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

**Amardeep Singh[1,2,3] and Aneil F. Agrawal[1]**

[1]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada, M5S 3B2

[2]Department of Integrative Biology, University of Texas at Austin, Austin, TX, 78712

[3]E-mail: amardeep.singh@utexas.edu

**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 $\pi_N/\pi_S$, 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 $\pi_N/\pi_S$ in an attempt to infer differences in the form of selection acting on sex-biased versus unbiased genes. We take a

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

94

95 **Methods**

96 Obtaining RNA-seq Data and Read Mapping

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

111 Differential Exon Usage Analysis

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

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

120 $$\log(y_{ij}) = \mu + W_i + X_j + Z_{ij}$$

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

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

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

142 Gene Expression Analysis

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

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

166 Population Genomic Data

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

175 converted to release 6 of the *Drosophila* reference genome using the *UCSC liftOver* (Kent et al.
176 2002) tool.

177 Estimating $\pi_N/\pi_S$ and Tajima's *D*

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

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

197 Gene Ontology (GO) Enrichment Analysis

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

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

207 Statistical Analysis

208 We used linear regression analysis to examine the relationship between population
209 genomic measures (i.e., Tajima's *D* and $\pi_N/\pi_S$) and the fixed effects of sex-averaged gene
210 expression, sex-bias in gene expression (measured as the absolute value the $\log_2$FC of male to
211 female gene expression) and SDIU. To improve the distributional properties for statistical
212 analysis, we analyzed $\log(\pi_N/\pi_S)$ rather than $\pi_N/\pi_S$. This required the exclusion of genes where
213 $\pi_N = 0$, which only represented 6.01% and 5.51% of genes expressed in the body and head
214 respectively. For both Tajima's *D* and $\log(\pi_N/\pi_S)$, we fit a regression model using the '*lmperm'*
215 function of the *R* package *permuco* (v.1.1.0; Frossard and Renaud 2019) which uses a Freedman-
216 Lane permutation method (Freedman and Lane 1983) and type III Wald's chi-square test to
217 estimate *P*-values for fixed effects. *P*-values were estimated after 10,000 permutated model fits.

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

223

224 **Results and Discussion**

225 Identity and Functional Properties of Genes Exhibiting Sexually Dimorphic Exon Usage

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

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

244

245 **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%) |

246 [1]These data refer to the union of genes expressed, tested and exhibiting sexually dimorphic
247 isoform usage found in either head, body or both.

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

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

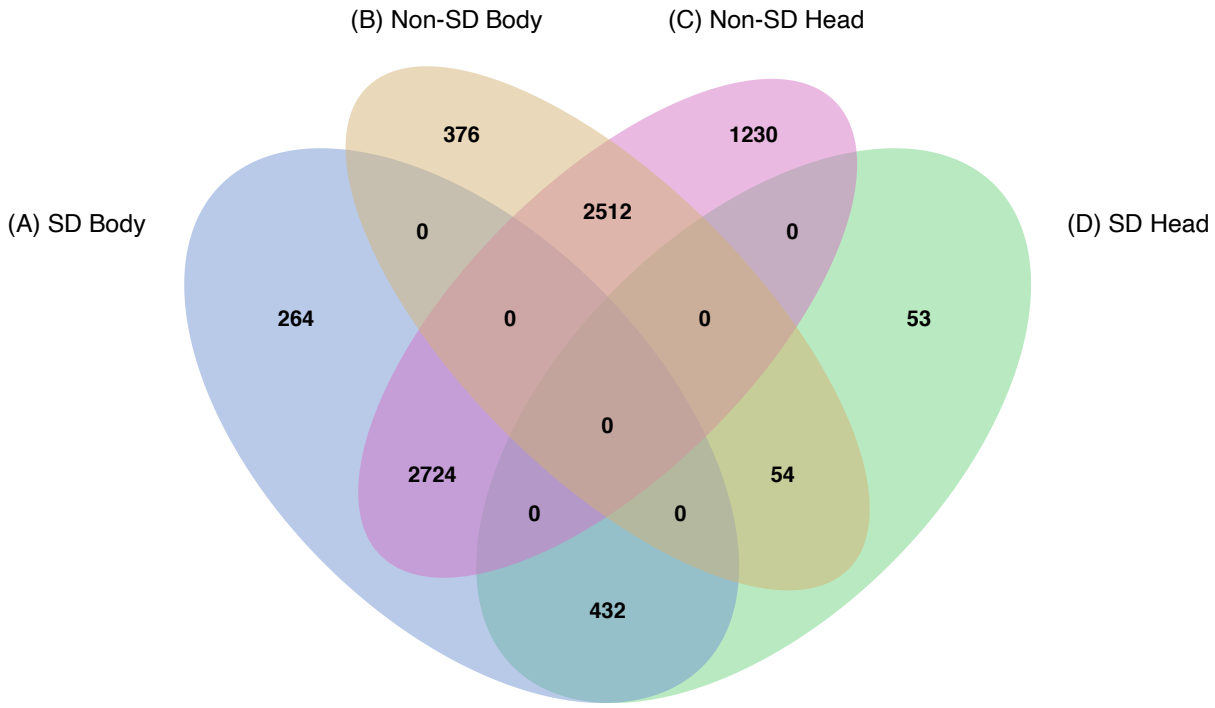



**Figure 1:** Venn diagrams of overlap between genes found to exhibit sexually dimorphic (SD) exon usage when expressed in **(A)** body and **(D)** head tissue and genes with no evidence of sexually dimorphic exon usage when expressed in **(B)** body and **(C)** head tissue. Only genes that were tested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

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

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

278 Coincidence of two forms of sexually dimorphism in expression

279 The majority of expression studies have examined variation in total expression for a
280 gene. Studies of sex-biased gene expression (SBGE) have shown repeatedly that SBGE is highly
281 tissue specific (e.g., Parisi et al. 2004; Yang et al. 2006; Huylmans and Parsch 2014; Dutoit et al.
282 2018); both our data and other studies (e.g., Telonis-Scott et al. 2009; Chang et al. 2011) indicate
283 that the same is true for sex differences in isoform usage. Because both of these forms of sexual
284 dimorphism are thought to be related to resolving intra-locus sexual conflict, we are interested in
285 understanding the relationship between SBGE and SDIU.

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

293 To further explore the relationship between SDIU and SBGE, we divided genes into bins
294 representing different levels of sex-bias using defined ranges of $\log_2$FC in gene expression, and
295 only considered genes that could be assigned to bins with reasonably high certainty (see
296 methods). Within each bin, we then calculated the proportion of isoform dimorphic to non-
297 dimorphic genes in each quantile bin. For genes expressed in the body, there is a reduction in the
298 proportion of isoform dimorphic genes among sex-biased genes relative to unbiased genes
299 (Figure 2C). Conversely, for genes expressed in head tissue, female-biased and male-biased
300 genes are enriched for SDIU (Figure 2D). The fly body results are in line with Rogers et al.
301 (2020) who reported non-significant trends towards a negative relationship between SDIU and
302 SBGE in gonadal tissue of three bird species.

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

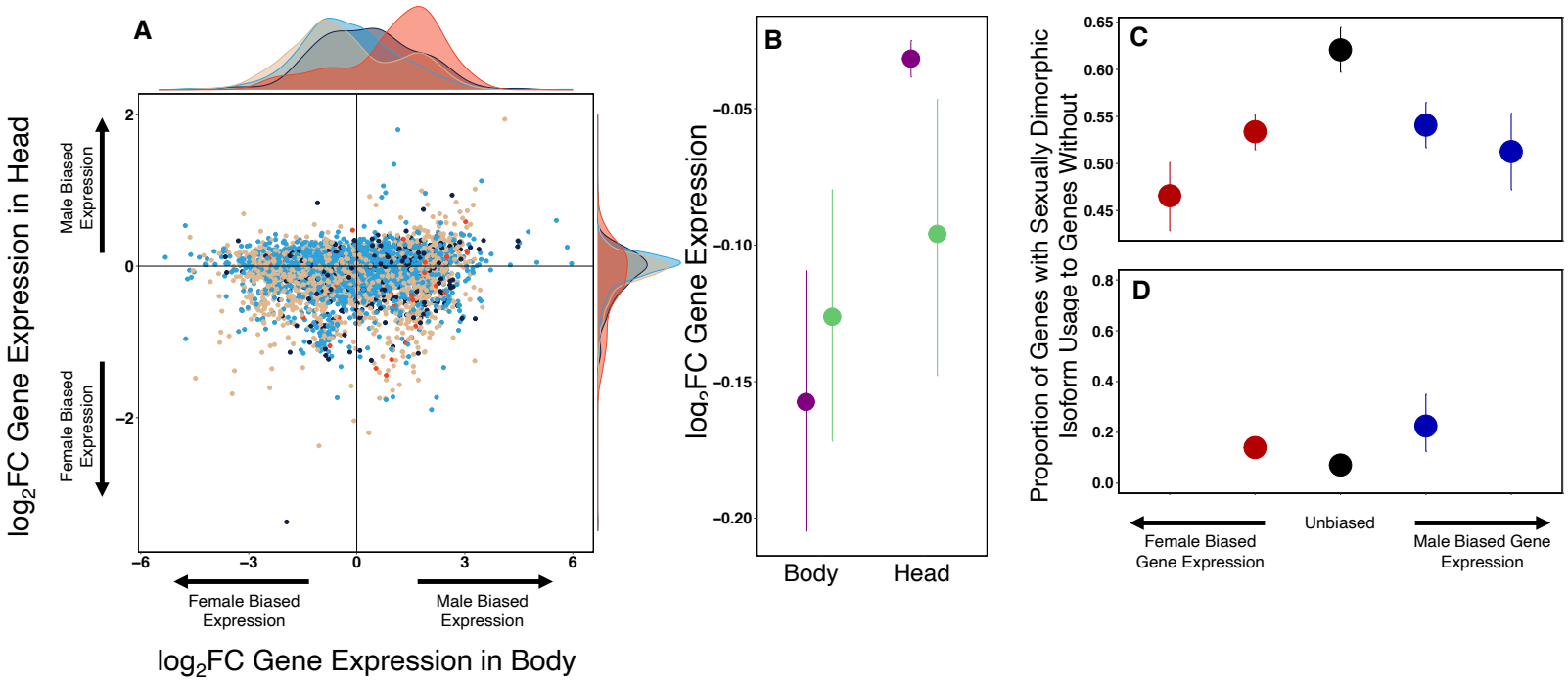



**Figure 2:** Patterns of sex-bias in gene expression (SBGE) and sexual dimorphism in isoform usage (SDIU) for genes expressed in the body and head tissue. **(A)** Scatter plot and density distributions of $\log_2$ fold change ($\log_2$FC) in male to female gene expression of genes exhibiting SDIU only 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.

337 Given that the X chromosome spends a disproportionate amount of its time in females,
338 and because of its hemizygous expression in males, there has been considerable interest in
339 whether it is enriched for genes that experience sexually antagonistic selection contributing to
340 intralocus conflict or contributing to sexual dimorphism (Rice 1987; Rowe et al. 2018; Frank and
341 Patten 2020) We therefore examined the genomic distribution of SDIU and SBGE genes. In the
342 body data, there is no evidence of an enrichment of genes exhibiting SDIU on the X
343 chromosome (Figure 3A). In contrast, SBGE is strongly associated with the X chromosome, with
344 female-biased genes being significantly overrepresented on the X chromosome relative to
345 unbiased genes whereas male biased genes are significantly underrepresented on the X
346 chromosome (Figure 3C). This observation of a "demasculinization" of the X chromosome is
347 consistent with previous studies of SBGE in *D. melanogaster* and several other species (Parisi et
348 al. 2003; Ranz et al. 2003; Khil et al. 2004; Reinke et al. 2004; Sturgill et al. 2007; Meisel et al.
349 2012; Albritton et al. 2014). We find no relationship between SBGE and SDIU with respect to
350 genomic location (Figure 3D).

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

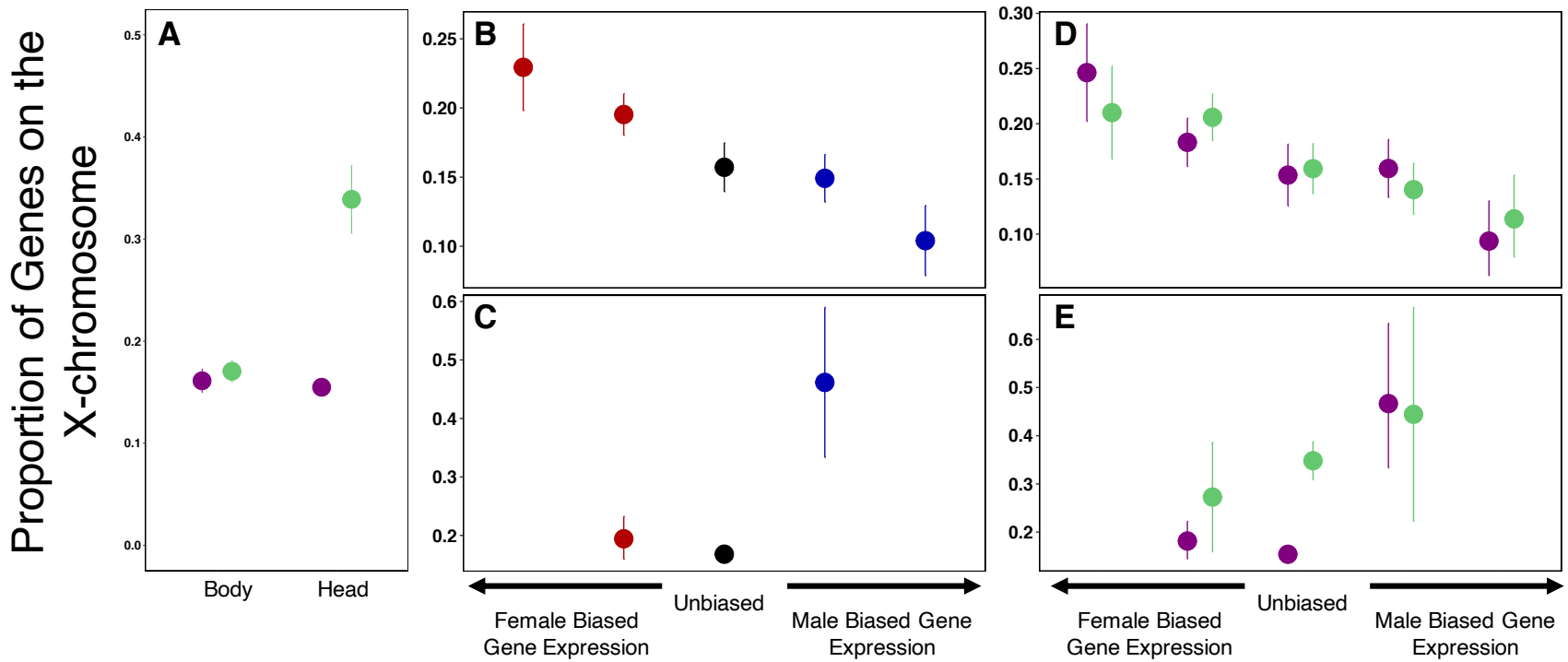



**Figure 3:** Proportion of genes exhibiting sexually dimorphic isoform usage (SDIU) and sex-biased gene expression (SBGE) that are X-linked. **(A)** The proportion of total SDIU (green) and non-SDIU (purple) genes that are found on the X chromosome when expressed in body or head tissue. **(B-C)** The proportion of genes found on the X chromosome in bins of $\log_2$FC 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 $\log_2$FC 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.

369 Population genomic patterns associated with SDIU and SBGE

370 To explore patterns of genetic diversity at genes with and without SDIU, we used two
371 population genetic metrics – Tajima's *D* and $\pi_N/\pi_S$ – to look for clues about the form of
372 selection that have acted at these genes. Tajima's *D* is a useful summary statistic of the site
373 frequency spectrum; selective sweeps tend to reduce Tajima's *D* whereas balancing selection
374 increases it (Charlesworth and Charlesworth 2010). Recent studies exploring the relationship
375 between Tajima's *D* and sexually dimorphic gene expression have been mixed: some studies
376 have found a decrease in Tajima's *D* at sex-biased genes (Wright et al. 2018, 2019) while others
377 have reported the opposite (Dutoit et al. 2018) or a more mixed relationship (Pröschel et al.
378 2006; Sayadi et al. 2019). Tajima's *D* at genes exhibiting SDIU is significantly reduced relative
379 to genes that do not on the basis of non-overlapping 95% confidence intervals (CIs) for genes
380 expressed in body and head tissue (Figure 4A). This result could indicate that balancing selection
381 is less common among genes with versus without SDIU; this would be consistent with the idea
382 that the evolution of sexually dimorphic isoform usage may represent at least a partial resolution
383 of sexual conflict. The Tajima's *D* result could also indicate that selective sweeps are more
384 common among genes with SDIU versus those without; this would be consistent with idea that
385 dimorphic isoform usage removes sex-specific constraints, allowing a greater fraction of
386 mutations to be beneficial.

387 Previous studies have found that some patterns of molecular variation are correlated with
388 expression level. For example, highly expressed genes tend to have lower sequence variation
389 presumably due to stronger purifying selection (e.g., Pál et al. 2001; Subramanian and Kumar
390 2004; Carneiro et al. 2012; Paape et al. 2013; Williamson et al. 2014; Ho et al. 2019). To
391 visualize whether expression level might be a confounding factor, all genes (for a given tissue)
392 were first binned into equal quantiles of sex-averaged gene expression; within these bins
393 Tajima's *D* tends to be lower for SDIU than non-SDIU genes (Figure 4B; Figure 4C). As others
394 have done (Sayadi et al. 2019; Wright et al. 2019), we also looked for a relationship between
395 Tajima's *D* and sex-biased gene expression but found no obvious pattern (Figure 4E; Figure 4F).

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

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

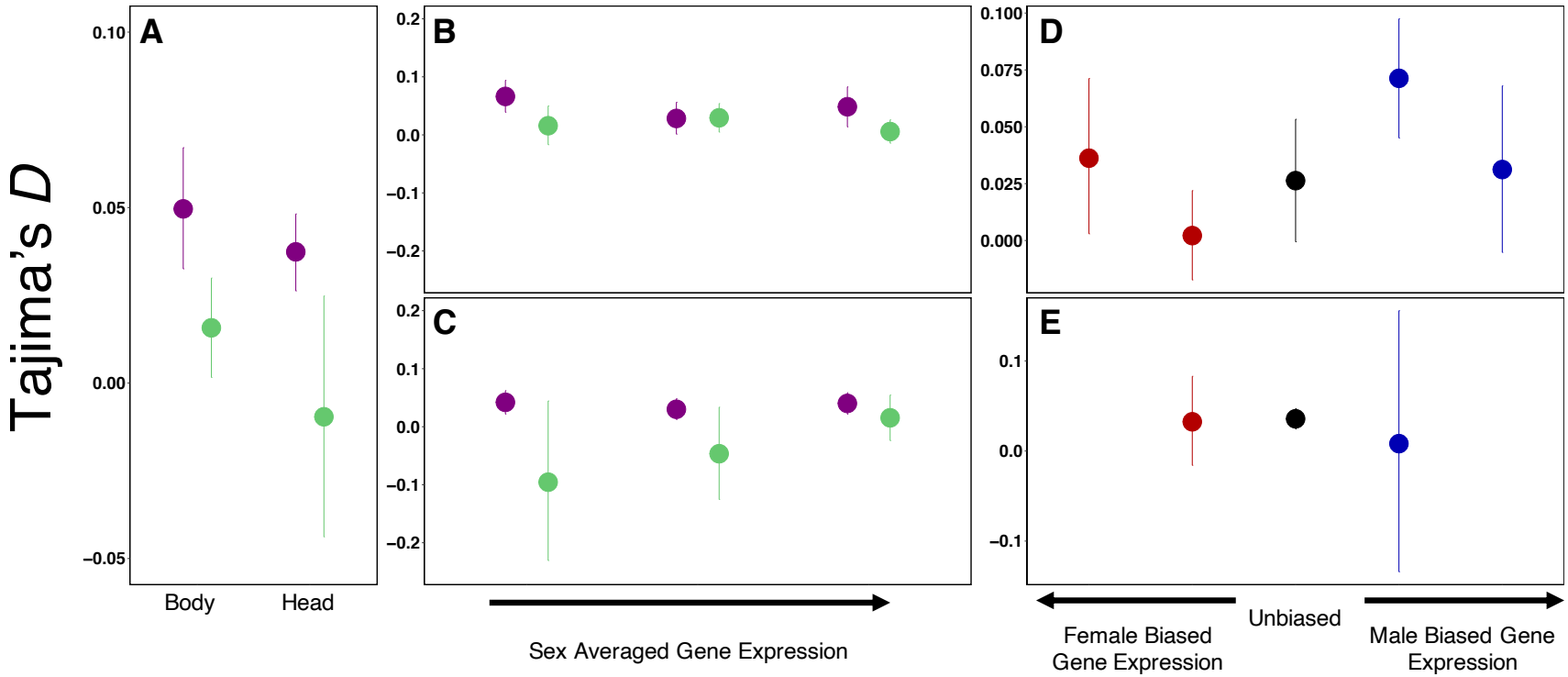



**Figure 4:** Patterns of Tajima's *D* and sexual dimorphism in isoform usage (SDIU). Mean Tajima's *D* for genes exhibiting SDIU (green) or not (purple) **(A)** averaged across all genes expressed in body and head tissue. **(B, C)** Mean Tajima's *D* for genes expressed in **(B)** body or **(C)** head 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 $\log_2$FC 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.

**Table 2:** Parameter estimates from linear 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 Tajima's D in body and head tissue. *P*-values were obtained by a Wald's Type III Chi-square test. Bold terms indicate statistically significant effects at $\alpha$ = 0.05.

| Tissue | Effect | Estimate | Standard Error | *P* |
|---|---|---|---|---|
| Body Tissue | **Intercept** | **0.061** | **0.014** | **< 0.001** |
| | **Sex-averaged Gene Expression**[1] | **$-2.01 \times 10^{-6}$** | **$9.01 \times 10^{-7}$** | **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 | $-6.53 \times 10^{-7}$ | $4.70 \times 10^{-7}$ | 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 |

[1]Gene expression averaged across both sexes.
[2]Measured as $|\text{Log}_2\text{FC male:female gene expression}|$.
[3]Indicator of whether or not a gene exhibited statistically significant evidence of SDIU.

424 We also examined $\pi_N/\pi_S$, using the ratio of nucleotide diversity at 0-fold non-
425 synonymous sites ($\pi_N$) to 4-fold synonymous sites ($\pi_S$), which can be useful in identifying
426 differences in the strength of purifying selection. Overall, we do not find a significant difference
427 in $\pi_N/\pi_S$ between genes exhibiting SDIU relative to other genes expressed in either head or
428 body tissue (Figure 5A). $\pi_N/\pi_S$ tends to be lower in genes that are more highly expressed (Figure
429 5B; Figure 5C), but there are no obvious differences between SDIU and non-SDIU genes when
430 they are binned into quantiles of sex-averaged expression. This pattern of reduced $\pi_N/\pi_S$ in more
431 highly expressed genes has been observed in other studies and has been interpreted as resulting
432 from stronger purifying selection acting at highly expressed genes (e,g., Pál et al. 2001;
433 Subramanian and Kumar 2004; Carneiro et al. 2012; Paape et al. 2013; Williamson et al. 2014;
434 Ho et al. 2019).

435 In contrast to SDIU, SBGE is related to $\pi_N/\pi_S$, with higher $\pi_N/\pi_S$ values associated with
436 sex-biased genes, at least for genes expressed in the body (Figure 5D). Studies in several species
437 including seed beetles (Sayadi et al. 2019), several bird species (Harrison et al. 2015; Dutoit et
438 al. 2018) , guppies (Wright et al. 2018), and humans (Gershoni and Pietrokovski 2014) have
439 shown an inflation of $\pi_N/\pi_S$ at sex-biased genes. Elevated $\pi_N/\pi_S$ for sex-biased genes may
440 indicate weaker purifying selection. There also appears to be a negative relationship between
441 $\pi_N/\pi_S$ and overall gene expression (i.e., sex-averaged gene expression) whereby highly
442 expressed genes appear to have lower values of $\pi_N/\pi_S$. To test these relationships statistically,
443 we fit linear models similar to those described above separately for heads and bodies. Regression
444 analysis with the body dataset finds a statistically significant negative relationship between
445 $\pi_N/\pi_S$ and sex-averaged gene expression as well as SBGE, but no significant effect of SDIU on
446 $\pi_N/\pi_S$ (Table 3).

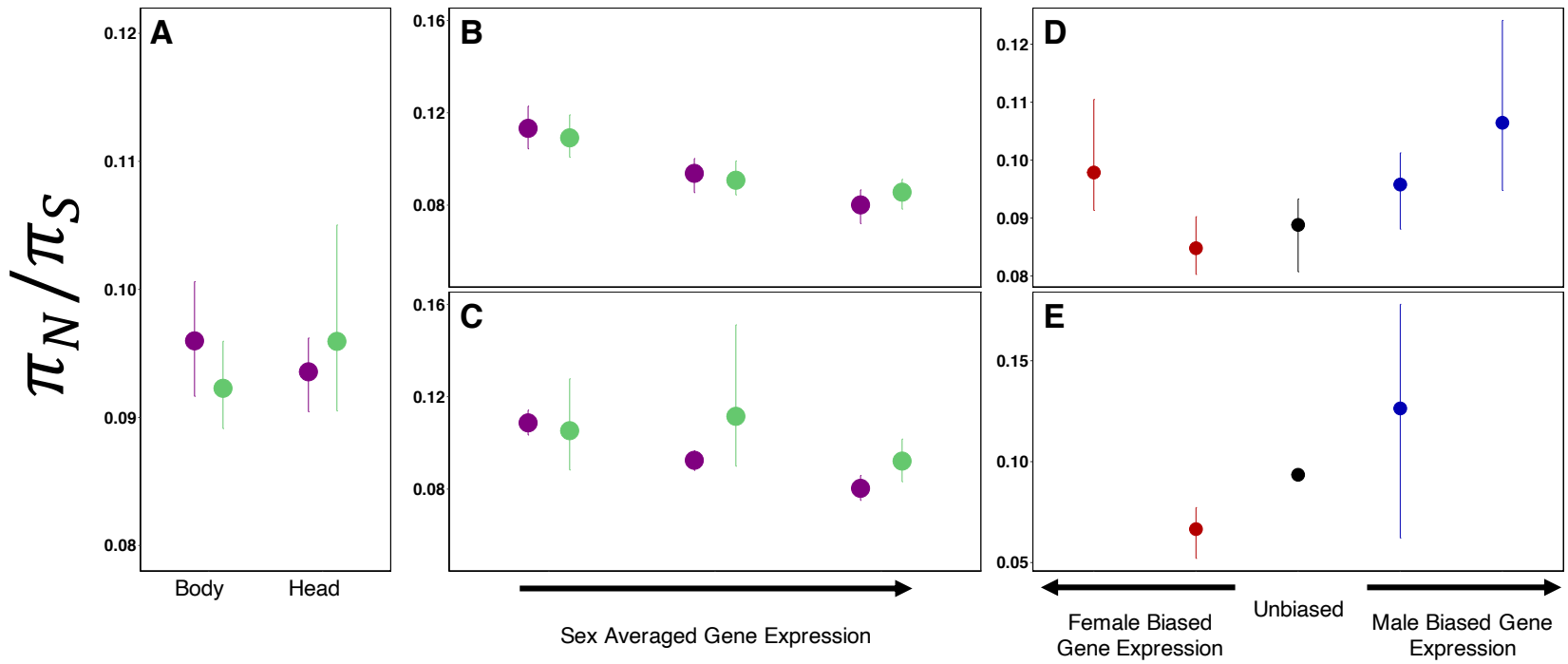



**Figure 5:** Patterns of $\pi_N/\pi_S$ and sexual dimorphism in isoform usage (SDIU). Median $\pi_N/\pi_S$ for genes exhibiting SDIU (green) or not (purple) **(A)** averaged across all genes expressed in body and head tissue. **(B, C)** Median $\pi_N/\pi_S$ for genes expressed in **(B)** body or **(C)** head tissue averaged across genes within quantiles of sex-averaged gene expression. **(D, E)** Median $\pi_N/\pi_S$ for all genes expressed in **(D)** body or **(E)** head tissue averaged across all genes within bins of $\log_2$FC 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 $\pi_N/\pi_S$ 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 | *P* |
|---|---|---|---|---|
| Body Tissue | **Intercept** | -2.350 | **0.004** | **< 0.001** |
| | **Sex-averaged Gene Expression**[1] | **$-2.33 \times 10^{-5}$** | **$3.24 \times 10^{-6}$** | **< 0.001** |
| | **Absolute value of SBGE**[2] | **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 Expression**[1] | **$-4.43 \times 10^{-6}$** | **$91.35 \times 10^{-6}$** | **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 [1]Gene expression averaged across both sexes.
462 [2]Measured as |$\text{Log}_2$FC male:female gene expression|.
463 [3]Indicator 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 $\pi_N/\pi_S$, 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 $\pi_N/\pi_S$, 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.

516 **Literature Cited**

517 Albritton, S. E., A. L. Kranz, P. Rao, M. Kramer, C. Dieterich, and S. Ercan. 2014. Sex-biased
518 gene expression and evolution of the X chromosome in nematodes. Genetics 197:865–883.

519 Andrews, S. 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data.

520 Aris-Brosou, S., and L. Excoffier. 1996. The impact of population expansion and mutation rate
521 heterogeneity on DNA sequence polymorphism. Mol. Biol. Evol. 13:494–504.

522 Arnqvist, G., and L. Rowe. 2005. Sexual Conflict. Princeton University Press, Princeton NJ.

523 Ast, G. 2004. How did alternative splicing evolve? Nat. Rev. Genet. 5:773–782.

524 Baker, D. A., T. Nolan, B. Fischer, A. Pinder, A. Crisanti, and S. Russell. 2011. A
525 comprehensive gene expression atlas of sex- and tissue-specificity in the malaria vector,
526 *Anopheles gambiae*. BMC Genomics 12:296.

527 Barberan-Soler, S., and A. M. Zahler. 2008. Alternative splicing regulation during *C. elegans*
528 development: Splicing factors as regulated targets. PLoS Genet. 4:e1000001.

529 Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate : A Practical and
530 Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57:289–300.

531 Blekhman, R., J. C. Marioni, P. Zumbo, M. Stephens, and Y. Gilad. 2010. Sex-specific and
532 lineage-specific alternative splicing in primates. Genome Res. 20:180–189.

533 Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina
534 sequence data. Bioinformatics 30:2114–2120.

535 Bonduriansky, R., and S. F. Chenoweth. 2009. Intralocus sexual conflict. Trends Ecol. Evol.
536 24:280–288.

537 Bourgon, R., R. Gentleman, and W. Huber. 2010. Independent filtering increases detection
538 power for high-throughput experiments. Proc. Natl. Acad. Sci. U. S. A. 107:9546–9551.

539 Braverman, J. M., R. R. Hudson, N. L. Kaplan, C. H. Langley, and W. Stephan. 1995. The
540 hitchhiking effect on the site frequency spectrum of DNA polymorphisms. Genetics
541 140:783–796.

542 Carneiro, M., F. W. Albert, J. Melo-Ferreira, N. Galtier, P. Gayral, J. A. Blanco-Aguiar, R.
543 Villafuerte, M. W. Nachman, and N. Ferrand. 2012. Evidence for widespread positive and
544 purifying selection across the european rabbit (*Oryctolagus cuniculus*) genome. Mol. Biol.
545 Evol. 29:1837–1849.

546 Chang, P. L., J. P. Dunham, S. V. Nuzhdin, and M. N. Arbeitman. 2011. Somatic sex-specific
547 transcriptome differences in *Drosophila* revealed by whole transcriptome sequencing. BMC
548 Genomics 12:364.

549 Charlesworth, B., and D. Charlesworth. 2010. Elements of Evolutionary Genetics. 1st ed.
550 Roberts and Company Publishers, Greenwood Village, CO.

551 Cox, R. M., and R. Calsbeek. 2009. Sexually Antagonistic Selection, Sexual Dimorphism, and
552 the Resolution of Intralocus Sexual Conflict. Am. Nat. 173:176–187.

553 Cutter, A. D. 2019. A Primer of Molecular Population Genetics. Oxford University Press,
554 Oxford.

555 Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker,
556 G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, and R. Durbin. 2011. The variant call
557 format and VCFtools. Bioinformatics 27:2156–2158.

558 Dapper, A. L., and M. J. Wade. 2020. Relaxed Selection and the Rapid Evolution of
559 Reproductive Genes. Trends Genet. 36:640–649. Elsevier Ltd.

560 Dean, R., and J. E. Mank. 2016. Tissue specificity and sex-specific regulatory variation permit
561 the evolution of sex-biased gene expression. Am. Nat. 188:E74–E84.

562 Dobin, A., C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson,
563 and T. R. Gingeras. 2013. STAR: Ultrafast universal RNA-seq aligner. Bioinformatics
564 29:15–21.

565 Dos Santos, G., A. J. Schroeder, J. L. Goodman, V. B. Strelets, M. A. Crosby, J. Thurmond, D.
566 B. Emmert, W. M. Gelbart, N. H. Brown, T. Kaufman, M. Werner-Washburne, R. Cripps,
567 K. Broll, L. S. Gramates, K. Falls, B. B. Matthews, S. Russo, P. Zhou, M. Zytkovicz, B.
568 Adryan, H. Attrill, M. Costa, S. Marygold, P. McQuilton, G. Millburn, L. Ponting, R.
569 Stefancsik, S. Tweedie, and G. Grumbling. 2015. FlyBase: Introduction of the *Drosophila*

570 *melanogaster* Release 6 reference genome assembly and large-scale migration of genome
571 annotations. Nucleic Acids Res. 43:D690–D697.

572 Dutoit, L., C. F. Mugal, P. Bolívar, M. Wang, K. Nadachowska-Brzyska, L. Smeds, H. P. Yazdi,
573 L. Gustafsson, and H. Ellegren. 2018. Sex-biased gene expression, sexual antagonism and
574 levels of genetic diversity in the collared flycatcher (*Ficedula albicollis*) genome. Mol.
575 Ecol. 27:3572–3581.

576 Eden, E., R. Navon, I. Steinfeld, D. Lipson, and Z. Yakhini. 2009. *GOrilla*: A tool for discovery
577 and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics 10:48.

578 Ellegren, H., and J. Parsch. 2007. The evolution of sex-biased genes and sex-biased gene
579 expression. Nat. Rev. Genet. 8:689–698.

580 Ewels, P., M. Magnusson, S. Lundin, and M. Käller. 2016. MultiQC: Summarize analysis results
581 for multiple tools and samples in a single report. Bioinformatics 32:3047–3048.

582 Frank, S. A., and M. M. Patten. 2020. Sexual antagonism leads to a mosaic of X-autosome
583 conflict. Evolution. 74:495–498.

584 Freedman, D., and D. Lane. 1983. A Nonstochastic Interpretation of Reported Significance
585 Levels. J. Buisness Econ. Stat. 1:292–298.

586 Frossard, J., and O. Renaud. 2019. *permuco*: Permutation Tests for Regression, (Repeated
587 Measures) ANOVA/ANCOVA and Comparison of Signals.

588 Gershoni, M., and S. Pietrokovski. 2014. Reduced selection and accumulation of deleterious
589 mutations in genes exclusively expressed in men. Nat. Commun. 5:4438.

590 Gibilisco, L., Q. Zhou, S. Mahajan, and D. Bachtrog. 2016. Alternative Splicing within and
591 between *Drosophila* Species, Sexes, Tissues, and Developmental Stages. PLoS Genet.
592 12:e1006464.

593 Harrison, P. W., A. E. Wright, F. Zimmer, R. Dean, S. H. Montgomery, M. A. Pointer, and J. E.
594 Mank. 2015. Sexual selection drives evolution and rapid turnover of male gene expression.
595 Proc. Natl. Acad. Sci. U. S. A. 112:4393–4398.

596 Hartl, D. L., and A. G. Clark. 2007. Principles of Population Genetics. 4th ed. Sinauer,

Sunderland, MA.

Hartley, S. W., and J. C. Mullikin. 2016. Detection and visualization of differential splicing in RNA-Seq data with JunctionSeq. Nucleic Acids Res. 44:e127.

Hersh, E., J. A. Madjidian, S. Andersson, M. Strandh, W. S. Armbruster, and A. Lankinen. 2015. Sexual antagonism in the pistil varies among populations of a hermaphroditic mixed-mating plant. J. Evol. Biol. 28:1321–1334.

Ho, E. K. H., M. Bartkowska, S. I. Wright, and A. F. Agrawal. 2019. Population genomics of the facultatively asexual duckweed *Spirodela polyrhiza*. New Phytol. 224:1361–1371.

Huang, W., A. Massouras, Y. Inoue, J. Peiffer, M. Ràmia, A. M. Tarone, L. Turlapati, T. Zichner, D. Zhu, R. F. Lyman, M. M. Magwire, K. Blankenburg, M. A. Carbone, K. Chang, L. L. Ellis, S. Fernandez, Y. Han, G. Highnam, C. E. Hjelmen, J. R. Jack, M. Javaid, J. Jayaseelan, D. Kalra, S. Lee, L. Lewis, M. Munidasa, F. Ongeri, S. Patel, L. Perales, A. Perez, L. L. Pu, S. M. Rollmann, R. Ruth, N. Saada, C. Warner, A. Williams, Y. Q. Wu, A. Yamamoto, Y. Zhang, Y. Zhu, R. R. H. Anholt, J. O. Korbel, D. Mittelman, D. M. Muzny, R. A. Gibbs, A. Barbadilla, J. S. Johnston, E. A. Stone, S. Richards, B. Deplancke, and T. F. C. Mackay. 2014. Natural variation in genome architecture among 205 *Drosophila melanogaster Genetic* Reference Panel lines. Genome Res. 24:1193–1208.

Huylmans, A. K., and J. Parsch. 2014. Population- and sex-biased gene expression in the excretion organs of *Drosophila melanogaster*. G3 Genes, Genomes, Genet. 4:2307–2315.

Innocenti, P., and E. H. Morrow. 2010. The Sexually Antagonistic Genes of *Drosophila melanogaster*. PLoS Biol. 8:e1000335.

International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. Nature 409:860–921.

Jakšić, A. M., and C. Schlötterer. 2016. The interplay of temperature and genotype on patterns of alternative splicing in *Drosophila melanogaster*. Genetics 204:315–325.

Kent, W. J., C. W. Sugnet, T. S. Furey, K. M. Roskin, T. H. Pringle, A. M. Zahler, and a. D. Haussler. 2002. The Human Genome Browser at UCSC. Genome Res. 12:996–1006.

624 Khil, P. P., N. A. Smirnova, P. J. Romanienko, and R. D. Camerini-Otero. 2004. The mouse X
625 chromosome is enriched for sex-biased genes not subject to selection by meiotic sex
626 chromosome inactivation. Nat. Genet. 36:642–646.

627 Kidwell, J. F., M. T. Clegg, F. M. Stewart, and T. Prout. 1977. Regions of stable equilibria for
628 models of differential selection in the two sexes under random mating. Genetics 85:171–
629 183.

630 Lack, J. B., C. M. Cardeno, M. W. Crepeau, W. Taylor, R. B. Corbett-Detig, K. A. Stevens, C.
631 H. Langley, and J. E. Pool. 2015. The *Drosophila* genome nexus: A population genomic
632 resource of 623 *Drosophila melanogaster* genomes, including 197 from a single ancestral
633 range population. Genetics 199:1229–1241.

634 Lack, J. B., J. D. Lange, A. D. Tang, R. B. Corbett-Detig, and J. E. Pool. 2016. A thousand fly
635 genomes: An expanded *Drosophila* genome nexus. Mol. Biol. Evol. 33:3308–3313.

636 Lande, R. 1980. Sexual Dimorphism, Sexual Selection, and Adaptation in Polygenic Characters.
637 Evolution. 34:292–305.

638 Long, Y., G. Song, J. Yan, X. He, Q. Li, and Z. Cui. 2013. Transcriptomic characterization of
639 cold acclimation in larval zebrafish. BMC Genomics 14:612.

640 Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion
641 for RNA-seq data with DESeq2. Genome Biol. 15:1–21.

642 MacKay, T. F. C., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles, D. Zhu, S. Casillas, Y.
643 Han, M. M. Magwire, J. M. Cridland, M. F. Richardson, R. R. H. Anholt, M. Barrón, C.
644 Bess, K. P. Blankenburg, M. A. Carbone, D. Castellano, L. Chaboub, L. Duncan, Z. Harris,
645 M. Javaid, J. C. Jayaseelan, S. N. Jhangiani, K. W. Jordan, F. Lara, F. Lawrence, S. L. Lee,
646 P. Librado, R. S. Linheiro, R. F. Lyman, A. J. MacKey, M. Munidasa, D. M. Muzny, L.
647 Nazareth, I. Newsham, L. Perales, L. L. Pu, C. Qu, M. Ràmia, J. G. Reid, S. M. Rollmann,
648 J. Rozas, N. Saada, L. Turlapati, K. C. Worley, Y. Q. Wu, A. Yamamoto, Y. Zhu, C. M.
649 Bergman, K. R. Thornton, D. Mittelman, and R. A. Gibbs. 2012. The *Drosophila*
650 *melanogaster* Genetic Reference Panel. Nature 482:173–178.

651 Mank, J. E. 2017. Population genetics of sexual conflict in the genomic era. Nat. Rev. Genet.

652 18:721–730.

653 McIntyre, L. M., L. M. Bono, A. Genissel, R. Westerman, D. Junk, M. Telonis-Scott, L.
654 Harshman, M. L. Wayne, A. Kopp, and S. V. Nuzhdin. 2006. Sex-specific expression of
655 alternative transcripts in *Drosophila*. Genome Biol. 7:R79.

656 Meisel, R. P., J. H. Malone, and A. G. Clark. 2012. Disentangling the relationship between sex-
657 biased gene expression and X-linkage. Genome Res. 22:1255–1265.

658 Mironov, A. A., J. W. Fickett, and M. S. Gelfand. 1999. Frequent alternative splicing of human
659 genes. Genome Res. 9:1288–1293.

660 Modrek, B., and C. Lee. 2002. A genomic view of alternative splicing. Nat. Genet. 30:13–19.

661 Modrek, B., A. Resch, C. Grasso, and C. Lee. 2001. Genome-wide detection of alternative
662 splicing in expressed sequences of human genes. Nucleic Acids Res. 29:2850–2859.

663 Nei, M., and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of
664 restriction endonucleases. Proc. Natl. Acad. Sci. U. S. A. 76:5269–5273.

665 Osada, N., R. Miyagi, and A. Takahashi. 2017. Cis- and trans- regulatory effects on gene
666 expression. Genetics 206:2139–2148.

667 Paape, T., T. Bataillon, P. Zhou, T. J. Y. Kono, R. Briskine, N. D. Young, and P. Tiffin. 2013.
668 Selection, genome-wide fitness effects and evolutionary rates in the model legume
669 *Medicago truncatula*. Mol. Ecol. 22:3525–3538.

670 Page, A. J., B. Taylor, A. J. Delaney, J. Soares, T. Seemann, J. A. Keane, and S. R. Harris. 2016.
671 SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb.
672 genomics 2:e000056.

673 Pál, C., B. Papp, and L. D. Hurst. 2001. Highly Expressed Genes in Yeast Evolve Slowly.
674 2Genetics 158:227–231.

675 Parisi, M., R. Nuttall, P. Edwards, J. Minor, D. Naiman, J. Lü, M. Doctolero, M. Vainer, C.
676 Chan, J. Malley, S. Eastman, and B. Oliver. 2004. A survey of ovary-, testis-, and soma-
677 biased gene expression in *Drosophila melanogaster* adults. Genome Biol. 5:R40.

678 Parisi, M., R. Nuttall, D. Naiman, G. Bouffard, J. Malley, J. Andrews, S. Eastman, and B. Oliver.

679 2003. Paucity of genes on the *Drosophila* X chromosome showing male-biased expression.
680 Science. 299:697–700.

681 Parsch, J., and H. Ellegren. 2013. The evolutionary causes and consequences of sex-biased gene
682 expression. Nat. Rev. Genet. 14:83–87.

683 Pröschel, M., Z. Zhang, and J. Parsch. 2006. Widespread adaptive evolution of *Drosophila* genes
684 with sex-biased expression. Genetics 174:893–900.

685 Quinlan, A. R., and I. M. Hall. 2010. BEDTools: A flexible suite of utilities for comparing
686 genomic features. Bioinformatics 26:841–842.

687 Ramani, A. K., J. A. Calarco, Q. Pan, S. Mavandadi, Y. Wang, A. C. Nelson, L. J. Lee, Q.
688 Morris, B. J. Blencowe, M. Zhen, and A. G. Fraser. 2011. Genome-wide analysis of
689 alternative splicing in *Caenorhabditis elegans*. Genome Res. 21:342–348.

690 Ranz, J. M., C. I. Castillo-Davis, C. D. Meiklejohn, and D. L. Hartl. 2003. Sex-dependent gene
691 expression and evolution of the *Drosophila* transcriptome. Science. 300:1742–1745.

692 Reinke, V., I. S. Gil, S. Ward, and K. Kazmer. 2004. Genome-wide germline-enriched and sex-
693 biased expression profiles in *Caenorhabditis elegans*. Development 131:311–323.

694 Revil, T., D. Gaffney, C. Dias, J. Majewski, and L. A. Jerome-Majewska. 2010. Alternative
695 splicing is frequent during early embryonic development in mouse. BMC Genomics 11:399.

696 Rice, W. R. 1984. Sex Chromosomes and the Evolution of Sexual Dimorphism. Evolution.
697 38:735–742.

698 Rice, W. R. 1987. The Accumulation of Sexually Antagonistic Genes as a Selective Agent
699 Promoting the Evolution of Reduced Recombination Between Primitive Sex Chromosomes.
700 Evolution. 41:911–914.

701 Rogers, T. F., D. H. Palmer, and A. E. Wright. 2020. Sex-Specific Selection Drives the
702 Evolution of Alternative Splicing in Birds. Mol. Biol. Evol. 38:519–530.

703 Rowe, L., S. F. Chenoweth, and A. F. Agrawal. 2018. The Genomics of Sexual Conflict. Am.
704 Nat. 192:274–286.

705 Sayadi, A., A. Martinez Barrio, E. Immonen, J. Dainat, D. Berger, C. Tellgren-Roth, B. Nystedt,

706 and G. Arnqvist. 2019. The genomic footprint of sexual conflict. Nat. Ecol. Evol. 3:1725–
707 1730.

708 Sorek, R., R. Shamir, and G. Ast. 2004. How prevalent is functional alternative splicing in the
709 human genome? Trends Genet. 20:68–71.

710 Stewart, A. D., A. Pischedda, and W. R. Rice. 2010. Resolving intralocus sexual conflict:
711 Genetic mechanisms and time frame. J. Hered. 101:94–99.

712 Sturgill, D., Y. Zhang, M. Parisi, and B. Oliver. 2007. Demasculinization of X chromosomes in
713 the *Drosophila* genus. Nature 450:238–241.

714 Subramanian, S., and S. Kumar. 2004. Gene expression intensity shapes evolutionary rates of the
715 proteins encoded by the vertebrate genome. Genetics 168:373–381.

716 Tajima, F. 1989. Statistical Method for Testing the Neutral Mutation Hypothesis by DNA
717 Polymorphism. Genetics 123:585–595.

718 Tan, M. H., K. F. Au, A. L. Yablonovitch, A. E. Wills, J. Chuang, J. C. Baker, W. H. Wong, and
719 J. B. Li. 2013. RNA sequencing reveals a diverse and dynamic repertoire of the *Xenopus*
720 *tropicalis* transcriptome over development. Genome Res. 23:201–216.

721 Telonis-Scott, M., A. Kopp, M. L. Wayne, S. V. Nuzhdin, and L. M. McIntyre. 2009. Sex-
722 specific splicing in *Drosophila*: Widespread occurrence, tissue specificity and evolutionary
723 conservation. Genetics 181:421–434.

724 Trabzuni, D., A. Ramasamy, S. Imran, R. Walker, C. Smith, M. E. Weale, J. Hardy, and M.
725 Ryten. 2013. Widespread sex differences in gene expression and splicing in the adult human
726 brain. Nat. Commun. 4: 2771.

727 Williamson, R. J., E. B. Josephs, A. E. Platts, K. M. Hazzouri, A. Haudry, M. Blanchette, and S.
728 I. Wright. 2014. Evidence for Widespread Positive and Negative Selection in Coding and
729 Conserved Noncoding Regions of Capsella grandiflora. PLoS Genet. 10:e1004622.

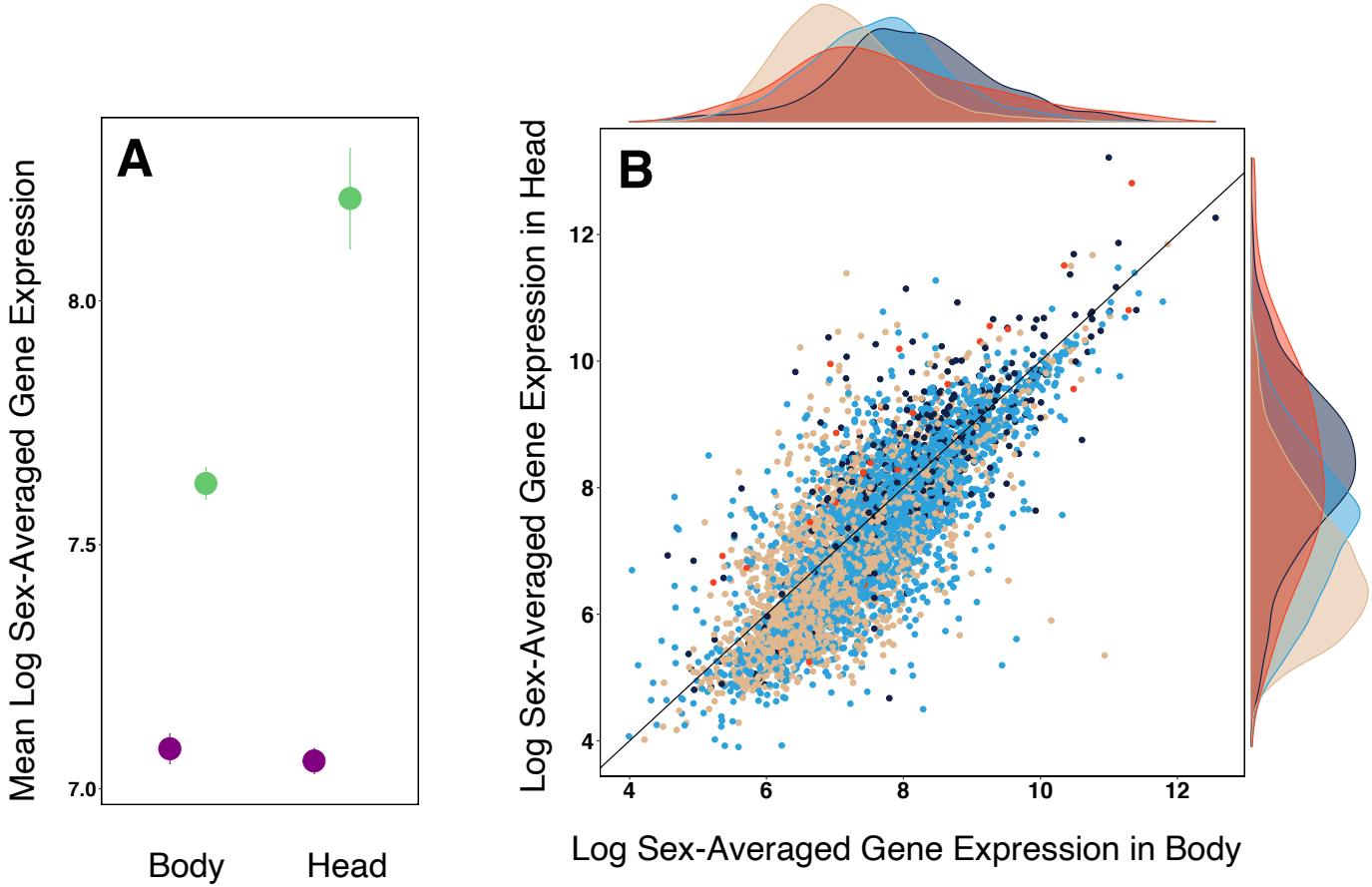
730 Wright, A. E., M. Fumagalli, C. R. Cooney, N. I. Bloch, F. G. Vieira, S. D. Buechel, N. Kolm,
731 and J. E. Mank. 2018. Male-biased gene expression resolves sexual conflict through the
732 evolution of sex-specific genetic architecture. Evol. Lett. 2:52–61.

Wright, A. E., T. F. Rogers, M. Fumagalli, C. R. Cooney, and J. E. Mank. 2019. Phenotypic sexual dimorphism is associated with genomic signatures of resolved sexual conflict. Mol. Ecol. 28:2860–2871.

Yang, X., E. E. Schadt, S. Wang, H. Wang, A. P. Arnold, L. Ingram-Drake, T. A. Drake, and A. J. Lusis. 2006. Tissue-specific expression and regulation of sexually dimorphic genes in mice. Genome Res. 16:995–1004.

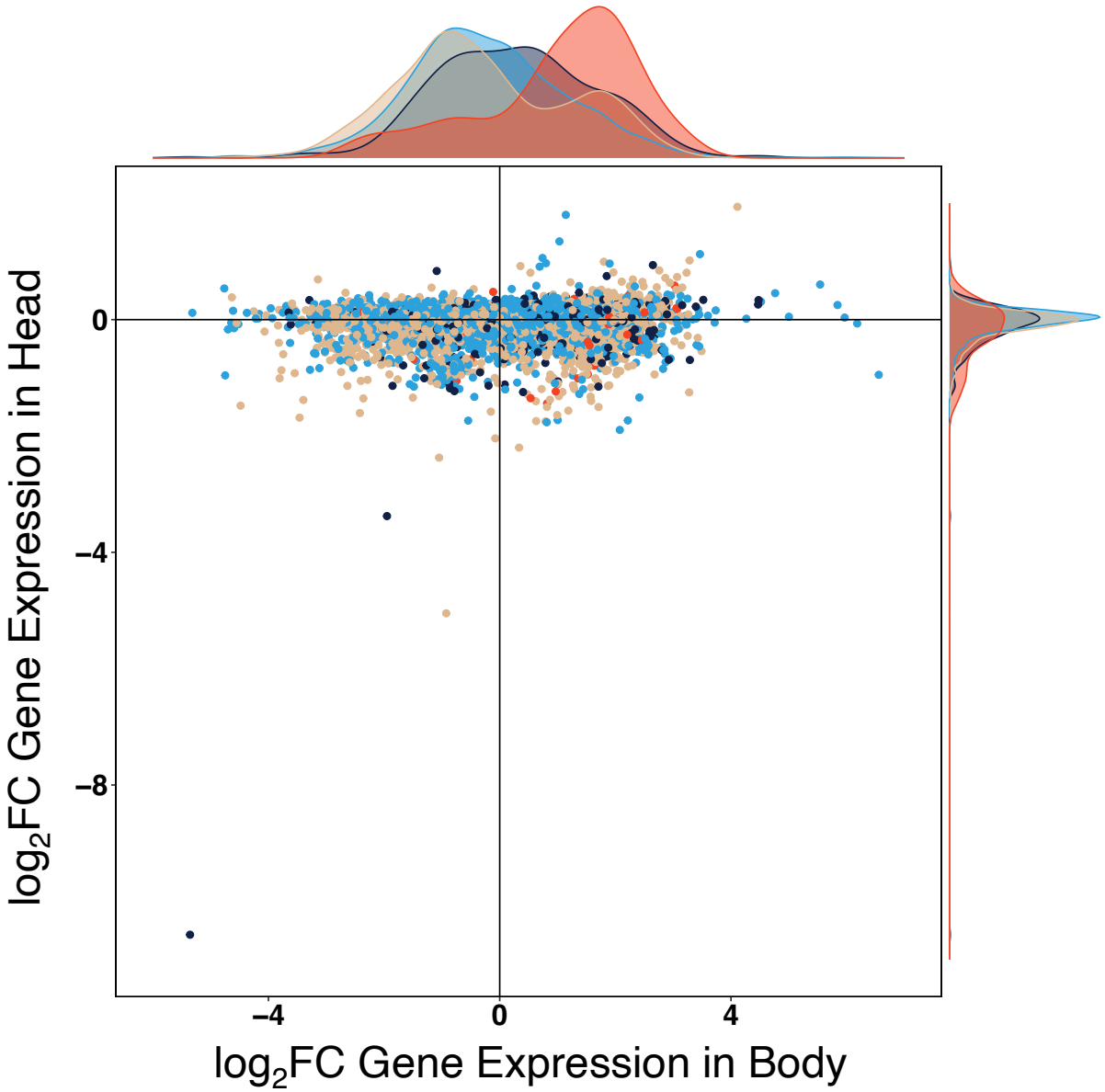
Zemp, N., R. Tavares, A. Muyle, D. Charlesworth, G. A. B. Marais, and A. Widmer. 2016. Evolution of sex-biased gene expression in a dioecious plant. Nat. Plants 2:16168.

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.




752

753 **Figure S2:** Scatter plot and density distributions of the $\log_2$ fold change ($\log_2$FC) in male to
754 female gene expression of genes exhibiting sexual dimorphism in isoform usage (SDIU) only in
755 bodies (light blue), only in heads (red), in both heads and bodies (dark blue) or in neither heads
756 nor bodies (beige) when expressed in bodies (x-axis) or heads (y-axis). Density plots above and
757 to the right of the figure correspond to the $\log_2$FC in gene expression in bodies and heads
758 respectively.

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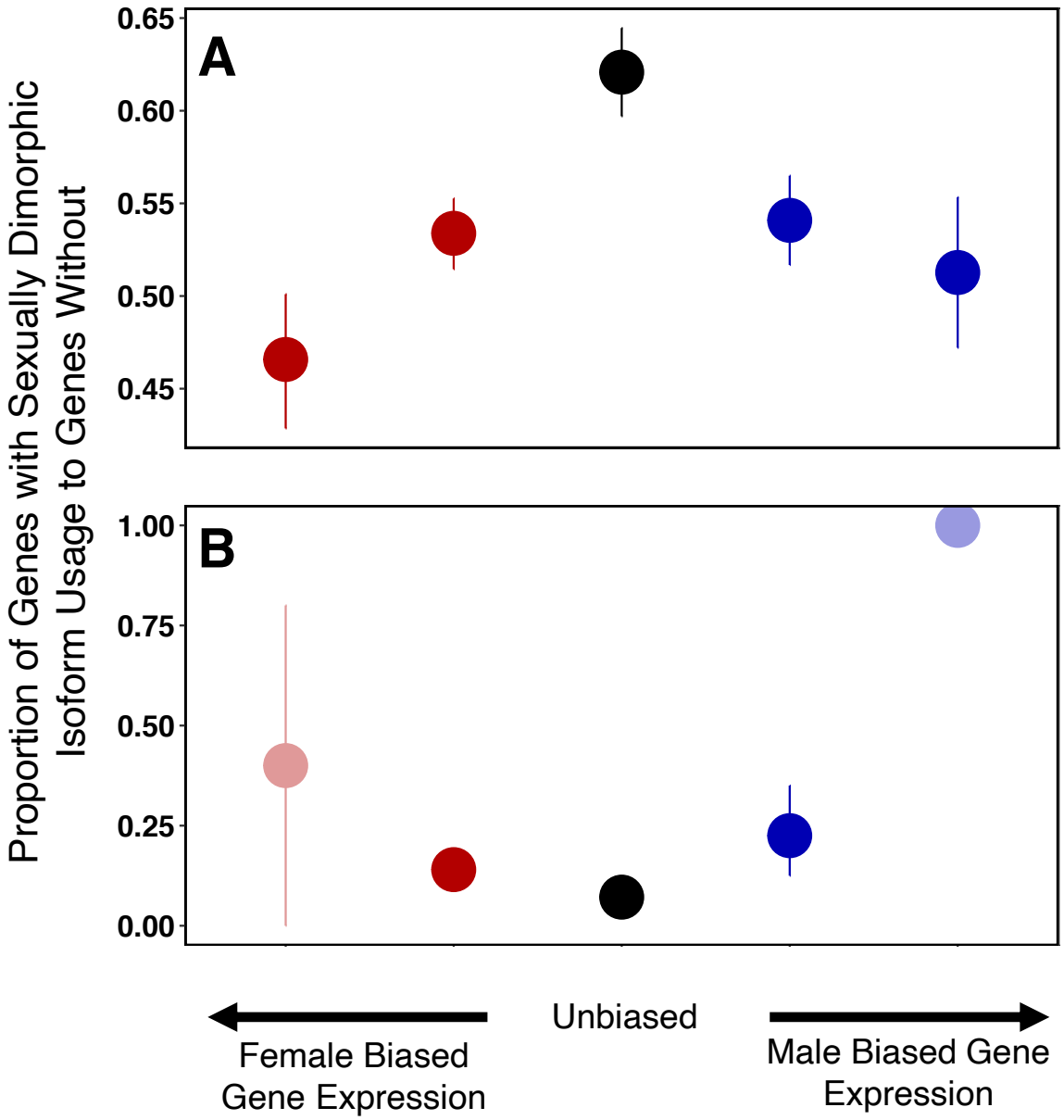


**Figure S3:** The proportion of genes exhibiting SDIU to genes not exhibiting SDIU in bins of log2FC in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue) expression when expressed in **(C)** bodies or **(D)** heads. Faded points indicate bins with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

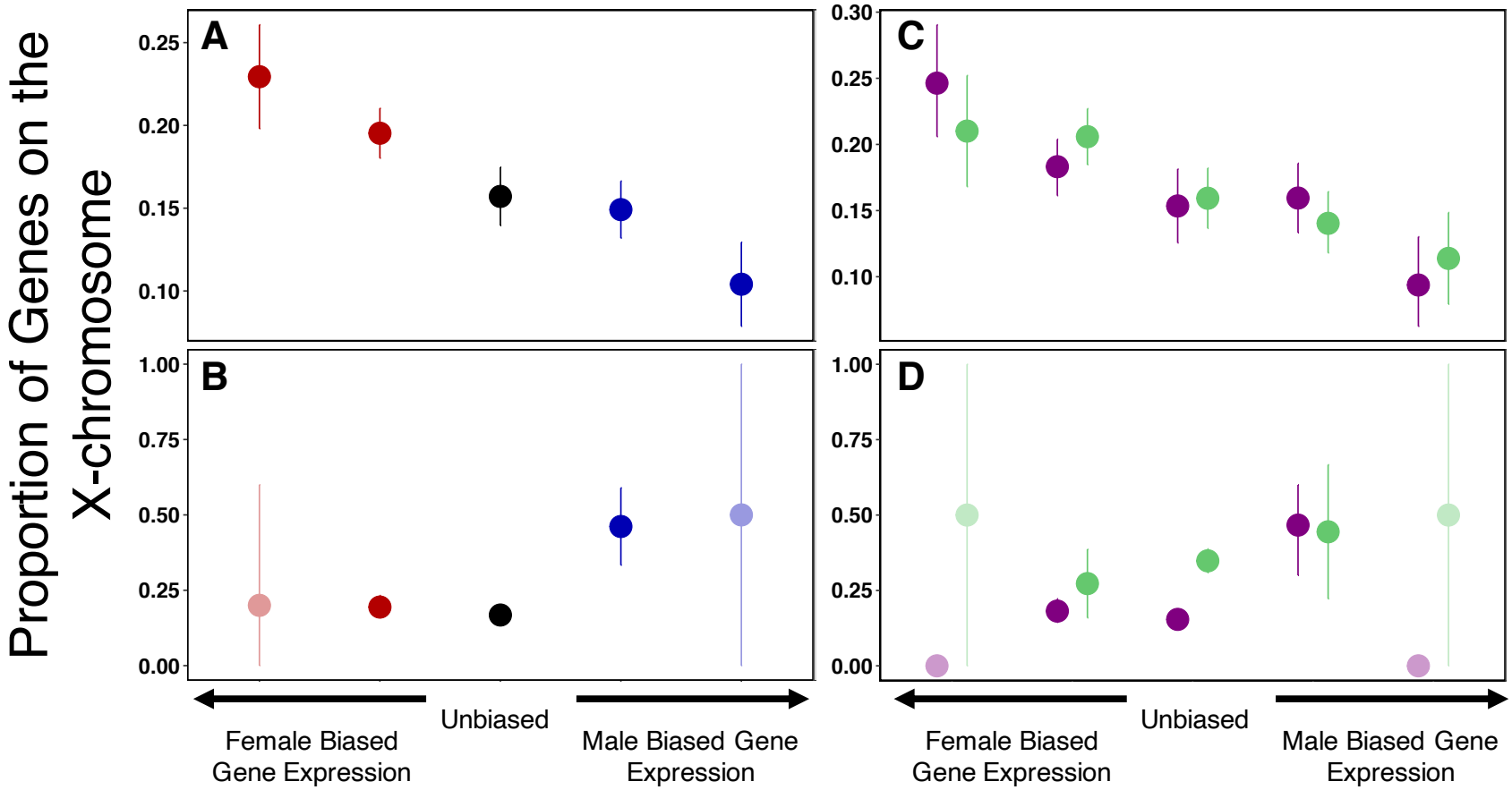


**Figure S4:** Proportion of genes exhibiting sexually dimorphic isoform usage (SDIU) and sex-biased gene expression (SBGE) that are X-linked. **(A-B)** The proportion of genes found on the X chromosome in bins of $\log_2$FC in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue) expression when expressed in **(A)** bodies or **(B)** heads. **(C-D)** The proportion of genes exhibiting SDIU (green) and not exhibiting SDIU (purple) found on the X chromosome in bins of $\log_2$FC 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 **(C)** bodies or **(D)** heads. Faded points indicate bins with fewer than 5 genes. Error bars are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

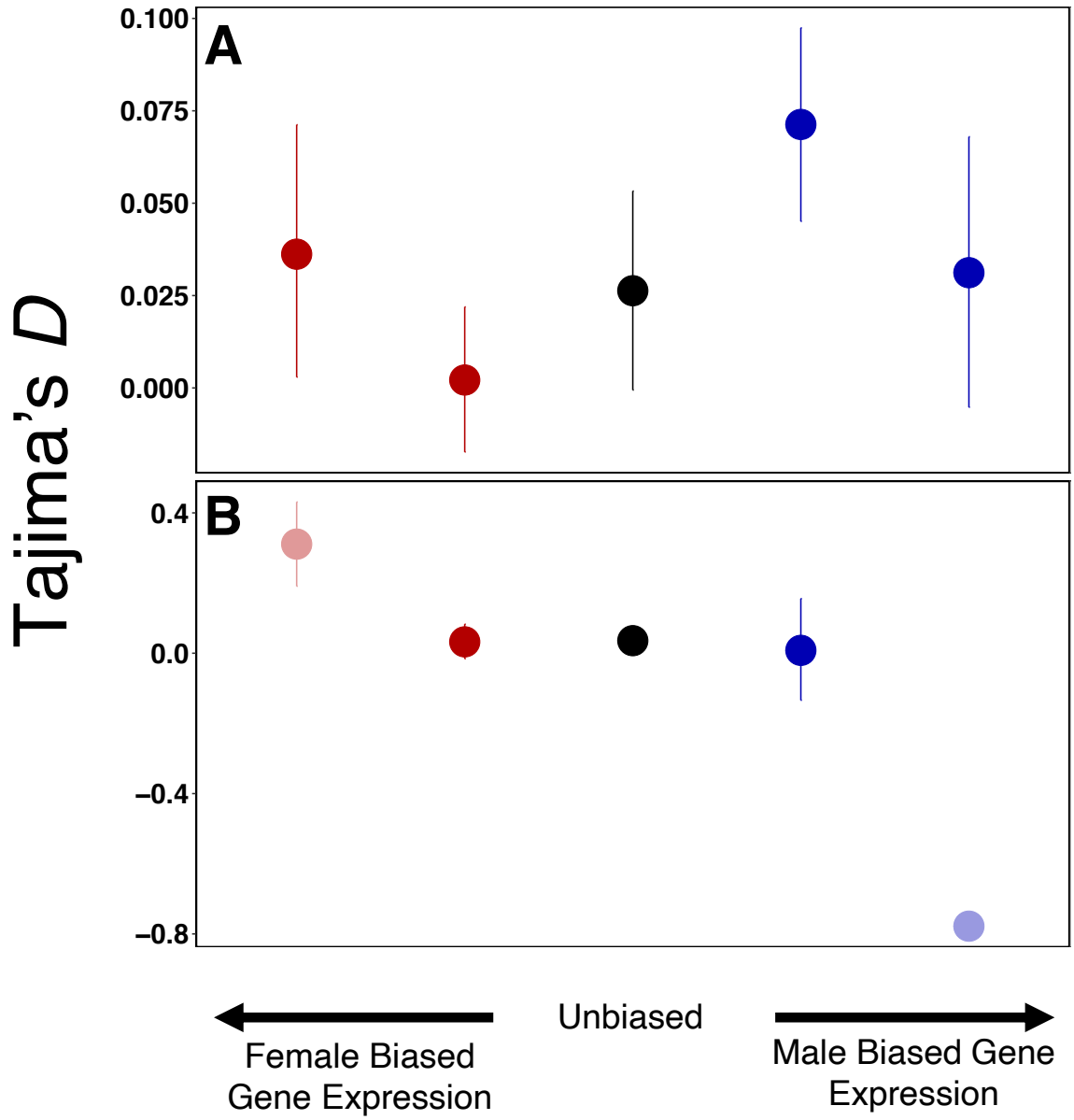


**Figure S5: (A, B)** Mean Tajima's *D* for all genes expressed in **(A)** body or **(B)** head tissue averaged across all genes within bins of $\log_2$FC in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue). Faded points indicate bins with fewer than 5 genes. Error bars are 95% confidence intervals estimated from 10,000 bootstraps resampling estimates.

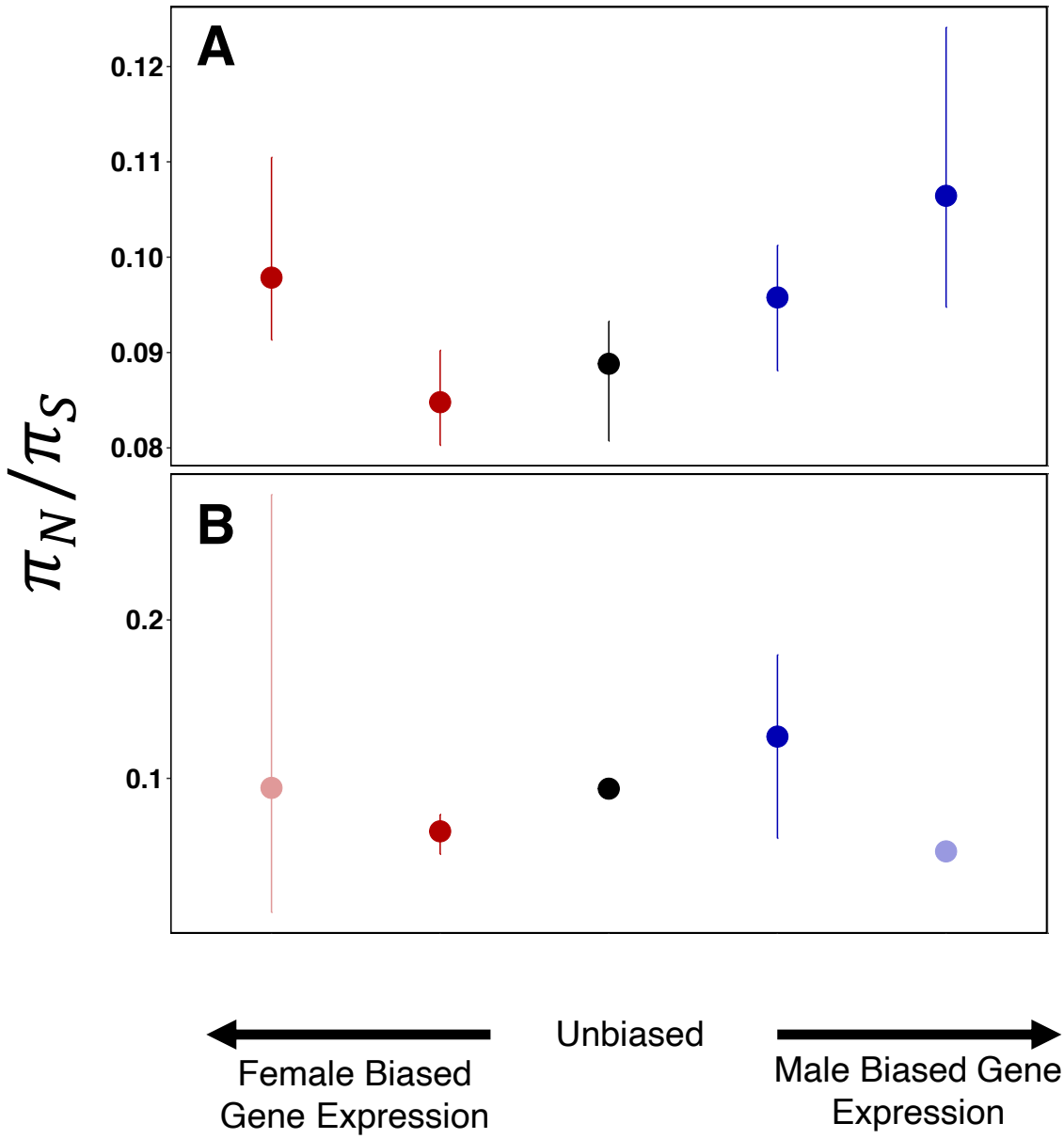


**Figure S6: (A, B)** Median $\pi_N/\pi_S$ for all genes expressed in **(A)** body or **(B)** head tissue averaged across all genes within bins of $\log_2$FC in gene expression (see Methods) ranging from female-biased (red), unbiased (black) and male-biased (blue). Faded points indicate bins with fewer 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 |
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| 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 |

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 $\pi_N/\pi_S$. $\pi_N/\pi_S$ 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 | *P* |
|---|---|---|---|---|
| Body Tissue | **Intercept** | **2.700** | **0.112** | **< 0.001** |
| | **Sex-averaged Gene Expression[1]** | **$-4.29 \times 10^{-5}$** | **$5.55 \times 10^{-6}$** | **< 0.001** |
| | **Absolute value of SBGE[2]** | **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 Expression[1]** | **$-4.68 \times 10^{-6}$** | **$2.39 \times 10^{-6}$** | **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 |

[1]Gene expression averaged across both sexes.
[2]Measured as $|\text{Log}_2\text{FC male:female gene expression}|$.
[3]Indicator of whether or not a gene exhibited statistically significant evidence of SDIU.