Intergenomic conflict revealed by patterns of sex-biased gene expression

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Intergenomic conflict can affect the distribution of genes across eukaryotic genomes. Because the phenotypic optima of males and females often differ, the fitness consequences of newly arisen alleles might not be concordant between the sexes and can be sexually antagonistic – genetic variants favored in one sex are deleterious in the other. In this article, we demonstrate that previously unexplained patterns of sex-biased gene expression in Drosophila melanogaster might have evolved by sexual antagonism, and that the majority of sex-biased expression is due to adaptive changes in males, implying that males experience stronger selection than females.

Introduction

The ultimate fate of new mutations is inextricably linked to the evolutionary forces acting on them. Selective pressures commonly differ between males and females, and might actually operate in opposing directions [1]. The evolution of sex-limited gene-expression is expected as a result of this ‘sexually antagonistic’ genomic tug of war, leaving a characteristic genetic footprint that depends on the degree of dominance on an allele and which of the two sexes benefits [2–4]. The X chromosome is predicted to be a haven for sexually antagonistic variation because dominant alleles that benefit females are under positive selection during two-thirds of their evolution, and rare recessive male-benefiting alleles are masked in females [3].

Because changes in gene regulation, as opposed to coding sequence, are important generators of phenotypic diversity [5], and coding sequence and regulatory evolution are often decoupled [6,7], microarrays (i.e. gene expression profiles) offer a window into the evolutionary processes shaping the genome. Studies have revealed that an amazing proportion of the genome is sex-biased in expression (i.e. expression level is sexually dimorphic) [8–13] and that a significant number of genes show newly derived sex-limitation between recently diverged species [11]. Male- and female-biased genes also exhibit idiosyncratic X-linkage patterns: male-biased genes are strongly deficient, whereas female-biased genes are slightly over-abundant [10,13]. However, inferences about the processes driving patterns of sex-biased expression have been constrained by the lack of a conceptual framework in which to (i) identify sex-specific adaptive change; and (ii) translate gene expression data into the classic population genetic constructs of recessive and dominant mutations. Both are fundamental to testing whether patterns of genomic divergence reflect sexually-antagonistic selection.

In this article, we consider published cDNA microarray data [11] under alternative models of adaptive change, with attention to whether shifts in expression correspond to recessive or dominant substitutions. Within this framework, we analyzed thousands of sex-biased genes,
including > 800 genes that appear to have evolved recent sex-limited expression in either *Drosophila melanogaster* or its sister taxon *D. simulans*. Our results demonstrate that previous assumptions about sex-biased expression are too simplistic – sexually dimorphic profiles often reflect downregulation in one sex but more commonly arise from upregulation in the other sex. We also show that the majority of shifts to sex-biased expression are due to adaptive change in males, including cases of increased expression (producing a male-biased gene) and decreased expression (producing a female-biased gene), implying that males experience stronger selection than females. Finally, analysis of the genomic distribution of loci reveals that previously unexplained genomic patterns of sex-biased expression might have evolved by sexual antagonism – a process that is often cited [14–21] but rarely directly tested.

**Framework for detecting sexual antagonism from microarray data**

Tests of intergenic conflict require information about how the sex-biased expression originated (Figure 1). The adaptive changes underlying male- and female-biased gene expression might reflect selection for increased or decreased expression in one of the two sexes (not just downregulation in the disfavored sex as has been assumed [22,23]). To determine whether sexually dimorphic expression profiles reflect shifts in male or female expression (Figure 1), a baseline expression value was established from non-sex-biased genes. Comparing male and female levels of expression in a sex-biased gene with this baseline revealed which sex had undergone a selectively driven shift in expression (see the supplementary material online). We also conducted additional analyses restricted to sex-biased genes that are lineage specific, where the shift in expression would reflect a change in one of the two sexes from a presumably non-sex biased ancestral condition. To determine the direction of evolutionary divergence, we compared the average expression in the sex-biased species with the average expression in the non-sex-biased sister species (supplementary material online).

Testing the sexual antagonism hypothesis also requires knowledge about the dominance of alleles involved in the evolution of sex-biased expression. Alleles that act antagonistically and affect gene expression can have a range of effects. An allele might cause a small change in gene expression when heterozygous (causing little, if any, phenotypic change), or it might cause a more dramatic phenotypic change. Depending on the magnitude of expression change, and hence expression of the antagonistic phenotype, sex-limited expression will be selectively favored to offset the fitness costs associated with these sexually antagonistic alleles, thereby producing differences in expression between males and females. The magnitude of sex-biases (i.e. the ratio of expression between the sexes) should therefore provide information about the relative dominance of alleles causing expression shifts: large sex-biases should reflect relatively dominant substitutions, whereas small sex-biases might reflect substitutions with lesser effects on heterozygote fitness (supplementary material online).

**Sex-specific adaptive change**

Tests of sexual antagonism require information about how the sex-biased expression originated (i.e. directionality of change). Comparing male and female expression values in sex-biased genes with average expression values for non-sex-biased genes enabled us to identify the direction of evolution that has produced sex-biased expression (Figure 1). Both male- and female-biased genes are highly expressed relative to non-sex-biased genes (Figure 2). This suggests that male-biased (female-biased) genes reflect male-benefiting (female-benefiting) expression changes in the majority of cases (Figure 1). Quantitatively, female-biased genes show a less marked (and non-significant) trend of upregulation (Figure 2), which suggests that directional selection on males for expression divergence is either more common or stronger than selection on females.

**Evidence of intergenic conflict**

The genomic distributions of both male- and female-biased genes appear to support the sexual antagonism hypothesis. Male-biased genes showed a significant decline on the X chromosome, whereas female-biased genes exhibited increased X-linkage, as expression fold increased (Figure 3). The robustness of these patterns was largely confirmed by separate analyses of recently evolved sex-biased genes in *D. melanogaster* or *D. simulans* (i.e. genes that exhibited sexually dimorphic expression in one of the two species; see supplementary material online). The patterns suggest that relatively dominant substitutions are favored on the X chromosome when they benefit males, but are disfavored on the X chromosome when they benefit females. The stronger pattern for male-biased genes might reflect the greater majority of male-biased genes that have arisen owing to selection for higher expression (Figure 2).

**Concluding remarks**

Microarray experiments on *Drosophila* and *Caenorhabditis elegans* have revealed a pattern where male-biased
genes (i.e. genes more highly expressed in males) are deficient and female-biased genes are overabundant on the X chromosome, prompting some researchers to doubt the efficacy of sexual antagonism in shaping the genome [10,11]. However, this conclusion is based on unverified assumptions about the adaptive significance of sex-biased genes. Our analyses show that the relative contributions of male and female adaptive divergence to the formation of sex-biased gene expression and the dominance coefficients of sexually antagonistic alleles will profoundly influence the genomic patterns of sex-biased genes. By considering that patterns of sex-limited expression can reflect antagonism over expression level (i.e. males and females have different expression optima), genomic distributions of sex-biased genes conform to predictions of the sexual antagonism model. These findings also provide corroborative support for studies indicating that cis-regulatory changes primarily contribute to evolutionary divergence in expression [24,25]; the genetic signature of sexual antagonism would not be observable if trans-regulatory changes predominated.

Because the adaptive significance of sex-biased expression divergence cannot be dealt with on a gene-by-

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**Figure 2.** Distributions of sex-biased genes relative to non-sex-biased genes in (a) female-biased genes and (b) male-biased genes. A baseline level of expression was established from median expression levels for non-sex-biased genes. Each sex-biased gene was categorized into one of four groups based on its direct influence of expression difference from the baseline (depicted by arrows, representing higher or lower expression levels relative to the baseline): (i) expression in both sexes below; (ii) one sex is marginally above and the other markedly below; (iii) one sex is marginally below and the other markedly above; and (iv) expression in both sexes is above. When downregulation and upregulation contribute equally to the formation of sex biases, below-expressed genes should be as abundant as above-expressed genes. Asymmetric contribution by one form of expression change (i.e. up- or downregulation) is apparent when below-expressed (red) or above-expressed genes (blue) predominate. Results for both Cy-5 and Cy-3 dyes (microarray platform) were independently determined and averaged from data provided by Jose Ranz (raw data from Ranz et al. [11]); although there is a dye effect, it does not affect one sex more than the other. Significance was determined by performing Chi-square tests. *P<0.05; **P<0.01; ***P<0.001.
gene basis, a baseline level of expression determined from non-sex-biased genes had to be used to identify the general trend of upregulation in sex-biased genes (Figure 2). The use of average expression of non-sex-biased genes as a benchmark might not be ideal. Nevertheless, separate analysis of lineage-specific sex-biased genes (supplementary material online), comparing their expression level with that of the non-biased orthologous gene in the other species (which can give a more accurate measure of the ancestral state), also showed the same general patterns (Figure S1 in the supplementary material online), suggesting that our conclusions are robust to the assumptions required. Similarly, our inference of allelic dominance based on sex-bias magnitude is a rough classification of dominance. However, there is a body of data and theory that supports the basis of our dominance classifications (supplementary material online). Finally, although we assumed that the evolution of sex-biased gene expression is a derived state (the validity of which cannot be confirmed because of the lack of phylogenetic information), a violation of this assumption is expected to merely distort the signal of sexual antagonism. The emergence of clear patterns of X-linkage in accordance with our predictions is therefore particularly provocative in light of the expected noise.

Although our findings support the hypothesis of sexual antagonism, other processes can also influence the genomic distribution of sex-biased genes. For example, the frequency of X-linked male-biased genes (Figure 3) is lower than the expected 16% (the X represents ~20% of the Drosophila genome; 16% of genes in this microarray data set were X-linked) at both high and low
expression-fold differences between the sexes. One potential factor influencing the distribution of male-biased genes is X-inactivation [26,27]; testis-expressed genes should be restricted to autosomes to maintain normal spermatogenetic function because the male X chromosome is inactive during meiosis. Fine temporal and spatial analyses of sex-biased gene expression in Drosophila testis (as described in mice [28]) should clarify the relative impact of this process on X-chromosome gene content.

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Supplementary data
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Supplementary material

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Publicly available microarray data (Ranz et al. [1]; http://www.sciencemag.org/cgi/content/full/300/5626/1742/DC1) from Drosophila melanogaster and D. simulans were downloaded and sorted into several gene classes: (i) non-sex-biased genes (D. melanogaster N = 1204; D. simulans N = 1249); (ii) male-biased genes (D. melanogaster N = 1316; D. simulans N = 1375); and (iii) female-biased genes (D. melanogaster N = 2072; D. simulans N = 1968). Genes exhibiting sex-bias were further subdivided into conserved (the direction of bias is the same for D. melanogaster and D. simulans) and lineage-specific sex-biased genes. Cytological positions for each gene were obtained through Flybase (www.flybase.org).

We identified a subset of cDNA probes (133) that are not associated with currently recognized genes or that lacked expression estimates. The remaining sample size included 4643 genes, roughly one third of the D. melanogaster genome. Raw fluorescence intensity values (per gene, per replicate) were kindly provided by Jose Ranz. Mean and median scores for each gene (measured separately for each sex and dye) did not deviate substantially, which is expected if microarray variance is normally distributed. However, there was an apparent dye effect. We therefore analyzed median values for each dye.

Additional analyses of the direction of adaptive change

A subset of genes (n = 878) of the thousands of sex-biased genes, identified by Ranz et al. [1], were sex-biased in only one of the two species, D. melanogaster or D. simulans. To confirm the result that sex-biased expression is primarily due to adaptive changes in males (see main text), we conducted additional analyses restricted to these sex-biased genes that are lineage specific, where the shift in expression would reflect a change in one of the two sexes from a presumably non-sex biased ancestral condition. To determine the direction of evolutionary divergence, we compared the average expression in the sex-biased species to the average expression in the non-sex-biased species. The difference in the average expression between non-sex biased genes and sex-biased genes can identify which of the two sexes has undergone an adaptive divergence. If the average expression of non-sex biased genes in greater than the average expression of sex-biased genes (i.e. the observed difference between non-sex biased and sex-biased gene expression reflects a reduction in expression in one of the two sexes), it suggests that the sex with lower expression has undergone an adaptive shift. However, a smaller average expression value of non-sex biased genes relative to sex-biased genes (i.e. the observed difference between non-sex biased and sex-biased gene expression reflects an increase in expression in one of the two sexes) would suggest adaptive divergence in the sex with higher expression.
The results from the lineage specific analyses were consistent with our conclusions based on the entire dataset (see main text) – namely, shifts to sex-biased expression predominately reflect adaptive changes in males. With the exception of a slight surplus of female-adaptive expression change in female-biased genes in *D. melanogaster*, all comparisons were in the predicted direction, but all were not significant with this reduced sample size.

To test whether these adaptive shifts in expression have had sexually antagonistic consequences, the genomic location of these sex-biased genes were considered. If adaptive divergence of male (female) expression is hindered (enhanced) on the X chromosome, the frequency of X-linkage in male- (female-) divergent genes is expected to decrease (increase) along with the magnitude of expression change (a function of sex-biased expression fold), owing to the presumed heterozygous phenotypic effects of alleles altering gene expression (see below for details). These predictions are supported by the results from the logistic regression analysis (Figure S1), indicating that patterns of X-linkage in sex-biased genes evolved by sexual antagonism.

The concordance of results from this different method of analysis (i.e. considering patterns of sex-biased expression that are lineage-specific) with those based on our previous analyses of all sex-biased genes supports our conclusion that sexually antagonistic selection has contributed to the genomic-linkage patterns of sex-biased genes (i.e. the overabundance of female-biased and deficiency of male-biased genes on the X chromosome). If the earlier results (see main text) were an artifact of the employed analysis (i.e. by assigning directionality in divergence by comparing sex-biased gene expression with median non-sex biased gene expression within species), then the predicted patterns of X-linked sex-biased genes would not have been independently observed (Figure S1). Furthermore, independent analysis of lineage-specific sex-biased genes also suggests that genomic structuring owing to sexually-antagonistic selection is an ongoing process in *Drosophila* evolution, as has been indicated by recent work [e.g. 2,3].
**Figure S1.** X-linkage as a function of fold difference in expression between the sexes for lineage-specific sex-biased genes. Sex-biased expression levels of X-linked and autosomal genes (shown in blue and green, respectively) were those reported by Ranz et al. [1]. The relationship between X-linkage and expression fold difference between the sexes (shown in red) was determined by logistic regression (JMP ver. 4.0; SAS Institute). Small sample size hinders statistical power however trends for each plot follow predicted patterns of the sexual antagonism model.

The relationship between gene expression and phenotypic dominance and recessivity

A general prediction of the physiological model of dominance [4] is that the magnitude of expression change in new alleles should be correlated with the probability that fitness effects will be present in heterozygous individuals (Figure S2). The magnitude of change required will be determined by the relationship (i.e. linear or nonlinear) between fitness and the amount of gene product, and this might vary according to gene categories. Nevertheless, the predicted positive correlation between expression divergence and degree of dominance should generally hold for all classes of genes, particularly when the direction of expression change causes an increase in gene product. Research over the past 15 years [5,6] has lent strong support to Wright’s model. For example, enzymes follow a diminishing returns function (thus, knockout alleles act recessively), whereas non-enzymatic genes (i.e. regulatory, structural, signaling and binding proteins) tend to show greater dosage sensitivity [7].
Figure S2. Hypothetical relationships between phenotypes (fitness) and gene dosage, according to Wright’s physiological theory of dominance (modified from Kondrashov and Koonin [7]). The red function shows a linear relationship between expression and phenotype. Alleles increasing or decreasing dose in such genes should generally have codominant effects on phenotype and fitness. Alternatively, the blue curve represents a diminishing return function of increasing gene dose. The dominance of alleles increasing or decreasing expression for such genes will be determined by the magnitude of dose change each allele causes, and by the relationship between phenotype and the wild type genotype (i.e. the location along the curve that describes the phenotype of wild type homozygotes).

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