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Paucity of Genes on the *Drosophila* X Chromosome Showing Male-Biased Expression

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Abstract

Sex chromosomes are primary determinants of sexual dimorphism in many organisms. These chromosomes are thought to arise via the divergence of an ancestral autosome pair and are almost certainly influenced by differing selection in males and females. Exploring how sex chromosomes differ from autosomes is highly amenable to genomic analysis. We examined global gene expression in *Drosophila melanogaster* and report a dramatic underrepresentation of X-chromosome genes showing high relative expression in males. Using comparative genomics, we find that these same X-chromosome genes are exceptionally poorly conserved in the mosquito *Anopheles gambiae*. These data indicate that the X chromosome is a disfavored location for genes selectively expressed in males.

The *Drosophila melanogaster* X chromosome is present in one dose in males and two in females. There are at least three aspects of this fact that might influence X-chromosome development over large time scales. First, the dose difference has led to a dosage-compensation mechanism based on elevated transcription of X-chromosome genes in males (1). However, some X-chromosome genes may be expressed at lower levels in males than females because of dose, especially in germ cells where dosage compensation has not been documented (2). This might be a disadvantageous condition for genes encoding abundant male-specific proteins. Second, as males are hemizygous, mutations in X-chromosome genes are immediately available for selection even if those mutations would be recessive in diploids (3, 4). This would promote accumulation of genes with functions favorable to males on the X chromosome. Third, two-thirds of the X chromosomes in a population reside in females. Positive selection in females would lead to X feminization, whereas selection against male-advantage genes deleterious to females would lead to demasculinization of the X chromosome.

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Genes showing sex-biased gene expression profiles are also likely to have sex-biased functions. To investigate how genes with sex-differential expression profiles are distributed among the chromosomes in *Drosophila*, we have used an Incyte microarray to assay the expression of 14,142 predicted transcripts in competitive hybridizations [available from the Gene Expression Omnibus (5) under accessions GPL20, and GSM2456-GSM2469]. Sex-bias is conservatively defined as greater than twofold and is justified by the observation that >99% of array elements are within twofold in control hybridizations (Fig. 1A). Recent work has shown that sex-biased gene expression is significant in *Drosophila* (6-8). This first report of sex-biased expression of the full (predicted) genome also strongly indicates that there is significant sex-biased expression—especially in gonads (Fig. 1, B to D).

To investigate how genes with sex-biased expression are distributed in the genome, we parsed the data according to chromosome location (Fig. 2). The X chromosome showed a significant departure from a random distribution of genes with male-biased expression. For example, in gonad (Fig. 2A), only 10% of the transcripts were male-biased (at a twofold cutoff), whereas 14 to 17% of the autosomal transcripts showed male-biased differential expression. This highly significant underrepresentation of X-chromosome genes with male-biased expression held at 4- and 10-fold cutoffs as well (Fig. 2A). Similarly, with no cutoff, 45% of X-chromosome genes were expressed preferentially in testis. Corresponding frequencies for the autosome arms were 57, 57, 58, and 56%. We also examined the expression profiles of whole male flies and male flies with gonads removed (Fig. 2, D and G). Although the number of genes showing male-biased expression is lower, we observed a similar underrepresentation of X-chromosome genes with male-biased expression. Thus, the underrepresentation of male-biased transcripts from the X chromosome is not restricted to gonads. There was no chromosome bias in the distribution of genes with female-biased expression, except for a greater number of female-biased genes on the X chromosome in gonad comparisons (Fig. 2B). Similarly, genes showing no sex-bias were also randomly distributed, except for a slight over-representation on the X chromosome 2L (Fig. 2, C, F, and I). Thus, there are multiple significant departures from random distributions of genes with male versus female bias involving the X chromosome. In addition, the nonrandom distributions involving the X chromosome are more extreme and especially pronounced for genes showing male-biased expression.

We also examined gene expression profiles using expressed sequence tags (ESTs). Testis and male-specific accessory gland ESTs are biased toward autosomal locations (8, 9), which suggests that the X chromosome is not a favored location for genes differentially expressed in the male soma. In contrast, the chromosomal distribution of ovary ESTs from the Berkeley *Drosophila* Genome Project (10) was random.

Most sex-biased expression occurs in the germ line. There are at least two possible global differences between X chromosomes in somatic versus germline tissues that might influence sex-biased gene expression. First, although chromatin-mediated dosage compensation occurs in the soma of *Drosophila*, that specific dosage-compensation mechanism does not function in germ cells (2). This enigma has resulted in the suggestion that there is no dosage compensation in the germ line. Thus, much of the underrepresentation of X-chromosome male-biased genes we observe could be a simple dose effect. This is much less likely for the somatic underrepresentation where X-chromosome dosage compensation is clearly present, but some genes do escape dosage compensation. Our analysis might be identifying those genes. Second, the X chromosomes of *Drosophila* and other animals become precociously condensed and inactivated in spermatocytes (11-14). If X-chromosome inactivation occurs early enough to alter steady-state transcript levels in spermatocytes, then this effect could explain the underrepresentation we observe. Indeed, in the only full-genome study of chromosomal bias to date, it was shown that *C. elegans* has essentially no X-chromosome genes showing male-

biased germ line-restricted expression (15). This correlates with an altered chromatin state in those germ cells (11, 12).

To test for dose effects and epigenetic mechanisms that might globally reduce male-biased X-chromosome gene expression in the testis, we used the microarray data to examine the overall distribution pattern. If X-chromosome gene expression were globally reduced in testis because of the absence of dosage compensation, or especially because of male X inactivation, then there would be a shift in the distribution toward female-biased expression. However, we found that male-biased expression in the testis was similarly underrepresented regardless of the bias cutoff used (Fig. 2A). Similarly, scatter plots of array intensities from the ovary versus testis experiments show no evidence of a global effect on X-chromosome expression. Many X-chromosome (Fig. 3A) and autosomal (Fig. 3B) transcripts fall neatly around 1:1 expression in testis and ovary (the median ratio is 0.94 for the X chromosome and 1.1 for each of the autosome arms). Thus, these data are inconsistent with gross twofold dosage effects or precocious X-chromosome inactivation in spermatocytes, which would result in even more extreme distribution shifts.

Sex-biased expression complicates the analysis of subtle global differences in X-chromosome gene expression. Therefore, to further examine whether there are global reductions in transcription, we turned to the analysis of genes encoding the ribosomal proteins, which are present in 1:1 stoichiometry. There are 38 annotated *Drosophila* ribosomal protein genes (16), 10 on the X chromosome and 28 on the autosomes. If there is global reduction in the expression of X-chromosome genes in testis because of dose or inactivation, then the X-chromosome ribosomal protein genes should show a twofold or greater reduction in expression relative to the autosomal members. The ribosomal proteins encoded on the X chromosome compared with those on the autosomes showed similar relative expression in ovary versus testis and gonadectomized flies (Fig. 3, C and D). Although some genes may be expressed solely based on dose or may be inactivated in males, these data clearly indicate that global effects fail to account for the observed underrepresentation of X-chromosome genes with male-biased expression. This is further supported by the underrepresentation of X-chromosome male-biased genes in the soma (Fig. 2), where male dosage compensation is known and where there is no male X inactivation. Therefore, underrepresentation is likely because of gene-by-gene effects over the course of sexchromosome divergence.

Demasculinization of the X chromosome through time can be examined by mapping male-biased genes onto the genome of a related organism with the same ancestral X chromosome. If genes with male-biased expression show net loss (due to movement, loss, or rapid divergence), and if the specific genes lost in the two lineages differ, then residual X-chromosome male-biased genes in one lineage should be underrepresented in the other. To test this, we looked for correlation between sex-biased gene expression profiles in *Drosophila* and chromosomal location in the distantly related mosquito, *Anopheles gambiae* (17).

Basic chromosomal structure is conserved between *Drosophila melanogaster* and *Anopheles gambiae*, although the X chromosome of the latter is smaller (18). Thus, we can test the idea of preferential loss of male-biased genes from the X chromosome. It is noteworthy that there have been translocations from X chromosome to autosome (and vice versa) in the two lineages, which have allowed us to determine if movement to an autosome “rescues” X-chromosome male-biased genes from loss. When we plotted the ratio of sex-biased expression in *Drosophila* gonads against the probability that a homolog exists in *Anopheles*, two clear correlations were observed. In agreement with traditional comparative studies on limited numbers of genes (19), genes highly expressed in males appear to change rapidly. More surprising is the strikingly tight correlation between degree of sex-biased expression and conservation.

Overall, 60% (8513) of the *Drosophila* transcripts represented on the array have homologs in *Anopheles* (18). When we tracked changes in linkage between the species, we found that conservation is directly related to sex-biased expression ratios and to chromosomal location. The poorest conservation is between *Drosophila* X-chromosome male-biased genes and the *Anopheles* X chromosome (Fig. 4A). Indeed, none of the X-chromosome genes showing greater than eightfold overexpression in *Drosophila* males are found on the *Anopheles* X chromosome, but this is not restricted to highly male-biased genes. There is a smooth inverse relationship between degree of male-biased expression and conservation. Translocation to an autosome clearly increases the probability of conservation. The only homologs of *Drosophila* genes with highly male-biased expression found on the *Anopheles* X chromosome are autosomal in *Drosophila* (Fig. 4B), and nearly 30% of *Drosophila* X chromosome, male-biased genes are conserved on an *Anopheles* autosome (Fig. 4C). The best-conserved male-biased genes are autosomal in both species (Fig. 4D). Thus, continued X linkage of a gene with male-biased expression in both lineages, presumably reflecting the configuration of the ancestral X chromosome, is highly disfavored. These data unambiguously indicate that X linkage lowers the effective “life-span” of a gene with male-biased expression. Movement to the autosomes can occur by translocation or by preferential retrotransposition of male-biased genes as has been recently shown (20).

It has been postulated that the X chromosome is a favored location for evolution of male advantage alleles because of the lack of a less advantageous second allele at that locus in hemizygotes (3). Indeed, in mammals it appears that the X chromosome is the favored location for male-biased expression, at least for a few genes expressed in primary spermatocytes (4). However, this may be because of compensation, in advance, for the precocious inactivation of those X chromosomes in preparation for meiosis (20). It certainly seems clear that the X chromosome is a poor location for male-biased gene expression in *C. elegans*, and in late spermatocytes of *Drosophila* and humans, where X-chromosome inactivation has been implicated (11, 12, 15, 20). However, X inactivation does not explain the paucity of X-chromosome genes showing male-biased expression in the *Drosophila* soma, or indeed in the bulk of spermatogenesis.

There are multiple forces shaping the X chromosome. Our data suggest that at least some of them result in demasculinization, because of net selection against extant, or poor net de novo creation of, male-biased genes. Although such antagonistic selection has been proposed as a force for masculinizing the X chromosome, or feminizing a chromosome with restricted passage through females, it is easily adapted to the idea of demasculinization. Because the X chromosome is present in females two-thirds of the time, there is pressure against genes with male-biased expression that are detrimental to females. However, it seems odd that those genes showing the most male-bias, and therefore generally showing the lowest expression in females, would be subjected to the strongest negative selection in females. Is leaky expression of genes directing male development sufficiently detrimental to females to be selected against? How can the X chromosome not be favored for de novo male-biased genes, when hemizyosity means that even normally recessive genes are “dominant” and thus subjected to immediate selection in males? Clearly, the sequencing and expression profiles of more organisms to develop better models of the ancestral X chromosome will greatly aid the unraveling of these and other long-standing questions of sex chromosome origin and divergence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Gene expression profiles. Control experiments performed with split samples (A). The twofold cutoff is indicated (red). Adult male versus female (B), testes versus ovaries (C) and gonadectomized male versus gonadectomized females (D). Intensities greater than twofold are indicated by blue (male-biased) and red (female-biased). The average of eight ratios is shown (B to D).

Fig 2.

Gene expression for major chromosome arms. Columns are male-biased (**A, D, G**), female-biased (**B, E, H**), or nonsex-differentially expressed (**C, F, I**). Rows represent comparing testes versus ovaries (**A to C**), whole adult males versus females (**D to F**) and gonadectomized males versus gonadectomized females (**G to I**). The percentage of genes with male, female, or non-biased expression are shown: less than twofold (open), greater than twofold (light), greater than fourfold (dark), and > 10-fold (black). Remaining genes were not reliably detected. Significant deviations (chi-square) are indicated for greater than twofold (** $P < 0.0001$; * $P < 0.005$).

Fig 3.

Expression parsed into chromosome arms (A and B). The relative distribution between the X chromosome (A) and a typical autosome arm (B) is shown. Expression of X-chromosome (red) and autosomal (black) genes encoding 38 ribosomal proteins: gonads (C), gonadectomized (D). No significant differences in regression lines (C). The difference in intercept is significant, but is well within the twofold range (D).

Fig 4.

Drosophila/Anopheles homology correlates expression and location. Percentage of *Drosophila*: X-chromosome genes with X chromosome (A), autosomal genes with X chromosome (B), X-chromosome genes with autosomal (C), and autosomal genes with autosomal (D) *Anopheles* homologs.