Sexual dimorphism in gene expression: coincidence and population genomics of two forms of differential expression in *Drosophila melanogaster*

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Abstract

In most species, selection favours different phenotypes in the two sexes. This type of sexual antagonism can be resolved through the evolution of sexual dimorphism. Sex differences in gene regulation is a proximate mechanism by which this resolution can be achieved. One form of differential gene regulation is sex differences in the amount a gene is expressed, so called sex-biased gene expression (SBGE). Less attention has been given to sexual dimorphism in isoform usage (SDIU), resulting from sex-specific alternative splicing, which may be another way in which conflict between the sexes is resolved. Here, we use RNA-seq data from two tissue types (heads and bodies) from 18 genotypes of adult *Drosophila melanogaster* to investigate SDIU. In our data, SBGE and SDIU are both much more prevalent in the body than the head. SDIU is less common among sex-biased than unbiased genes in the body, though the opposite pattern occurs in the head. SDIU, but not SBGE, is significantly associated with reduced values of Tajima's D , possibly indicating that such genes experience positive selection more frequently. SBGE, but not SDIU, is associated with increased π_N/π_S , possibly indicating weaker purifying selection. Together, these results are consistent with the idea that the SDIU and SBGE are alternative pathways towards the resolution of conflict between the sexes with distinct evolutionary consequences.

Introduction

In sexual populations, the sexes employ different reproductive strategies to maximize fitness and as result, shared phenotypes often exhibit divergent, sex-specific trait optima. Because the sexes largely share a genome, the genetic basis of such traits is often the same between the sexes. Thus, genetic variation underpinning such shared traits will often experience divergent, sexually antagonistic selection resulting in an evolutionary conflict between the sexes termed ‘intra-locus sexual conflict’ (Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009). Intra-locus sexual conflict has been well documented in a variety of plant (e.g., Hersh et al. 2015; Zemp et al. 2016) and animal (e.g., Arnqvist and Rowe 2005; Cox and Calsbeek 2009) species and may be an important mechanism in maintaining genetic variation (Kidwell et al. 1977; Rice 1984). Ultimately, intra-locus sexual conflict is thought to be resolved through the evolution of sexual dimorphism (Lande 1980; Rice 1984).

Gene expression can be viewed as a trait in its own right or as a proximate mechanism affecting ‘traditional’ traits (e.g., morphological, behavioural, or physiological). Over the last ~15 years, there have been an explosion of studies investigating sexual dimorphism in gene expression, typically called ‘sex-biased gene expression’, SBGE (Ellegren and Parsch 2007; Parsch and Ellegren 2013; Mank 2017). SBGE refers to sex differences in the abundance of all transcripts mapping to a given gene. In addition to dimorphism in total expression of a gene, the sexes may differ in other aspects of the transcriptome. Here we focus on sexual dimorphism in isoform usage (SDIU).

Differential isoform usage due to alternative splicing of mRNA transcripts results in the production of different isoforms from the expression of a single gene. Differential isoform usage is a widespread phenomenon among multicellular eukaryotic organisms (Ast 2004). For example, in humans, it is estimated that over half of all expressed genes exhibit alternative splicing (Mironov et al. 1999; International Human Genome Sequencing Consortium 2001). Because the function of proteins and other gene products depends on the specific exons retained in mRNA transcripts after transcription, differential isoform usage has the potential to greatly expand the functional repertoire of the genome. Indeed, in humans, the majority of genes exhibiting alternative splicing are thought to cause functionally significant alterations to gene products (Modrek et al. 2001; Modrek and Lee 2002; Sorek et al. 2004). Thus, differential

isoform usage can allow a single gene to alter or take on different functional roles across developmental stages (e.g., Gibilisco et al. 2016) tissue types (e.g., Barberan-Soler and Zahler 2008; Telonis-Scott et al. 2009; Revil et al. 2010; Ramani et al. 2011; Tan et al. 2013) and abiotic environments (Long et al. 2013; Jakšić and Schlötterer 2016). Across the sexes, differential isoform usage could thus allow the sexes to express their optimal trait values through the production of different gene products despite sharing a genome.

Widespread sexual dimorphism in isoform usage has been documented in humans (Trabzuni et al. 2013) and other primates (Blekhman et al. 2010). A recent study documented dimorphism in isoform usage in birds (Rogers et al. 2020). Among genes exhibiting SBGE, they found a more even usage of alternative isoforms in the sex where the gene was more highly expressed, a pattern that they interpreted as evidence of sex-specific selection driving SDIU. Several microarray and RNA-seq studies across *Drosophila* species have also investigated sexual dimorphism in isoform abundance and exon usage. These studies have generally supported a view that sexual dimorphism in isoform usage is widespread (e.g., McIntyre et al. 2006; Telonis-Scott et al. 2009; Chang et al. 2011), tissue-specific (Telonis-Scott et al. 2009), and evolutionarily conserved (Gibilisco et al. 2016).

Here, we further explore SDIU using publicly available RNA-seq data from two tissue types (head and body tissue) of 18 genotypes of adult *Drosophila melanogaster*. As SBGE and SDIU can represent different forms of sex-differential gene regulation, we are interested in their relationship. Resolution or mitigation of intra-locus sexual conflict may tend to require the evolution of both SBGE and SDIU; on the other hand, SDIU and SBGE may serve as alternative routes to mitigating sexual conflict so that the evolution of one form of sex-differential regulation obviates selection for the other. We investigate the co-occurrence of SDIU and SBGE, finding SDIU is less common among sex-biased than unbiased genes in the body, though the opposite pattern occurs in the head.

Our other avenue of investigation focuses on population genetic properties of genes exhibiting sex-differential regulation. This follows in spirit from several previous studies, including in seed beetles (Sayadi et al. 2019) and several bird species (Wright et al. 2019), that have examined population genomic properties such as Tajima's D and π_N/π_S in an attempt to infer differences in the form of selection acting on sex-biased versus unbiased genes. We take a

similar approach but use it to compare how the two different forms of expression dimorphism—SBGE and SDIU—are associated with the population genomic metrics used in searching for signatures of sexual conflict resolution. Specifically, we investigate how SBGE and SDIU are related to Tajima’s D and π_N/π_S , finding a reduction in Tajima’s D at genes exhibiting SDIU and a positive relationship between π_N/π_S and SBGE.

Methods

Obtaining RNA-seq Data and Read Mapping

We obtained publicly available raw RNA-seq data from the DNA Data Bank of Japan Sequence Read Archive (Accession number: DRA002265). Detailed methods on animal husbandry, RNA extraction and sequencing can be found in Osada et al. (2017). Briefly, males from 18 randomly chosen inbred lines of the *Drosophila* Genomic Resource Panel (DGRP; MacKay et al. 2012; Huang et al. 2014) were crossed to females from a single inbred strain from a West African population. RNA was extracted from heads and bodies (i.e., whole body minus heads) of 100 virgin F1 males and females from each cross. RNA from female heads and bodies was obtained and sequenced from two replicates of each of the 18 DGRP lines while only a single replicate was available for males. We therefore arbitrarily retained sequence data only for female samples labelled as ‘replicate 1’. Reads were inspected for quality using *FastQC* (v0.11.9; Andrews 2010) and *MultiQC* (v0.8.3; Ewels et al. 2016). Reads were trimmed for adapter content using *Trimmomatic* (v0.39; Bolger et al. 2014). RNA-seq reads were then mapped to the *Drosophila melanogaster* reference genome (release 6.32; Dos Santos et al. 2015) using *STAR* (v2.7.1a; Dobin et al. 2013) with default settings.

Differential Exon Usage Analysis

To identify a list of genes exhibiting SDIU, we performed differential exon usage analysis using the R package *JunctionSeq* (v1.17; Hartley and Mullikin 2016). Briefly, *JunctionSeq* splits genes into annotated exons and splice junction ‘counting bins’ and counts the number of reads that overlap each counting bin within an annotated gene. To test for differential exon usage between the sexes, a generalized linear model (GLM) with logarithmic link function was fit at each gene feature (i.e., exon or splice junction) to assess sex differences in the number

of reads that map to a particular gene feature relative to the total number of reads that map to the gene to which the feature belongs. Specifically, for each gene, *JunctionSeq* fits the model

$$\log(y_{ij}) = \mu + W_i + X_j + Z_{ij}$$

where y_{ij} is the normalized count of reads from the i^{th} sex mapping to the j^{th} gene feature/counting bin. μ is the experiment wide, mean normalized gene expression, W_i reflects the expected number of reads mapping to the focal gene in sex i , X_j reflects the proportion of reads mapping to counting bin j , and Z_{ij} is an interaction term that reflects differences between the sexes in the proportion of reads mapping to counting bin j . A significant effect of the interaction term was taken as evidence of SDIU. To test the significance of the interaction term, the above model was compared to a null model that was identical, but which omits the interaction term.

We restricted our analyses to genes with at least two annotated exons and to exons where at least an average of 50 reads mapped in each sex. To correct for multiple testing, P -values were adjusted using the Benjamini and Hochberg procedure (Benjamini and Hochberg 1995). Genes were considered to show evidence for sexual dimorphism in isoform usage if the sexes exhibited statistically significant differential usage of a single exon (or more) within a gene using an adjusted P -value cut off of $P < 0.01$.

Ideally, genotype would be included in the model as a random effect but *JunctionSeq* does not allow this. This is unlikely to be a major issue here. Our dataset is perfectly balanced with each genotype being represented by only a single sample for each sex for each tissue (head and body data were analyzed separately). More importantly, our goal is to identify a list of genes enriched for SDIU for further analysis; our focus is not on the accuracy of P -values of individual genes. As a simple check of the *JunctionSeq* pipeline, we analyzed 10 datasets in which we switched the male/female labels for a randomly chosen half of the genotypes. In these permuted data sets, no genes were identified as having SDIU at an adjusted P -value cut off of $P < 0.01$.

Gene Expression Analysis

We estimated differential gene expression between the sexes using the R package *DESeq2* (v1.28.1; Love et al. 2014). Briefly, *DESeq2* estimates normalized read counts mapping to genes on a $\log_2$ scale and uses a GLM framework to test for differential gene expression

between experimental conditions. Before performing differential gene expression analysis, we performed a ‘pre-filtering’ procedure to remove genes that had very low numbers of reads mapping. Pre-filtering RNA-seq data has been shown to substantially increase power in detecting differential gene expression by reducing the burden of multiple testing but can also result in an increase in Type I error (i.e., increased false-positive rate) (Bourgon et al. 2010). To balance these competing issues, we removed any genes that had fewer than an average of 50 reads mapping from all 36 samples (i.e., 18 male and 18 female genotypes). We then estimated the $\log_2$ fold change ($\log_2FC$) in male to female gene expression for each annotated gene and retained only genes that were tested for differential exon usage. In downstream analyses, we assigned each gene to bins based on either their average expression of the gene or based on the $\log_2FC$ in male to female gene expression. To assign genes to quantile bins based on average expression, we determined the sex-averaged gene expression of each gene and binned genes into three equal quantiles of increasing sex-averaged gene expression. Separately, we assigned each gene into bins of sex-bias in gene expression. We assigned each gene to one of five bins of predefined ranges of $\log_2FC$ in gene expression ranging from highly female biased ($\log_2FC < -2$), moderately female biased ($-2 \leq \log_2FC \leq -0.5$), unbiased ($0.5 \leq \log_2FC \leq 0.5$), moderately male biased ($2 \geq \log_2FC \geq -0.5$) and strongly male biased ($\log_2FC \geq 2$). This procedure was conducted separately for genes expressed in heads and bodies. Genes for which the 95% confidence interval (CIs) in $\log_2FC$ overlapped with multiple bins were excluded from analyses using this binning procedure.

Population Genomic Data

We obtained whole genome sequences for 205 genotypes of the DGRP from the *Drosophila* Genome Nexus (DGN; Lack et al. 2015, 2016). FASTA files were obtained from the DGN website (<https://www.johnpool.net/genomes.html>) and were filtered to remove sites with evidence of high relatedness and recent admixture using Perl scripts provided on the DGN website. Two files were then created from a multi-FASTA file containing all 205 DGRP genotypes using *SNP-sites* (v2.5.1; Page et al. 2016). The first is a typical VCF file containing only biallelic sites. The second file was formatted identically to a VCF file but contained genotypic information at both variant and invariant sites. Coordinates for both VCF files were

converted to release 6 of the *Drosophila* reference genome using the *UCSC liftOver* (Kent et al. 2002) tool.

Estimating π_N/π_S and Tajima's D

To estimate π_N/π_S , per gene, we obtained a GFF file for the *D. melanogaster* genome from Ensembl (https://useast.ensembl.org/Drosophila_melanogaster/) and scored each coding site in the genome as 4-fold (i.e., synonymous sites) or 0-fold (i.e., non-synonymous sites) degenerate across the *Drosophila* genome using a Python script (script available at <https://github.com/tvkenet/Degeneracy>). We then parsed our VCF containing variant and invariant sites in the DGRP into two files that contained either only 4-fold sites or only 0-fold degenerate sites. Separately for files containing 4-fold and 0-fold degenerate sites, we estimated per site nucleotide diversity (Nei and Li 1979) using *VCFtools* (v0.1.17; Danecek et al. 2011). We then used *bedtools* (v2.25.0 Quinlan and Hall 2010) to map 4-fold and 0-fold degenerate sites to genes and estimated the mean π_N within a gene as the mean nucleotide diversity at 0-fold degenerate sites within each gene and π_S within a gene as the mean nucleotide diversity at 4-fold degenerate sites within each gene. Genes with fewer than 30 4-fold degenerate (i.e., synonymous) sites were filtered out from further analyses.

We used a similar approach to estimating gene-wide Tajima's D (Tajima 1989). Specifically, we identified biallelic SNPs at 4-fold degenerate sites in the DGRP VCF file produced above. We then calculated Tajima's D at each 4-fold degenerate site. 4-fold degenerate, segregating sites were then mapped to genes in the *D. melanogaster* genome and Tajima's D was averaged over all 4-fold degenerate sites within each gene. Genes with fewer than 30 4-fold degenerate sites were filtered out from further analyses.

Gene Ontology (GO) Enrichment Analysis

We tested for an enrichment of gene ontology (GO) terms among genes exhibiting statistically significant SDIU. This analysis was conducted using *GOrilla* (Eden et al. 2009) which uses a hypergeometric model to test for an enrichment of GO terms in a target set of genes relative to a user supplied background set of genes. This analysis was conducted separately for head and body tissue. In both analyses the target set of genes was compared to a background list that was composed of genes that had been tested in the differential exon usage analysis

conducted with *JunctionSeq* but did not exhibit statistically significant SDIU. P -values for GO terms were converted to Q -values and a Q -value threshold of 0.01 was used to assess statistical significance.

Statistical Analysis

We used linear regression analysis to examine the relationship between population genomic measures (i.e., Tajima's D and π_N/π_S) and the fixed effects of sex-averaged gene expression, sex-bias in gene expression (measured as the absolute value the $\log_2FC$ of male to female gene expression) and SDIU. To improve the distributional properties for statistical analysis, we analyzed $\log(\pi_N/\pi_S)$ rather than π_N/π_S . This required the exclusion of genes where $\pi_N = 0$, which only represented 6.01% and 5.51% of genes expressed in the body and head respectively. For both Tajima's D and $\log(\pi_N/\pi_S)$, we fit a regression model using the 'lmer' function of the *R* package *permuco* (v.1.1.0; Frossard and Renaud 2019) which uses a Freedman-Lane permutation method (Freedman and Lane 1983) and type III Wald's chi-square test to estimate P -values for fixed effects. P -values were estimated after 10,000 permuted model fits.

To ensure that the exclusion of genes with $\pi_N = 0$ did not fundamentally change the interpretation of the data, we performed a separate logistic regression analysis including these genes in which we treated π_N/π_S as a binary trait (i.e., $x = 0$ representing $\pi_N/\pi_S = 0$ and $x = 1$ representing $\pi_N/\pi_S = 1$). The results of this analysis are described in Table S3 and add further support to the interpretation of the main analysis.

Results and Discussion

Identity and Functional Properties of Genes Exhibiting Sexually Dimorphic Exon Usage

We find evidence of extensive sexual dimorphism in isoform usage (SDIU or 'isoform dimorphism') among multi-exon genes expressed in both head and body tissue of adult *Drosophila melanogaster*. Table 1 summarizes the results of our differential exon usage analysis. Of multi-exon genes tested, 3420 and 539 genes expressed in adult body and head tissue exhibit statistically significant SDIU respectively which accounts for 53.76% and 7.65% of all genes tested in bodies and heads respectively (Table 1). These data suggest that SDIU usage occurs in

approximately 30.33% and 6.06% of all genes annotated in the *D. melanogaster* genome and that are expressed in body and head tissue, respectively. Considering the union of genes expressed in either tissue, we detected SDIU in at least one of the tissues for 29.84% of genes. These data accord well with several previous studies that have found evidence of widespread SDIU both in this species (e.g., McIntyre et al. 2006; Telonis-Scott et al. 2009; Chang et al. 2011) as well as other species of *Drosophila* (Gibilisco et al. 2016). McIntyre et al. (2006) reported that 68% of all multi-transcript genes expressed in whole body tissue exhibited significant sex-bias in the expression of one or more alternative isoforms in a microarray study using *D. melanogaster*. Similarly, a microarray study in this species by Telonis-Scott et al. (2009) found that ~58% of multi-exon genes tested in whole body tissue exhibited SDIU. The lower percentage we report here (~50% of tested genes exhibiting SDIU versus ~60-70%) may reflect differences in technical and statistical methodologies, including the choice of false discovery rates.

Table 1: Summary of the differential exon usage analysis between the sexes.

	Genes Expressed	Genes Tested	Genes with Sexually Dimorphic Isoform Usage (% of total genes expressed)
Head	8904	7006	540 (6.06%)
Body	11,277	6362	3420 (30.33%)
¹ Head and Body	11,825	7646	3528 (29.84%)

¹These data refer to the union of genes expressed, tested and exhibiting sexually dimorphic isoform usage found in either head, body or both.

These results suggest heads and bodies differ substantially in the frequency of SDIU. Although the majority of sexually dimorphic genes in head tissue was also sexually dimorphic in body tissue, there are many genes exhibiting SDIU in body tissue where we did not detect dimorphism in head tissue (Figure 1). An important caveat is that the power to detect differential isoform usage is correlated with overall gene expression, indeed there is a substantial increase in overall gene expression for genes in which we detect SDIU compared to those we do not (Figure S1A). If this were the main driver of tissue differences we observe, then genes with similar

255 expression levels in both tissues should exhibit similar frequencies of SDIU. However, even for
256 genes with similar expression levels in head and body tissue, many more genes appear to exhibit
257 SDIU in body tissue than in head tissue (Figure S1B). In addition, these results comport well
258 with previously published data. For example, Telonis-Scott et al. (2009) reported reduced levels
259 of SDIU in genes when expressed in head tissue compared to carcass (i.e., head- and gonad-less
260 bodies) and gonadal tissue.

261
262 **Figure 1:** Venn diagrams of overlap between genes found to exhibit sexually dimorphic (SD)
263 exon usage when expressed in (A) body and (D) head tissue and genes with no evidence of
264 sexually dimorphic exon usage when expressed in (B) body and (C) head tissue. Only genes that
265 were tested for SDIU in both body and head are represented here.

266
267 Genes exhibiting SDIU are enriched for a variety of gene ontology (GO) terms, though
268 there are far more enriched GO terms for the genes identified as exhibiting SDIU in the body
269 (Table S1) compared to the head (Table S2). For sexually dimorphic genes expressed in head
270 tissue, there is an enrichment for GO terms related to cellular (including neuron specific)
271 components and processes as well as genes related to behaviour. Especially notable GO terms
272 enriched in SDIU genes in the body are GO terms associated with development and reproduction

including genes related to ‘*oogenesis*’, ‘*gamete generation*’ and ‘*germ cell generation*’ as well as genes related to cellular and organelle components and cell metabolism. The enrichment of genes related to reproduction and development may not be surprising considering much of the variation in isoform usage between the sexes has previously been reported as driven by gene expression in gonadal tissue (Telonis-Scott et al. 2009; Gibilisco et al. 2016).

Coincidence of two forms of sexually dimorphism in expression

The majority of expression studies have examined variation in total expression for a gene. Studies of sex-biased gene expression (SBGE) have shown repeatedly that SBGE is highly tissue specific (e.g., Parisi et al. 2004; Yang et al. 2006; Huylmans and Parsch 2014; Dutoit et al. 2018); both our data and other studies (e.g., Telonis-Scott et al. 2009; Chang et al. 2011) indicate that the same is true for sex differences in isoform usage. Because both of these forms of sexual dimorphism are thought to be related to resolving intra-locus sexual conflict, we are interested in understanding the relationship between SBGE and SDIU.

SBGE is widespread both in heads and bodies, though there is greater variation among genes in SBGE in body tissue relative to head tissue (Figure 2A). These results are consistent with previously published data in a number of species that find that sex-bias in gene expression is generally more extensive in gonadal than in somatic tissue including brain tissue (e.g., Innocenti and Morrow 2010; Baker et al. 2011; Dutoit et al. 2018). In the head, SDIU genes are, on average, significantly more female-biased in expression than non-SDIU genes, but there is no difference in the body (Figure 2B).

To further explore the relationship between SDIU and SBGE, we divided genes into bins representing different levels of sex-bias using defined ranges of $\log_2FC$ in gene expression, and only considered genes that could be assigned to bins with reasonably high certainty (see methods). Within each bin, we then calculated the proportion of isoform dimorphic to non-dimorphic genes in each quantile bin. For genes expressed in the body, there is a reduction in the proportion of isoform dimorphic genes among sex-biased genes relative to unbiased genes (Figure 2C). Conversely, for genes expressed in head tissue, female-biased and male-biased genes are enriched for SDIU (Figure 2D). The fly body results are in line with Rogers et al. (2020) who reported non-significant trends towards a negative relationship between SDIU and SBGE in gonadal tissue of three bird species.

The reduction in isoform dimorphism at highly sex-biased genes expressed in the body could be because SBGE and SDIU provide alternative routes to the resolution of sexual conflict and thus the evolution of one reduces the selective pressure for the evolution of the other. On the other hand, the opposite pattern emerges in genes expressed in the head with an apparent increase in the proportion of genes exhibiting SDIU at sex-biased genes relative to unbiased genes. One hypothesis is that the resolution of intra-locus conflict may often require both forms of expression dimorphism whereas those genes experiencing sexually concordant selection will evolve neither, creating a positive association between SDIU and SBGE. A somewhat more mechanistic hypothesis for the positive association is that just as SBGE is more likely to evolve at genes that exhibit lower pleiotropic constraint (Dean and Mank 2016), SDIU may also be able to more easily evolve at such genes. In birds, however, Rogers et al (2020) found that genes exhibiting SDIU exhibited greater tissue breadth in gene expression (used as an indicator of pleiotropic constraint) than non-SDIU genes. A third, more mundane, explanation is that the positive association of SBGE and SDIU in heads exists because of how we quantify gene/isoform expression (i.e., counting reads mapping to genes); we may be biased towards finding a positive association between SBGE and SDIU because we are observing the same phenomenon in different ways. For example, if both males and females express a single isoform of a gene at the same level but females also express an additional isoform that is not expressed by males, we would detect both SBGE and SDIU at this gene. However, this methodological bias must not be very strong as there are many unbiased genes that exhibit SDIU (704 in head and 405 in body) and the association between SBGE and SDIU is negative in the body.

Figure 2: Patterns of sex-bias in gene expression (SBGE) and sexual dimorphism in isoform usage (SDIU) for genes expressed in the body and head tissue. **(A)** Scatter plot and density distributions of $\log_2$ fold change ($\log_2$ FC) in male to female gene expression of genes exhibiting SDIU only in bodies (light blue), only in heads (red), in both heads and bodies (dark blue) or in neither heads nor bodies (beige) when expressed in bodies (x-axis) or heads (y-axis). Density plots above and to the right of the figure correspond to the $\log_2$ FC gene expression in bodies and heads respectively. Some genes with extreme sex-bias in expression were omitted from scatter plot, see Supplemental Figure S2 for plots with all genes. **(B)** Mean $\log_2$ FC in male to female gene expression at genes exhibiting SDIU (green) and those that do not exhibit SDIU (purple) when expressed in bodies or heads. **(C-D)** The proportion of genes exhibiting SDIU to genes not exhibiting SDIU in bins of $\log_2$ FC in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue) expression when expressed in **(C)** bodies or **(D)** heads. Genes in the most strongly sex-biased bins were excluded from plot of genes expressed in heads **(D)** because too few genes (i.e., fewer than 5) were present in these bins; their inclusion strengthens the pattern (see Supplemental Figure S3 for plots including omitted bins). Error bars in panels B-D are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

Given that the X chromosome spends a disproportionate amount of its time in females, and because of its hemizygous expression in males, there has been considerable interest in whether it is enriched for genes that experience sexually antagonistic selection contributing to intralocus conflict or contributing to sexual dimorphism (Rice 1987; Rowe et al. 2018; Frank and Patten 2020). We therefore examined the genomic distribution of SDIU and SBGE genes. In the body data, there is no evidence of an enrichment of genes exhibiting SDIU on the X chromosome (Figure 3A). In contrast, SBGE is strongly associated with the X chromosome, with female-biased genes being significantly overrepresented on the X chromosome relative to unbiased genes whereas male biased genes are significantly underrepresented on the X chromosome (Figure 3C). This observation of a “demasculinization” of the X chromosome is consistent with previous studies of SBGE in *D. melanogaster* and several other species (Parisi et al. 2003; Ranz et al. 2003; Khil et al. 2004; Reinke et al. 2004; Sturgill et al. 2007; Meisel et al. 2012; Albritton et al. 2014). We find no relationship between SBGE and SDIU with respect to genomic location (Figure 3D).

These patterns are quite different for the head data. In contrast to the body data, there are a significantly higher proportion of X-linked genes exhibiting SDIU in the head relative to non-SDIU genes (Figure 3A). With respect to SBGE, both male- and female-biased genes are overrepresented on the X relative to unbiased genes, with the strongest overrepresentation among male-biased genes (Figure 3C). The over-representation of SDIU genes on the X chromosome occurs primarily among unbiased genes rather than sex-biased genes (Figure 3E), indicating that this pattern is not driven by a relationship between SDIU and SBGE.

Figure 3: Proportion of genes exhibiting sexually dimorphic isoform usage (SDIU) and sex-biased gene expression (SBGE) that are X-linked. (A) The proportion of total SDIU (green) and non-SDIU (purple) genes that are found on the X chromosome when expressed in body or head tissue. (B-C) The proportion of genes found on the X chromosome in bins of $\log_2FC$ in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue) expression when expressed in (B) bodies or (C) heads. (D-E) The proportion of genes exhibiting SDIU (green) and not exhibiting SDIU (purple) found on the X chromosome in bins of $\log_2FC$ in gene expression (see Methods) ranging from female-biased (left most pair of bins), unbiased (middle bin) and male-biased (right most pair of bins) expression when expressed in (D) bodies or (E) heads. Genes in the most strongly sex-biased bins were excluded from plot of genes expressed in heads (i.e., panels C and E) because too few genes (i.e., fewer than 5) were present in these bins (see Supplemental Figure S4 for plots including omitted bins). Error bars are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

Population genomic patterns associated with SDIU and SBGE

To explore patterns of genetic diversity at genes with and without SDIU, we used two population genetic metrics – Tajima’s D and π_N/π_S – to look for clues about the form of selection that have acted at these genes. Tajima’s D is a useful summary statistic of the site frequency spectrum; selective sweeps tend to reduce Tajima’s D whereas balancing selection increases it (Charlesworth and Charlesworth 2010). Recent studies exploring the relationship between Tajima’s D and sexually dimorphic gene expression have been mixed: some studies have found a decrease in Tajima’s D at sex-biased genes (Wright et al. 2018, 2019) while others have reported the opposite (Dutoit et al. 2018) or a more mixed relationship (Pröschel et al. 2006; Sayadi et al. 2019). Tajima’s D at genes exhibiting SDIU is significantly reduced relative to genes that do not on the basis of non-overlapping 95% confidence intervals (CIs) for genes expressed in body and head tissue (Figure 4A). This result could indicate that balancing selection is less common among genes with versus without SDIU; this would be consistent with the idea that the evolution of sexually dimorphic isoform usage may represent at least a partial resolution of sexual conflict. The Tajima’s D result could also indicate that selective sweeps are more common among genes with SDIU versus those without; this would be consistent with idea that dimorphic isoform usage removes sex-specific constraints, allowing a greater fraction of mutations to be beneficial.

Previous studies have found that some patterns of molecular variation are correlated with expression level. For example, highly expressed genes tend to have lower sequence variation presumably due to stronger purifying selection (e.g., Pál et al. 2001; Subramanian and Kumar 2004; Carneiro et al. 2012; Paape et al. 2013; Williamson et al. 2014; Ho et al. 2019). To visualize whether expression level might be a confounding factor, all genes (for a given tissue) were first binned into equal quantiles of sex-averaged gene expression; within these bins Tajima’s D tends to be lower for SDIU than non-SDIU genes (Figure 4B; Figure 4C). As others have done (Sayadi et al. 2019; Wright et al. 2019), we also looked for a relationship between Tajima’s D and sex-biased gene expression but found no obvious pattern (Figure 4E; Figure 4F).

To examine these relationships in a more statistically rigorous framework, we fit linear models, separately for body and head tissue, modeling Tajima’s D as a function of sex-averaged gene expression, sex-bias in gene expression (measured as the absolute value of the $\log_2$ fold

399 change of male to female gene expression) and sexual dimorphism in isoform usage (i.e., genes
400 that do or do not exhibit statistically significant SDIU). Consistent with the patterns described
401 above, for the body data, there is a significant negative relationship between Tajima's D and
402 SDIU (Table 2). There is also a significant negative relationship with sex-averaged gene
403 expression but no significant relationship with sex-bias in gene expression. For genes expressed
404 in head tissue, the patterns are similar but marginally non-significant (Table 2), although the
405 direction of the effect of average gene expression and SDIU are in the same direction as for the
406 body data. The head dataset includes many fewer SDIU genes, so power is reduced.

Figure 4: Patterns of Tajima's D and sexual dimorphism in isoform usage (SDIU). Mean Tajima's D for genes exhibiting SDIU (green) or not (purple) (A) averaged across all genes expressed in body and head tissue. (B, C) Mean Tajima's D for genes expressed in (B) body or (C) head tissue within quantiles of sex-averaged gene expression. (D, E) Mean Tajima's D for genes expressed in (D) body or (E) head tissue averaged across genes within bins of log₂FC in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue). Genes in the most strongly sex-biased bins were excluded from plot of genes expressed in heads (E) because too few genes (i.e., fewer than 5) were present in these bins (see Supplemental Figure S5 for plots including omitted bins). Error bars are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

417 **Table 2:** Parameter estimates from linear model fit to test the fixed effects of overall gene expression, sex-bias in gene expression
 418 (i.e., log₂ fold change in male to female gene expression) and sexual dimorphism in isoform usage (SDIU) on Tajima's D in body and
 419 head tissue. *P*-values were obtained by a Wald's Type III Chi-square test. Bold terms indicate statistically significant effects at $\alpha =$
 420 0.05.

Tissue	Effect	Estimate	Standard Error	<i>P</i>
Body Tissue	Intercept	0.061	0.014	< 0.001
	Sex-averaged Gene Expression¹	-2.01 x 10⁻⁶	9.01 x 10⁻⁷	0.025
	Absolute value of SBGE ²	-0.005	0.007	0.469
	Sexual Dimorphism in Isoform Usage (SDIU)³	-0.031	0.013	0.022
Head Tissue	Intercept	0.034	0.008	< 0.001
	Sex-averaged Gene Expression	-6.53 x 10 ⁻⁷	4.70 x 10 ⁻⁷	0.154
	Absolute value of SBGE ¹	0.025	0.025	0.301
	Sexual Dimorphism in Isoform Usage (SDIU)	-0.045	0.024	0.064

421 ¹Gene expression averaged across both sexes.

422 ²Measured as |Log₂FC male:female gene expression|.

423 ³Indicator of whether or not a gene exhibited statistically significant evidence of SDIU.

We also examined π_N/π_S , using the ratio of nucleotide diversity at 0-fold non-synonymous sites (π_N) to 4-fold synonymous sites (π_S), which can be useful in identifying differences in the strength of purifying selection. Overall, we do not find a significant difference in π_N/π_S between genes exhibiting SDIU relative to other genes expressed in either head or body tissue (Figure 5A). π_N/π_S tends to be lower in genes that are more highly expressed (Figure 5B; Figure 5C), but there are no obvious differences between SDIU and non-SDIU genes when they are binned into quantiles of sex-averaged expression. This pattern of reduced π_N/π_S in more highly expressed genes has been observed in other studies and has been interpreted as resulting from stronger purifying selection acting at highly expressed genes (e.g., Pál et al. 2001; Subramanian and Kumar 2004; Carneiro et al. 2012; Paape et al. 2013; Williamson et al. 2014; Ho et al. 2019).

In contrast to SDIU, SBGE is related to π_N/π_S , with higher π_N/π_S values associated with sex-biased genes, at least for genes expressed in the body (Figure 5D). Studies in several species including seed beetles (Sayadi et al. 2019), several bird species (Harrison et al. 2015; Dutoit et al. 2018), guppies (Wright et al. 2018), and humans (Gershoni and Pietrokovski 2014) have shown an inflation of π_N/π_S at sex-biased genes. Elevated π_N/π_S for sex-biased genes may indicate weaker purifying selection. There also appears to be a negative relationship between π_N/π_S and overall gene expression (i.e., sex-averaged gene expression) whereby highly expressed genes appear to have lower values of π_N/π_S . To test these relationships statistically, we fit linear models similar to those described above separately for heads and bodies. Regression analysis with the body dataset finds a statistically significant negative relationship between π_N/π_S and sex-averaged gene expression as well as SBGE, but no significant effect of SDIU on π_N/π_S (Table 3).

Figure 5: Patterns of π_N/π_S and sexual dimorphism in isoform usage (SDIU). Median π_N/π_S for genes exhibiting SDIU (green) or not (purple) (A) averaged across all genes expressed in body and head tissue. (B, C) Median π_N/π_S for genes expressed in (B) body or (C) head tissue averaged across genes within quantiles of sex-averaged gene expression. (D, E) Median π_N/π_S for all genes expressed in (D) body or (E) head tissue averaged across all genes within bins of $\log_2FC$ in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue). Genes in the most strongly sex-biased bins were excluded from plot of genes expressed in heads (E) because too few genes (i.e., fewer than 5) were present in these bins (see Supplemental Figure S6 for plots including omitted bins). Error bars are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

457 **Table 3:** Parameter estimates from linear model fit to test the fixed effects of overall gene expression (i.e., sex averaged gene
 458 expression), sex-bias in gene expression (i.e., $\log_2$ fold change in male to female gene expression) and sexual dimorphism in isoform
 459 usage (SDIU) on π_N/π_S in body and head tissue. *P*-values were obtained by a Wald's Type III Chi-square test. Bold terms indicate
 460 statistically significant effects at $\alpha = 0.05$.

Tissue	Effect	Estimate	Standard Error	<i>P</i>
Body Tissue	Intercept	-2.350	0.004	< 0.001
	Sex-averaged Gene Expression¹	-2.33 x 10⁻⁵	3.24 x 10⁻⁶	< 0.001
	Absolute value of SBGE²	0.004	0.014	0.002
	Sexual Dimorphism in Isoform Usage (SDIU) ³	-0.037	0.029	0.154
Head Tissue	Intercept	-2.383	0.022	< 0.001
	Sex-averaged Gene Expression¹	-4.43 x 10⁻⁶	91.35 x 10⁻⁶	0.003
	Absolute value of SBGE ²	0.032	0.066	0.618
	Sexual Dimorphism in Isoform Usage (SDIU) ³	0.053	0.067	0.426

461 ¹Gene expression averaged across both sexes.

462 ²Measured as $|\log_2 \text{FC male:female gene expression}|$.

463 ³Indicator of whether or not a gene exhibited statistically significant evidence of SDIU.

An abundance of caution is warranted in interpreting the population genomic patterns reported here as evidence for or against particular forms of selection acting at genes exhibiting SDIU or SBGE. Population demography (Hartl and Clark 2007; Cutter 2019), genome-wide variation in rates of recombination (Braverman et al. 1995) and mutation (Aris-Brosou and Excoffier 1996) are all well recognized as factors that influence patterns of genetic diversity. Nonetheless this study provides insights into the properties of genes exhibiting two different forms of gene regulation: sexually dimorphic isoform usage and sex-biased gene expression and documents population genetic patterns at such genes.

SDIU and SBGE are two different ways to differentially regulate how genes are expressed in the two sexes and are thought to be mechanisms by which sexual conflict can be resolved (Bonduriansky and Chenoweth 2009; Stewart et al. 2010). As is well known for SBGE, it appears that SDIU is also widespread in *Drosophila melanogaster*, particularly among genes expressed in body tissue. Genes exhibiting SDIU are functionally diverse and involved in processes that are likely hotspots for sexual conflict including over development and reproduction. The negative association between SDIU and SBGE in the body suggests that to some extent when one of these mechanisms evolves, it may negate the selective pressure to evolve the other. However, given that we observe the opposite pattern in heads (i.e., co-occurrence of SBGE and SDIU), it is premature to draw a strong inference.

The relationship between SDIU and SBGE in the present study suggests that these two forms of gene expression regulation experience different forms of selection. Genes exhibiting SDIU are negatively related to Tajima's D but do not exhibit a strong relationship to π_N/π_S , a pattern that is consistent with SDIU genes being more frequently subject to selective sweeps or less frequently subject to balancing selection compared to non-SDIU genes. In contrast, SBGE is not associated with Tajima's D , but is negatively associated with π_N/π_S , which may indicate that that sex-biased genes experience weaker purifying selection, perhaps because selection is weak in the sex with reduced expression (Dapper and Wade 2020). Though divergent selection between the sexes is likely to drive the evolution of both SBGE and SDIU, the population genomic consequences following the evolution of these alternative forms of dimorphism appears quite different.

Acknowledgements: We wish to thank Tyler Kent for providing a script to identify *N*-fold codon degeneracy as well as Julia Kreiner, and James Santangelo for helpful discussion and bioinformatics advice. Funding was provided in the form of an Ontario Graduate Scholarship to A.S. and an NSERC discovery grant to A.F.A.

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743 Supplemental Figures and Tables

744

745 **Figure S1:** Patterns of overall gene expression and sexual dimorphism in isoform usage (SDIU) for genes expressed in the body and
 746 head tissue. **(A)** The mean of the log sex-averaged gene expression of genes exhibiting sexually dimorphic isoform usage (SDIU;
 747 green) or no SDIU (purple) for genes expressed in the body and head. Error bars are 95% confidence intervals estimated from 10,000
 748 bootstraps resampling estimates. **(B)** Scatter plot and density distributions of log sex-averaged gene expression of genes exhibiting
 749 SDIU only in bodies (light blue), only in heads (red), in both heads and bodies (dark blue) or in neither heads nor bodies (beige) when
 750 expressed in bodies (x-axis) or heads (y-axis). Density plots above and to the right of the figure correspond to the log sex-averaged
 751 gene expression in bodies and heads respectively.

Figure S2: Scatter plot and density distributions of the log₂ fold change (log₂FC) in male to female gene expression of genes exhibiting sexual dimorphism in isoform usage (SDIU) only in bodies (light blue), only in heads (red), in both heads and bodies (dark blue) or in neither heads nor bodies (beige) when expressed in bodies (x-axis) or heads (y-axis). Density plots above and to the right of the figure correspond to the log₂FC in gene expression in bodies and heads respectively.

Figure S3: The proportion of genes exhibiting SDIU to genes not exhibiting SDIU in bins of log2FC in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue) expression when expressed in (C) bodies or (D) heads. Faded points indicate bins with fewer than 5 genes. Error bars in panels B-D are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

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769 **Figure S4:** Proportion of genes exhibiting sexually dimorphic isoform usage (SDIU) and sex-biased gene expression (SBGE) that are
 770 X-linked. **(A-B)** The proportion of genes found on the X chromosome in bins of $\log_2FC$ in gene expression (see Methods) ranging
 771 from female-biased (red), unbiased (black) and male-biased (blue) expression when expressed in **(A)** bodies or **(B)** heads. **(C-D)** The
 772 proportion of genes exhibiting SDIU (green) and not exhibiting SDIU (purple) found on the X chromosome in bins of $\log_2FC$ in gene
 773 expression (see Methods) ranging from female-biased (left most pair of bins), unbiased (middle bin) and male-biased (right most pair
 774 of bins) expression when expressed in **(C)** bodies or **(D)** heads. Faded points indicate bins with fewer than 5 genes. Error bars are 95%
 775 confidence intervals estimated from 10,000 bootstraps resampling estimates.

Figure S5: (A, B) Mean Tajima's D for all genes expressed in (A) body or (B) head tissue averaged across all genes within bins of $\log_2FC$ in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue). Faded points indicate bins with fewer than 5 genes. Error bars are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

Figure S6: (A, B) Median π_N/π_S for all genes expressed in (A) body or (B) head tissue averaged across all genes within bins of $\log_2FC$ in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue). Faded points indicate bins with fewer than 5 genes. Error bars are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

795 **Table S1:** Gene Ontology (GO) categories enriched for genes exhibiting sexually dimorphic exon usage when expressed in body
 796 tissue of 18 male and 18 female genotypes from the *Drosophila* Genetic Resource Population (DGRP). Statistical significance was
 797 assessed at an FDR Q -value < 0.01 .

GO Term	Description	Enrichment	FDR Q -value
Component			
GO:0005737	Cytoplasm	1.18	1.29E-11
GO:0005829	Cytosol	1.25	9.20E-08
GO:0044464	Cell Part	1.04	4.77E-07
GO:0005886	Plasma Membrane	1.22	2.64E-05
GO:0044430	Cytoskeletal Part	1.30	2.67E-05
GO:0043226	Organelle	1.07	1.15E-04
GO:0005819	Spindle	1.64	1.03E-04
GO:0016020	Membrane	1.14	1.30E-04
GO:0043228	Non-Membrane-Bounded Organelle	1.18	1.82E-04
GO:0043232	Intracellular Non-Membrane-Bounded Organelle	1.18	1.64E-04
GO:0044424	Intracellular Part	1.04	1.86E-04
GO:0043229	Intracellular Organelle	1.06	2.42E-04
GO:0005654	Nucleoplasm	1.36	3.41E-04
GO:0005634	Nucleus	1.10	3.27E-04
GO:0097458	Neuron Part	1.26	5.68E-04
GO:0005575	Cellular Component	1.02	7.72E-04
GO:0043227	Membrane-Bounded Organelle	1.06	1.35E-03
GO:0005938	Cell Cortex	1.35	1.43E-03
GO:0048471	Perinuclear Region of Cytoplasm	1.52	1.40E-03
GO:0099568	Cytoplasmic Region	1.32	4.53E-03
GO:0044456	Synapse Part	1.32	4.74E-03

GO:0044444	Cytoplasmic Part	1.06	5.03E-03
GO:0043231	Intracellular Membrane-Bounded Organelle	1.06	1.03E-02
GO:0035770	Ribonucleoprotein Granule	1.41	1.27E-02
GO:0070069	Cytochrome Complex	1.74	1.25E-02
GO:0036464	Cytoplasmic Ribonucleoprotein Granule	1.41	1.69E-02
GO:0120038	Plasma Membrane Bounded Cell Projection Part	1.26	1.81E-02
GO:0044463	Cell Projection Part	1.26	1.75E-02
GO:0044459	Plasma Membrane Part	1.17	1.74E-02

Function

GO:0005515	Protein Binding	1.16	1.07E-11
GO:0016301	Kinase Activity	1.31	5.87E-04
GO:0005488	Binding	1.05	1.52E-03
GO:0015318	Inorganic Molecular Entity Transmembrane Transporter Activity	1.30	3.59E-03
GO:0016773	Phosphotransferase Activity, Alcohol Group as Acceptor	1.29	3.94E-03
GO:0005516	Calmodulin Binding	1.85	3.42E-03
GO:0019787	Ubiquitin-Like Protein Transferase Activity	1.34	4.15E-03
GO:0030554	Adenyl Nucleotide Binding	1.18	7.76E-03
GO:0032559	Adenyl Ribonucleotide Binding	1.18	7.69E-03
GO:0016772	Transferase Activity, Transferring Phosphorus-Containing Groups	1.22	9.54E-03

Process

GO:0048519	Negative Regulation of Biological Process	1.24	9.11E-14
GO:0048523	Negative Regulation of Cellular Process	1.25	2.36E-12
GO:0065007	Biological Regulation	1.11	4.34E-12
GO:0032502	Developmental Process	1.18	4.69E-12
GO:0050789	Regulation of Biological Process	1.11	1.23E-10
GO:0048583	Regulation of Response to Stimulus	1.24	2.27E-09
GO:0050794	Regulation of Cellular Process	1.11	4.27E-09

GO:0010646	Regulation of Cell Communication	1.25	7.35E-09
GO:0023051	Regulation of Signaling	1.25	6.53E-09
GO:0009966	Regulation of Signal Transduction	1.26	1.10E-08
GO:0051128	Regulation of Cellular Component Organization	1.24	1.89E-08
GO:0022414	Reproductive Process	1.21	1.41E-07
GO:0032879	Regulation of Localization	1.30	2.68E-07
GO:0048518	Positive Regulation of Biological Process	1.15	2.94E-07
GO:0048856	Anatomical Structure Development	1.18	2.79E-07
GO:0048585	Negative Regulation of Response to Stimulus	1.33	3.85E-07
GO:0050793	Regulation of Developmental Process	1.24	5.17E-07
GO:0003006	Developmental Process Involved in Reproduction	1.25	2.03E-06
GO:0022412	Cellular Process Involved in Reproduction in Multicellular Organism	1.23	1.92E-06
GO:0032501	Multicellular Organismal Process	1.17	2.28E-06
GO:0048869	Cellular Developmental Process	1.20	2.21E-06
GO:0009987	Cellular Process	1.05	2.78E-06
GO:0048522	Positive Regulation of Cellular Process	1.15	2.72E-06
GO:0009653	Anatomical Structure Morphogenesis	1.22	2.75E-06
GO:0009968	Negative Regulation of Signal Transduction	1.33	2.88E-06
GO:0023057	Negative Regulation of Signaling	1.31	5.66E-06
GO:0010648	Negative Regulation of Cell Communication	1.31	7.30E-06
GO:0051246	Regulation of Protein Metabolic Process	1.24	7.43E-06
GO:0010605	Negative Regulation of Macromolecule Metabolic Process	1.23	7.30E-06
GO:0051239	Regulation of Multicellular Organismal Process	1.22	8.45E-06
GO:0016043	Cellular Component Organization	1.11	8.98E-06
GO:0060255	Regulation of Macromolecule Metabolic Process	1.13	9.89E-06
GO:1902531	Regulation of Intracellular Signal Transduction	1.29	1.45E-05
GO:0051493	Regulation of Cytoskeleton Organization	1.40	1.73E-05
GO:0007610	Behavior	1.29	1.80E-05
GO:0009892	Negative Regulation of Metabolic Process	1.21	3.00E-05

GO:0051172	Negative Regulation of Nitrogen Compound Metabolic Process	1.24	3.50E-05
GO:0032268	Regulation of Cellular Protein Metabolic Process	1.23	3.98E-05
GO:0022603	Regulation of Anatomical Structure Morphogenesis	1.34	4.49E-05
GO:0071840	Cellular Component Organization or Biogenesis	1.10	5.01E-05
GO:2000026	Regulation of Multicellular Organismal Development	1.23	5.20E-05
GO:0007010	Cytoskeleton Organization	1.27	5.32E-05
GO:0065008	Regulation of Biological Quality	1.16	6.34E-05
GO:0031324	Negative Regulation of Cellular Metabolic Process	1.23	6.82E-05
GO:0051049	Regulation of Transport	1.32	8.06E-05
GO:0051960	Regulation of Nervous System Development	1.28	1.00E-04
GO:0045595	Regulation of Cell Differentiation	1.26	1.18E-04
GO:0006996	Organelle Organization	1.16	1.17E-04
GO:0051171	Regulation of Nitrogen Compound Metabolic Process	1.12	1.59E-04
GO:0080090	Regulation of Primary Metabolic Process	1.12	1.58E-04
GO:0019222	Regulation of Metabolic Process	1.11	1.64E-04
GO:0006928	Movement of Cell or Subcellular Component	1.22	1.80E-04
GO:0010647	Positive Regulation of Cell Communication	1.25	1.84E-04
GO:0023056	Positive Regulation of Signaling	1.25	1.80E-04
GO:0048609	Multicellular Organismal Reproductive Process	1.30	1.88E-04
GO:0007017	Microtubule-Based Process	1.28	2.27E-04
GO:0070647	Protein Modification by Small Protein Conjugation or Removal	1.30	2.46E-04
GO:0033043	Regulation of Organelle Organization	1.23	3.19E-04
GO:0009967	Positive Regulation of Signal Transduction	1.25	3.23E-04
GO:0007276	Gamete Generation	1.32	3.48E-04
GO:0008589	Regulation of Smoothened Signaling Pathway	1.49	3.82E-04
GO:0051248	Negative Regulation of Protein Metabolic Process	1.35	4.38E-04
GO:0048584	Positive Regulation of Response to Stimulus	1.22	4.49E-04
GO:0032269	Negative Regulation of Cellular Protein Metabolic Process	1.35	5.82E-04
GO:0051093	Negative Regulation of Developmental Process	1.34	7.20E-04

GO:0070507	Regulation of Microtubule Cytoskeleton Organization	1.61	7.40E-04
GO:0010604	Positive Regulation of Macromolecule Metabolic Process	1.17	7.39E-04
GO:0048646	Anatomical Structure Formation Involved in Morphogenesis	1.27	7.30E-04
GO:0001932	Regulation of Protein Phosphorylation	1.32	7.94E-04
GO:0045596	Negative Regulation of Cell Differentiation	1.46	7.90E-04
GO:0007281	Germ Cell Development	1.31	7.79E-04
GO:0048149	Behavioral Response to Ethanol	1.64	8.20E-04
GO:0048477	Oogenesis	1.36	8.14E-04
GO:0007292	Female Gamete Generation	1.36	8.03E-04
GO:0060284	Regulation of Cell Development	1.25	9.37E-04
GO:0031323	Regulation of Cellular Metabolic Process	1.10	1.03E-03
GO:0045880	Positive Regulation of Smoothened Signaling Pathway	1.61	1.03E-03
GO:0030029	Actin Filament-Based Process	1.32	1.04E-03
GO:0030036	Actin Cytoskeleton Organization	1.35	1.13E-03
GO:0051130	Positive Regulation of Cellular Component Organization	1.25	1.15E-03
GO:0051656	Establishment of Organelle Localization	1.37	1.18E-03
GO:0080134	Regulation of Response to Stress	1.27	1.29E-03
GO:0009893	Positive Regulation of Metabolic Process	1.15	1.36E-03
GO:0051173	Positive Regulation of Nitrogen Compound Metabolic Process	1.17	1.35E-03
GO:0032886	Regulation of Microtubule-Based Process	1.56	1.40E-03
GO:0044087	Regulation of Cellular Component Biogenesis	1.23	1.44E-03
GO:0016567	Protein Ubiquitination	1.32	1.81E-03
GO:0016331	Morphogenesis of Embryonic Epithelium	1.41	1.82E-03
GO:1902532	Negative Regulation of Intracellular Signal Transduction	1.37	1.83E-03
GO:0017145	Stem Cell Division	1.49	1.87E-03
GO:0048468	Cell Development	1.22	1.89E-03
GO:0051241	Negative Regulation of Multicellular Organismal Process	1.33	2.06E-03
GO:0051961	Negative Regulation of Nervous System Development	1.41	2.46E-03
GO:0048285	Organelle Fission	1.51	2.44E-03

GO:0042326	Negative Regulation of Phosphorylation	1.50	2.57E-03
GO:0032446	Protein Modification by Small Protein Conjugation	1.30	2.91E-03
GO:0034248	Regulation of Cellular Amide Metabolic Process	1.32	3.23E-03
GO:0051640	Organelle Localization	1.29	3.27E-03
GO:0007391	Dorsal Closure	1.41	3.46E-03
GO:1903829	Positive Regulation of Cellular Protein Localization	1.70	3.47E-03
GO:0001933	Negative Regulation of Protein Phosphorylation	1.49	3.66E-03
GO:0050767	Regulation of Neurogenesis	1.27	3.67E-03
GO:0010563	Negative Regulation of Phosphorus Metabolic Process	1.45	3.78E-03
GO:0045936	Negative Regulation of Phosphate Metabolic Process	1.45	3.74E-03
GO:0010629	Negative Regulation of Gene Expression	1.20	3.76E-03
GO:0022604	Regulation of Cell Morphogenesis	1.36	3.84E-03
GO:0022402	Cell Cycle Process	1.19	4.29E-03
GO:0051179	Localization	1.09	4.31E-03
GO:0042325	Regulation of Phosphorylation	1.26	4.61E-03
GO:0016334	Establishment or Maintenance of Polarity of Follicular Epithelium	1.65	4.81E-03
GO:0009890	Negative Regulation of Biosynthetic Process	1.22	4.78E-03
GO:0031327	Negative Regulation of Cellular Biosynthetic Process	1.22	4.74E-03
GO:0010608	Posttranscriptional Regulation of Gene Expression	1.30	4.90E-03
GO:0031325	Positive Regulation of Cellular Metabolic Process	1.15	5.46E-03
GO:0019220	Regulation of Phosphate Metabolic Process	1.25	5.42E-03
GO:0051174	Regulation of Phosphorus Metabolic Process	1.25	5.37E-03
GO:0008150	Biological Process	1.01	5.44E-03
GO:0031399	Regulation of Protein Modification Process	1.23	5.59E-03
GO:0009886	Post-Embryonic Animal Morphogenesis	1.22	5.57E-03
GO:0006796	Phosphate-Containing Compound Metabolic Process	1.15	5.56E-03
GO:0031400	Negative Regulation of Protein Modification Process	1.40	6.04E-03
GO:0051129	Negative Regulation of Cellular Component Organization	1.31	6.12E-03
GO:0097435	Supramolecular Fiber Organization	1.32	6.09E-03

GO:0050768	Negative Regulation of Neurogenesis	1.49	6.56E-03
GO:0010721	Negative Regulation of Cell Development	1.46	6.80E-03
GO:0048731	System Development	1.27	7.63E-03
GO:0030534	Adult Behavior	1.36	7.70E-03
GO:0007051	Spindle Organization	1.39	7.70E-03
GO:0010558	Negative Regulation of Macromolecule Biosynthetic Process	1.22	7.94E-03
GO:2000113	Negative Regulation of Cellular Macromolecule Biosynthetic Process	1.22	7.88E-03
GO:0007411	Axon Guidance	1.29	7.85E-03
GO:0051301	Cell Division	1.31	8.36E-03
GO:0098655	Cation Transmembrane Transport	1.34	8.60E-03
GO:0060341	Regulation of Cellular Localization	1.32	9.07E-03
GO:0006811	Ion Transport	1.22	9.46E-03
GO:0007389	Pattern Specification Process	1.20	9.70E-03

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805 **Table S2:** Gene Ontology (GO) categories enriched for genes exhibiting sexually dimorphic exon usage when expressed in head
 806 tissue of 18 male and 18 female genotypes from the *Drosophila* Genetic Resource Population (DGRP). Statistical significance was
 807 assessed at an FDR Q -value < 0.01 .

GO Term	Description	Enrichment	FDR Q -value
Component			
GO:0005829	Cytosol	1.96	8.51E-06
GO:0044444	Cytoplasmic Part	1.28	2.27E-03
GO:0097458	Neuron Part	1.87	2.06E-03
GO:0005886	Plasma Membrane	1.69	2.08E-03
GO:0044430	Cytoskeletal Part	2.15	3.32E-03
Function			
GO:0005515	Protein Binding	1.36	6.14E-03
Process			
GO:0003008	System Process	2.12	2.24E-03
GO:0007010	Cytoskeleton Organization	2.20	1.25E-03
GO:0007610	Behavior	2.02	8.34E-04
GO:0032501	Multicellular Organismal Process	1.52	1.19E-03
GO:0030036	Actin Cytoskeleton Organization	2.70	2.87E-03
GO:0032879	Regulation of Localization	1.85	5.35E-03
GO:0044089	Positive Regulation of Cellular Component Biogenesis	2.69	5.54E-03
GO:0051049	Regulation of Transport	2.15	4.86E-03

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Table S3: Parameter estimates from a logistic regression model fit to test the fixed effects of overall gene expression, sex-bias in gene expression (i.e., $\log_2$ fold change in male to female gene expression) and sexual dimorphism in isoform usage (SDIU) on π_N/π_S . π_N/π_S was modelled as a binary trait wherein genes are binned as either $\pi_N/\pi_S = 0$ or $\pi_N/\pi_S > 0$ in body and head tissue. Bold terms indicate statistically significant effects at $\alpha = 0.05$.

Tissue	Effect	Estimate	Standard Error	<i>P</i>
Body Tissue	Intercept	2.700	0.112	< 0.001
	Sex-averaged Gene Expression¹	-4.29 x 10⁻⁵	5.55 x 10⁻⁶	< 0.001
	Absolute value of SBGE²	0.137	0.062	0.028
	Sexual Dimorphism in Isoform Usage (SDIU) ³	0.095	0.113	0.401
Head Tissue	Intercept	2.907	0.068	< 0.001
	Sex-averaged Gene Expression¹	-4.68 x 10⁻⁶	2.39 x 10⁻⁶	0.050
	Absolute value of SBGE ²	-0.157	0.169	0.353
	Sexual Dimorphism in Isoform Usage (SDIU) ³	-0.135	0.195	0.489

¹Gene expression averaged across both sexes.

²Measured as $|\log_2 \text{FC male:female gene expression}|$.

³Indicator of whether or not a gene exhibited statistically significant evidence of SDIU.