Sex-specific effects of cis-regulatory variants in *Drosophila melanogaster*

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Abstract

Sexual dimorphism at the level of gene expression is common and well-documented, but much less is known about how different cis-regulatory alleles interact with the different trans-regulatory environments present in males and females. Here we show that sex-specific effects of cis-regulatory variants are common in Drosophila.
A hallmark of dioecious organisms is sexual dimorphism, phenotypic differences between males and females of a species such as size, coloration, and behavior. Differences in these organism-level exophenotypes are governed by sexual dimorphism in underlying endophenotypes including the regulation of gene expression (reviewed in Williams and Carroll 2009). Gene regulation is central to sexual dimorphism because males and females carry the same genome, except for their sex chromosomes. Indeed, the extent to which the genome is differently expressed in the two sexes is quite striking -- estimates in *Drosophila* suggest that approximately half of the genes in the genome are expressed differently in males and females (Gnad and Parsch 2006; Innocenti and Morrow 2010; Jin *et al.* 2001).

Mechanistically, the regulation of gene expression is governed by the interaction of *cis-*regulatory DNA sequences at each gene with *trans-*regulatory proteins and RNAs present in each cell (reviewed in Wray *et al.* 2003); the same *cis-*acting sequences have different activities in the different *trans-*regulatory environments of males and females. But, do sex-specific differences in the *trans-*regulatory environment generally have similar effects on alternative *cis-*regulatory alleles of a gene? Or, put another way, how often do *cis-*regulatory variants have sex-specific effects?

To address this question, we used pyrosequencing (Ahmadian *et al.* 2000) to measure relative allele-specific expression for 11 randomly selected autosomal genes in male and female F₁ progeny from reciprocal crosses between the highly inbred *D. melanogaster* lines *zhr* and *z30* (Begun and Aquadro 1993; Coolon *et al.* 2012; Ferree and Barbash 2009; Sawamura *et al.* 1993; Wu *et al.* 1995). Relative allele-specific expression in heterozygous genotypes provides a direct
read-out of relative cis-regulatory activity (Cowles et al. 2002; Wittkopp et al. 2004). These reciprocal crosses produced four genetically distinct progeny with identical autosomal genotypes (i.e., heterozygous for the zhr and z30 alleles at all autosomal loci) that differ in the identity of their sex chromosomes and/or the parent-of-origin for all of their chromosomes (Figure 1A). For each genotype, RNA and genomic DNA were extracted from 4 biological replicates containing 20 whole flies (7-10 days old) each and analyzed by pyrosequencing using gene specific primer sets (see Table S1) and protocols described in Wittkopp (2011).

Pairwise comparisons among these four genotypes resulted in six tests for differences in relative cis-regulatory activity between alleles of autosomal genes in different trans-regulatory backgrounds (Figure 1B-F). First, we compared female progeny from reciprocal crosses, which are genetically identical except for any epigenetic marks resulting from the maternal and paternal transmission of alleles known as genomic imprinting (Figure 1B). Next, we compared male progeny from reciprocal crosses, in which relative cis-regulatory activity could differ because of genomic imprinting and/or differences in X and Y chromosome genotypes; genetic differences between the zhr and z30 sex chromosomes have the potential to interact epistatically with cis-regulatory differences between the zhr and z30 alleles of the autosomal genes tested (Figure 1C). In the third and fourth comparisons, we examined male and female progeny from the same cross (Figure 1D,E). Differences in relative cis-regulatory activity of autosomal genes in these cases could be caused by epistatic effects of trans-acting variants located on the X and/or Y chromosomes and/or differences in the trans-regulatory environment between males and females resulting from sexual dimorphism (i.e. the same pairs of cis-regulatory variants react differently to the trans-regulatory environment of males and females resulting in a sex\*cis interaction).
Finally, in the fifth and sixth comparisons, we contrasted male progeny from one cross with female progeny from the reciprocal cross (Figure 1F,G). Differences in relative cis-regulatory activity of autosomal genes in these comparisons could come from genomic imprinting, epistatic effects of genetic differences on the sex chromosomes, and/or sexually dimorphic trans-regulation. In all cases, if relative activity of the zhr and z30 cis-regulatory alleles for autosomal genes is independent of the difference(s) in trans-acting environment, then relative allele-specific expression of these genes should be similar between the two genotypes compared. If, however, the cis- and trans-regulatory differences interact, relative allele-specific expression should differ between genotypes.

Measures of relative cis-regulatory activity ($Y_{ijk}$) were calculated from the pyrosequencing data as $\log_2(zhr/z30)$ for each gene ($i$) in each sex ($j$) from each cross ($k$), as described in Wittkopp (2011). These data were then fitted to the following linear model using proc MIXED in SAS v10.3 (Cary, NC): $Y_{ijk} = \mu + \text{Sex}_j(Gene_i) + \text{Cross}_k(\text{Sex}_j(Gene_i)) + \epsilon$. This model controlled for the differences in cis-regulatory activity among genes and allowed us to focus on the effects of different trans-regulatory backgrounds on relative cis-regulatory activity of the autosomal zhr and z30 alleles. We examined the effects of genomic imprinting, epistasis with trans-acting variants on the sex chromosomes, and sex* cis interaction with sexually dimorphic trans-regulatory environments on individual genes using the differences in least-squares means and 95% confidence intervals for these differences derived from this model. An interaction was considered statistically significant for a gene if the 95% confidence interval of the difference did not include zero. This is a conservative test for the absence of an interaction because it does not control for the increased false positive rate resulting from multiple testing.
Comparing females from reciprocal crosses (Figure 1B), we found no statistically significant evidence of genomic imprinting for any gene (Figure 2A), consistent with prior studies (Coolon et al. 2012; Wittkopp et al. 2006). In the comparison where relative cis-regulatory activity could be affected by either imprinting or genetic differences between X and/or Y-chromosomes (Figure 1C), one gene showed a statistically significant effect (Figure 2B). Given the absence of evidence for imprinting in the first comparison, we conclude that this difference most likely resulted from epistatic effects of one or more trans-acting loci that differ between the zhr and z30 alleles of one or both sex chromosomes. Previous studies provide mixed evidence for this type of epistasis: an intraspecific comparison of D. melanogaster females found no evidence for it among the eight genes tested (Wittkopp et al. 2008), whereas a study of interspecific Drosophila hybrids (D. yakuba and D. santomea) found evidence for it affecting 19 of the 22 genes tested (Llopart 2012). We observed much larger differences in relative cis-regulatory activity in all comparisons between males and females (Figure 1D-G), with significant differences observed for 6 of 11 genes tested in at least one of the four comparisons (Figure 2C-F). The statistical significance of the difference in relative cis-regulatory activity varied among comparisons for some genes, but the relative magnitude of the differences was generally consistent among genes in all four comparisons (Figure 2C-F). This is consistent with differences in trans-regulation between males and females that are similar in all four contrasts and primarily responsible for the differences in relative cis-regulatory activity observed. Statistical significance of the Sexj (Genei) and Crossk (Sexj (Genei)) terms in the full model provide further support for these conclusions (Table 1): after controlling for gene specific effects, differences between sexes (reflecting sexual dimorphism) explained much more of the total variation in relative cis-regulatory activity (F =
119) than the combined effects of genomic imprinting and epistasis with X- and Y-linked variation captured by the reciprocal crosses (F = 5).

Sexual dimorphism creates differences in gene expression between males and females (Gnad and Parsch 2006; Innocenti and Morrow 2010; Jin et al. 2001), and the data presented here show that these sex-specific trans-regulatory environments often interact differently with alternative cis-regulatory alleles of a gene. This suggests that many cis-regulatory polymorphisms have different effects in males and females. Interactions between sexually dimorphic trans-regulatory environments and species-specific cis-regulatory alleles also were recently observed between D. simulans and D. mauritiana using a different experimental design (MeikleJohn et al. 2013), indicating that these effects are not limited to cis-regulatory variants segregating within a species. Furthermore, while our observations are based on a small subset of the genome, the genes used are not enriched for particular functional groups, chromosomal location, or magnitude of cis-regulatory differences (data not shown) suggesting that the set is unbiased and that sex*cis-regulatory variant interactions are common. These types of interactions can result, for example, from cis-regulatory variants that affect binding sites for trans-regulatory factors that differ between the two sexes (Cooley et al. 2012; Williams and Carroll 2009), as was reported for the Drosophila desatF gene (Shirangi et al. 2009).

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**Literature Cited**


Table 1: Summary of effects from the general linear model

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex(gene)</td>
<td>21</td>
<td>75.82</td>
<td>3.61</td>
<td>119.23</td>
<td>&lt; 1E-25</td>
</tr>
<tr>
<td>cross(sex(gene))</td>
<td>22</td>
<td>3.08</td>
<td>0.14</td>
<td>4.63</td>
<td>1.40E-08</td>
</tr>
</tbody>
</table>

DF = degrees of freedom
Figure 1. Separating the effects of genomic imprinting, epistatic interactions and sexual dimorphism using reciprocal crosses. (A) Chromosomes present in the parental strains and F₁ offspring (excluding the “dot” 4th chromosome) are shown with chromosomes derived from zhr in black and chromosomes derived from z30 in grey. Note that all four types of offspring are heterozygous for all autosomes. (B-G) Six comparisons were performed, contrasting each type of offspring with each other type. For each genotypic type, only the sex chromosomes are shown. The source(s) of interactions potentially affecting relative cis-regulatory activity of autosomal genes in each comparison is shown, with “Imprinting” = genomic imprinting, “X” = epistatic interactions with variable X- or Y-linked loci, and “Sex” = sexually dimorphic trans-regulatory factors.

Figure 2. Relative cis-regulatory activity differed the most between males and females. For each of the six comparisons described in Figure 1B-G, the difference in relative cis-regulatory activity for each of the 11 genes tested is shown using the least-squares means (LS Means) and corresponding 95% confidence intervals derived from the general linear model described in the main text. Panels A-F in this figure correspond to panels B-G in Figure 1, respectively. In each case, the difference was considered to be statistically significant if zero was not contained within the 95% confidence interval. The potential causes of significant differences are indicated for each comparison, with “Imprinting” = genomic imprinting, “X” = epistatic interactions with variable X- or Y-linked loci, and “Sex” = sexually dimorphic trans-regulatory factors.
A

\[
\begin{array}{ccc}
\text{zhr} & \text{z30} & \text{zhr} \\
\varnothing & \text{♀} & \varnothing \\
\varnothing & \text{♀} & \varnothing \\
\end{array}
\]

\[
\begin{array}{ccc}
\text{z30} & \text{zhr} & \text{z30} \\
\varnothing & \varnothing & \varnothing \\
\varnothing & \varnothing & \varnothing \\
\end{array}
\]

B

1 vs. 3

Imprinting

C

2 vs. 4

X, imprinting

D

1 vs. 2

Sex, X

E

3 vs. 4

Sex, X

F

1 vs. 4

Sex, X, imprinting

G

2 vs. 3

Sex, X, imprinting