failure of males to pass beneficial X alleles directly to their sons predict polymorphism of SA alleles on the X. However, if females can distinguish between SA alleles of opposite signs at a given locus, female preference genes for female-benefiting alleles should eventually invade the population, leading to resolution of SA polymorphism in the direction of female-benefiting alleles (25). It seems reasonable that female-benefiting SA alleles are less

likely to be male-biased than male-benefiting SA alleles. Female choice for female-benefiting alleles may explain the observation that male-biased genes are underrepresented on the X (26) as well as the observation of differential migration of genes from the X to the autosomes in flies, accompanied by the overrepresentation of testis expression in newly autosomal genes (27). It is also possible that female-biased alleles accumulate on the X because the X spends more time in females than in males, and hence selection on the X, given additivity, is stronger in females than in males.

Materials and Methods

***Drosophila* Strains and Culture.** Nine isogenic lines of *D. melanogaster*, originally captured in an orchard in Winters, CA, and subjected to >20 generations of full sibling inbreeding, were used as parents. Lines were crossed in a full diallel design with reciprocals but without homozygous parents (72 F_1 progeny). Progeny were reared on dextrose medium from matings of 10 females and 10 males removed after 3 days, and maintained at 25°C with a 12:12-h light/dark cycle. Twenty virgin males and females were collected within 24 h from each replicate, transferred to fresh vials, and maintained for 3 days before RNA extraction. Crosses were distributed across subblocks because of the size of the experiment; four subblocks for each two blocks were performed with half the total number of crosses reared in each subblock as a partially balanced incomplete block design (see SI Fig. 4 for design). Subblocks were pooled into a single replicate.

RNA Sample Preparation. RNA was extracted from 20 whole 3-day posteclosion flies, snap-frozen in liquid nitrogen by using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA was purified by using RNeasy kit (Qiagen, Valencia, CA) and concentration determined by using a NanoDrop spectrophotometer; sample quality was examined by using an Agilent 2100 Bioanalyzer.

Microarray Design, Hybridization, and Signal Detection. The chip was synthesized on an Agilent platform [www.genomics.purdue.edu/services/droschip, AMADID 012798 (3)]. We considered only the 12,850 unique gene transcripts (represented by 12,936 probes).

Fluorescent cRNA was synthesized by using the Agilent low-RNA input fluorescent linear amplification kit following manufacturer's protocols. Labeled RNA was cleaned by using Qiagen RNeasy columns, and cRNA yield was quantified on a NanoDrop ND-1000 spectrophotometer. Seven hundred fifty nanograms of each labeled sample was pooled and hybridized to the arrays following the manufacturers protocol. Hybridizations were performed with males and females of the same genotype, labeled in contrasting dyes, hybridized to the same chip. We analyzed two independent biological replicates for each genotype and sex combination. Please see SI Fig. 4 for details of block design. For one replicate, males were labeled with Cy3 and females with Cy5; for the other, dyes were reversed. No technical replicates were performed because reliability of the Agilent platform is on average above 90% (L.M.M., L.M.B., L.H., A.K., M.L.W., and S.V.N., unpublished data, http://bioinformatics.ufl.edu/site/pages_for_research/Genomics_of_Sex_Dimorphism.htm).

Microarray experiments were carried out at the Interdisciplinary Center for Biotechnology Research Microarray Core, University of Florida. Hybridization occurred for 17 h at 60°C in accordance with the manufacturer's instructions, and arrays were scanned by using an Agilent Microarray scanner. Images were analyzed by using Imagene software version 6.0 at the Genomics Database Facility, Purdue University (West Lafayette, IN). Transcript abundance was estimated as the natural log of the spot mean minus the mean of the local background.

Density plots and 3D plot of the negative control grid, visual inspection of slides, between-replicates Spearman's correlation, and Cohen's κ-statistic (28) were computed for quality control. The resulting 144 slides had excellent agreement (weighted κ values 0.55–0.93, median 0.83 (28). Local imperfections in the slide (i.e., streaks, dust) were filtered before normalization by using natural log. The 90% value of the negative controls was set as the detection threshold. For a gene to be detected in a particular cross and sex combination, both replicates needed to be detected. Genes that were not detected in any slide were eliminated (9,350 probes representing 9,350 individual transcripts were analyzed).

Small Chromosomes. Thirty-three genes were evaluated from the nonrecombining 4th chromosome; of these, 2 were significant for GCA in males; in females, 10 other genes were significant (2 for GCA, 4 for SCA, and 4 for RSCA). Because this pattern seemed different from the other two autosomes, possibly because of low sequence variation (29), genes from the 4th chromosome were excluded from further analysis. Among five genes represented on the chip from the gene-poor Y chromosome, none were significant, consistent with low sequence variation on the Y (30).

Statistical Analyses. We tested differential expression among the two sexes using a linear model for each probe: $Y_{ijkn} = \mu + d_i + l_j + s_k + ls_{jk} + \varepsilon_{ijkn}$, where Y_{ijkn} is the transcript abundance for the analyzed probe, μ is the overall mean of the transcript abundance, d is the effect of dye ($i = 1, 2$), l is the effect of line ($j = 1, \ldots, 8$), s is the effect of sex ($k = 1, 2$), ls is for line by sex interaction, n is for the two replicates, and ε is the error. We tested the effect of differential expression between the sexes using an F test for the sex effect for each probe. After the calculation of nominal P values, we accounted for multiple tests by using a false discovery rate correction (31). For this and all subsequent analyses, we set the expected proportion of false discoveries at 0.10 in order balance type I and type II error (31).

To examine the mode of inheritance for each sex, we used a mixed effects linear model for a diallel design with both the genetic effects (GCA, SCA, RGCA, and RSCA) and replicate as random effects (19) and dye as a fixed effect. To test the individual genetic effects in this model, F tests were constructed for each of the genetic effects according to standard diallel analyses (19). We estimated variance components for the random genetic effects using a REML approach (19). A gene was significant for "any" genetic effect if any of the individual genetic effects was found to be significant. Heritability, CV_A, and genetic correlation were estimated as a function of the estimated variance components (19, 32, 33).

We used a nominal threshold of 0.01 for all χ^2 tests of association among lists. We used a χ^2 test to determine whether significance for a particular genetic effect (GCA, SCA, RGCA, RSCA, or "any" genetic effect) was more likely to occur on the X chromosome compared with the autosomes by comparing the proportion of significant genes for the genetic effect between the X and the autosomes, within and across sexes. Also, we performed χ^2 tests for association among lists and to determine whether findings in this study were consistent were previous work by identifying the genes in those studies that were significant in our set of 9,312 genes.

To compare nominal or ordinal responses, we used the κ-statistic, a chance-corrected measure of agreement (28), which is interpreted like the Pearson correlation coefficient for continuous variables. κ was used to compare the agreement between males and females for detection of significant genetic effects (28).

Interpretation of Genetic Architecture from Diallel. For details of interpretation of the diallel, please see ref. 19. In general, GCA may be thought of as an approximation of additive genetic variance, provided that additive-by-additive epistasis is very small ($\sigma^2_{GCA} = \sigma^2_A/2 + \sigma^2_{AA}/4 + \ldots$), whereas SCA is an approximation of

dominance variation ($\sigma^2_{SCA} = \sigma^2_A/2 + \sigma^2_{AA}/4 + \ldots$ (19). Assuming that the epistasis terms are small, GCA can be thought of as additive variation; similarly, RGCA is also a function of additive genetic variation. Hemizygosity of males precludes intralocus dominance on the X and also eliminates certain kinds of dominance epistasis ($D_X \times A_A$, $D_X \times A_X$, $D_X \times D_A$, and $D_X \times D_X$, where D and A indicate dominance or additive, respectively; subscript X or A indicates X chromosome or autosomes; see SI Table 4 for more details). All of the aforementioned nonadditive epistatic interaction terms involving the X chromosome are possible in females; however, to simplify our discussion, we focus on the additive and dominance terms.

Differences between reciprocal crosses allow detection of effects attributable to the X, to cytoplasmic (including maternal and epigenetic) effects, and/or to nuclear–cytoplasmic interactions. When reciprocal differences are considered for a given line across all crosses (RGCA), X or cytoplasmic effects are implicated. However, significant RGCA in females cannot be due to the X (because the X genotype is identical between reciprocal crosses) but, instead, must be due to cytoplasmic or epigenetic effects. Because only two genes have significant RGCA in females, these effects must be rare. The simplest explanation, then, of RGCA effects in males is a main effect of the X, either cis or trans, (69 genes, 46 on the X and 23 on the autosomes). Cross-specific differences in reciprocals (RSCA) may be due to interactions between the autosomes and the X or between nuclear and cytoplasmic effects (when reciprocal differences are cross-specific, RSCA) or epigenetic effects. Again, because the nuclear genotype is the same for reciprocal crosses in females, RSCA in females must be due to nuclear–cytoplasmic interactions (2,222 genes) or epigenetic effects. In males, RSCA could also be due to X–autosome interactions involving a single X allele and the autosomes (i.e., $A_X \times A_A$ or $A_X \times D_A$ epistasis) in addition to nuclear–cytoplasmic interactions. Only three genes have RSCA in males (two on the X, one autosomal). Interestingly, nuclear–cytoplasmic interactions are far more common in females than in males. Genes with significant RSCA in females are on the X (30%) relative to the autosomes (25%). The discrepancy between males and females for RSCA is curious and bears further investigation.

Heritability for gene expression was estimated by using the expression $2\sigma^2_{GCA}/2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{RSCA} + \sigma^2_{RGCA} + \sigma^2_{rep} + \sigma^2_{error})$ (19). CV_A was estimated as $100(\sqrt{2\sigma^2_{GCA}}/\bar{X})$ for each sex separately, where $\bar{X}$ was the mean expression for that sex (33). Only genes that had significant additive variation (i.e., were significant for GCA) were included in the estimation of heritability and CV_A. For autosomal genes with significant additive genetic variation in both sexes, the genetic correlation among the males and females was calculated (32). Our design does not permit comparable estimates of heritability or CV_A in males for X-linked genes and autosomal genes with significant X effects (i.e., significant RGCA), because hemizygosity will cause the amount of additive variation to be underestimated in males relative to females (for nine lines, nine genotypes are possible in males, whereas 36 are possible in females, ignoring reciprocals).

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Dataset 1. Full results of analyses (*n* = 9345). Each gene is indexed by a unique probeuid. In this table gene, full name, and chromosome are given for information. More complete annotation is located in SI Data Set 2. *F* tests are labeled with the convention f_<test name>_<sex>, nominal *P* values from the *F* test are labeled probf_<test>_<sex>. Significance is indicated as probf_<test>_<sex>. **"**sex_signficant" is an indicator of whether the amount of transcript is significantly different between the two sexes, and if so, "bias_toward" indicates in which direction (e.g., greater in males indicated by male); if not, cells are empty. "xsome_id" is an indicator, whereas "autosome" represents chromosome 2 or 3; 4 represents the 4th or dot chromosome; and *X* and *Y* represent the two sex chromosomes. "on_group" is an indicator of the detection of the transcript with values "both" for detected in both sexes, "male" if detected only in males and "fem". Significance is presented for each component of variance for each sex separately, for an FDR of 0.10. For example, "gca_male" would have a "y" if GCA in males were significant at an FDR of 0.10, or an "n" if not signficant. "any_sig_male" indicates whether or not any components of variance were significant in males; similarly, "any_sig_female" indicates whether or not any components of variance were significant in females. For these indicator variables, if genes are sex-limited (on in only one sex), cells are empty for the sex in which expression is not detected. "simple" applies only to genes on in both sexes, and indicates genes which were strictly additive in males and strictly nonadditive in females with y; other genes on in both sexes are indicated with n. The four tests (GCA,SCA,RGCA,RSCA) in that order are combined to give a pattern of significance as pattern_female and pattern_male. The value "y" indicates significance while the value "n" indicates nonsignificance at FDR 0.10 for that particular test. Thus "ynnn" is significant for GCA alone.

Dataset 2. Results of analyses for autosomal (*A*; chromosomes 2 and 3 only) and *X*-linked genes only where transcript is detected in both sexes (*n* = 8,607). Each gene is indexed by a unique probeuid. In this table gene, full name, chromosome, and cytological position are given for information. Significance at an FDR threshold of 0.10 is indicated as probf_<test>_<sex>. *F* tests are labeled with the convention f_<test name>_<sex>, nominal *P* values from the *F* test are labeled probf_<test>_<sex>. Significance is indicated as probf_<test>_<sex>. If expression is significantly different between the sexes , then "sex_significant" is has a "y" indicator and "bias_toward" is an indicator of whether the amount of transcript is greater in males or females. "xsome_id" is an indicator where "autosome" represents chromosome 2 or 3; 4 represents the 4th or dot chromosome; and *X* represents the *X* chromosome. Significance is presented for each component of variance for each sex separately, for an FDR of 0.10. For example, "gca_male" would have a "y" if GCA in males were significant at an FDR of 0.10, or an "n" if not significant. "any_sig_male" indicates whether or not any components of variance were significant in males; similarly, "any_sig_female" indicates whether or not any components of variance were significant in females. The four tests (GCA,SCA,RGCA,RSCA) in that order are combined to give a pattern of significance as pattern_female and pattern_male. The value "y" indicates significance while the value "n" indicates non_significance at FDR 0.10 for that particular test. Thus "ynnn" is significant for GCA alone.

Dataset 3. Abbreviated results plus full annotation information (*n* = 9,345). Each gene is indexed by a unique probeuid and the probe sequence is given. In this table gene, full name, and chromosome, and cytological position, Molecular_Functions, PhysicalMapEnd, PhysicalMapStart, Properties, Protein_Domains, Recombination_Map, Synonyms, GameFileID,

Full_Name, Gadfly. Annotation is from Flybase version 4.7 and was extracted using the program developed previously (McIntyre *et al.* 2006). In addition, relevant lists of significant results from Parisi *et al.* 2004 are identified by merging the gene names from the Affymetrix chip with the gene names identified here; presence on any of the lists is an indicator variable with the column header name corresponding to the nomenclature in the original paper (ovaries_38, testes_39, soma_40, male_biased_41, female_biased_42). Similarly, significant results from Mackay *et al.* 2005 are indicated by the name of the supplementary table in that paper (M, MF, MFC, F, C, CF; note that results with MF are the ones presented in our paper). If expression is significantly different between the sexes, then "sex_significant" is has a "y" indicator and "bias_toward" is an indicator of whether the amount of transcript is greater in males or females. The column "autosome" indicates if a gene is on chromosome 2 or 3, in which case the indicator is 1; if it is not (*i.e.* it is located on the *X*, *Y*, or the 4th, the indicator is 0. "xsome_id" is an indicator where "autosome" represents chromosome 2 or 3; 4 represents the 4th or dot chromosome; and *X* represents the sex chromosome. "on_group" is an indicator of the detection of the transcript with values "both" for detected in both sexes, "male" if detected only in males and "fem" if detected only in females. Significance is presented for each component of variance for each sex separately, for an FDR of 0.10. For example, "gca_male" would have a "y" if GCA in males were significant at an FDR of 0.10, or an "n" if not. "any_sig_male" indicates whether or not any components of variance were significant in males; similarly, "any_sig_female" indicates whether or not any components of variance were significant in females. For these indicator variables, if genes are sex-limited (on in only one sex), cells are empty for the sex in which the gene is not expressed. "simple" applies only to genes on in both sexes, and indicates genes which were strictly additive in males and strictly nonadditive in females with y; other genes on in both sexes are indicated with n. The four tests (GCA,SCA,RGCA,RSCA) in that order are combined to give a pattern of significance as pattern_female and pattern_male. The value "y" indicates significance while the value "n" indicates nonsignificance at FDR 0.10 for that particular test. Thus "ynnn" is significant for GCA alone.

Table 2. For genes expressed in both sexes (n = 8,607). Counts of genes for different combinations of modes of inheritance, for both sexes for all genes (left section of table). The total given for each effect (*i.e.*, GCA only) is for the total number of genes showing this effect in either sex. On the right side of the table are the counts for effects, for females only, for genes with any evidence for additive variation in males (right section of table; 1,570 genes with significant GCA, RGCA, or any combination of these modes of inheritance in males).

Table 3. Estimates of variance components. Each gene is indexed by a unique probeuid. In this table, gene, full name, and chromosome are given for information. More complete annotation is located in SI Data Set 2. If expression is significantly different between the sexes, then "sex_significant" is has a "y" indicator and "bias_toward" is an indicator of whether the amount of transcript is greater in males or females. "xsome_id" is an indicator where "autosome" represents chromosome 2 or 3; 4 represents the 4th or dot chromosome; and *X* represents the sex chromosome. Estimates are REML and named as follows: <sex>_est_<effect>. Heritability is estimated (Lynch and Walsh 1998) for each sex separately, and listed as <sex>_heritability. CV_A is estimated (Houle 1992) and listed as CV_A,_<sex>, Genetic correlation for those genes with GCA present in both sexes along with the standard error was calculated and is listed as RG and SERG respectively (Holland 2006).

Table 4. All possible modes of inheritance are listed, both within loci (additivity and dominance) and between loci (additive x additive, additive x dominance, and dominance x dominance epistasis) to indicate terms impossible for hemizygous males, but possible for diploid females. Letters *X* and *A* indicate effects coming from the *X* or the autosomes, respectively. Possible terms given the simple models of expression defined below are presented for expression from *X* linked genes for males and females, and then for expression from autosomal genes, for males and females. Sex differences in possible modes of inheritance for gene expression are summarized in the bolded rows. The terms listed as sex differences are possible in females but not in males due to hemizygosity of the *X* in males.