Two forms of sexual dimorphism in gene expression in *Drosophila melanogaster*: their coincidence and evolutionary genetics

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Impact Summary

Differences in how genes are expressed between the sexes allow males and females to differ, on average, in a variety of morphological, physiological, and behavioural traits. Using fruit fly data, we examine sexual dimorphism in two different forms of gene expression: (i) sex-biased gene expression (SBGE) quantifies sex differences in the total amount of expression from a gene and (ii) sexual dimorphic isoform usage (SDIU) indicates whether the sexes differ in their relative usage of different isoforms (structurally different proteins coded by the same gene). Based on whole body expression data, we find that these two forms of expression dimorphism co-occur at the same genes less often than expected by chance, possibly indicating that they represent alternative solutions to sexually antagonistic selection. A separate analysis indicates that patterns of quantitative genetic variation are more conducive to genes with SDIU evolving sex differences in total expression than those without SDIU. The observation that SBGE and SDIU are negatively associated suggests that genes with SDIU are not under sexually antagonistic selection for expression or that the variants that would allow for greater dimorphism have other negative effects. An analysis of patterns of DNA sequence variation at the population level suggests that genes with SDIU experience more adaptive evolution than those without. There is little evidence that SBGE is associated with adaptation, except for strongly male-biased genes.

Abstract

Sex differences in gene regulation is a proximate mechanism by which evolution can resolve sexually antagonistic phenotypic selection. One form of differential gene regulation is sex-biased gene expression (SBGE) in which the sexes differ in the amount a gene is expressed. Less attention has been given to sexual dimorphism in isoform usage (SDIU), resulting from sex differences in alternative splicing. Here, we use RNA-seq data from two tissue types (heads and bodies) from 18 genotypes of adult *Drosophila melanogaster* to investigate SDIU and SBGE. Both forms of expression dimorphism 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. With respect to body expression, we examine associations of SDIU and SBGE with several evolutionary genetic properties. The intersexual genetic correlation in expression (r_{mf}) increases from female-biased to unbiased to male-biased genes but is drastically reduced for genes with extreme male bias. r_{mf} is lower for genes with versus without SDIU, suggesting that genes with SDIU should be able to evolve greater levels of SBGE holding all else equal. SDIU has no detectable effect on relative non-synonymous diversity. In contrast, non-synonymous diversity is elevated for genes with extreme sex-bias (particularly male-bias) but other effects of SBGE are more subtle. SDIU is associated with reduced values of Tajima's *D*. After accounting for correlated variables, sex-bias tends to reduce Tajima's *D*. Finally, *DoS* is elevated for genes with SDIU, suggestive of higher rates of adaptive evolution. In contrast, SBGE is not associated with *DoS* after excluding genes with extreme male-bias (for which *DoS* is highly elevated). Together, these results show that both SDIU and SBGE are independently and distinctly associated with evolutionary genetic statistics, suggesting that selection acts somewhat differently on these two dimensions of expression dimorphism.

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; Delph et al.2022) 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 has been a strong interest in 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 examine sexual dimorphism in isoform usage (SDIU) in addition to SBGE.

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 ~95% of multi-exon genes exhibit alternative splicing (Pan et al. 2008). 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

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

112 Widespread sexual dimorphism in isoform usage has been documented in humans
113 (Trabzuni et al. 2013) and other primates (Blekhman et al. 2010) as well as in birds (Rogers et al.
114 2020). Several microarray and RNA-seq studies across *Drosophila* species have also investigated
115 sexual dimorphism in isoform abundance and exon usage. These studies have generally supported
116 a view that sexual dimorphism in isoform usage is widespread (e.g., McIntyre et al. 2006; Telonis-
117 Scott et al. 2009; Chang et al. 2011), tissue-specific (Telonis-Scott et al. 2009), and evolutionarily
118 conserved (Gibilisco et al. 2016).

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

128 Our other main avenue of investigation focuses on evolutionary genetic properties of
129 genes exhibiting sex-differential regulation. This follows from several previous studies,
130 including in flies (Griffin et al. 2013, Fraïsse et al. 2019), seed beetles (Sayadi et al. 2019) and
131 several bird species (Wright et al. 2019), that have examined quantitative and population
132 genomic properties in an attempt to infer evolutionary constraint or the form of selection acting
133 on sex-biased versus unbiased genes. We take a similar approach to examine how two different
134 forms of expression dimorphism—SBGE and SDIU—are associated with four evolutionary
135 genetic properties: intrasexual genetic correlation (r_{mf}) in expression, relative levels of
136 nonsynonymous diversity, Tajima's *D*, and the direction of selection (*DoS*).

137

138 **Methods**

139 Obtaining RNA-seq Data and Read Mapping

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

154 Differential Exon Usage Analysis

155 To identify a list of genes exhibiting SDIU, we performed differential exon usage
156 analysis using the R package *JunctionSeq* (v1.17; Hartley and Mullikin 2016). Briefly,
157 *JunctionSeq* splits genes into annotated exons and splice junction 'counting bins' and counts the
158 number of reads that overlap each counting bin within an annotated gene. To test for differential
159 exon usage between the sexes, a generalized linear model (GLM) with logarithmic link function
160 was fit at each gene feature (i.e., exon or splice junction) to assess sex differences in the number
161 of reads that map to a particular gene feature relative to the total number of reads that map to the
162 gene to which the feature belongs. Specifically, for each gene, *JunctionSeq* fits the model

163 $$log(y_{ij}) = \mu + W_i + X_j + Z_{ij}$$

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

172 *JunctionSeq* can test genes with at least two annotated exons. It applies a multiple-testing
173 correction method based on the Benjamini and Hochberg procedure (Benjamini and Hochberg
174 1995) to generate adjusted *P*-values. Genes were considered to show evidence for sexual
175 dimorphism in isoform usage if the sexes exhibited statistically significant differential usage of a
176 single exon (or more) within a gene using $p_{adj} < 0.05$.

177 Ideally, genotype would be included in the model as a random effect but *JunctionSeq*
178 does not allow this. This is unlikely to be a major issue here. Our dataset is perfectly balanced
179 with each genotype being represented by only a single sample for each sex for each tissue (head
180 and body data were analyzed separately). More importantly, our goal is to identify a list of genes
181 enriched for SDIU for further analysis; our focus is not on the accuracy of *p*-values of individual
182 genes. As a simple check of the *JunctionSeq* pipeline, we analyzed 10 datasets in which we
183 switched the male/female labels for a randomly chosen half of the genotypes. In these permuted
184 data sets, no genes were identified as having SDIU at $p_{adj} < 0.01$ whereas thousands of genes are
185 found in the true (unpermuted) data under the same criterion.

186 Sex-biased gene expression

187 We estimated differential gene expression between the sexes using the R package
188 *DESeq2* (v1.28.1; Love et al. 2014). Briefly, *DESeq2* estimates normalized read counts mapping
189 to genes on a $\log_2$ scale and uses a GLM framework to test for differential gene expression
190 between experimental conditions. Before performing differential gene expression analysis, we
191 performed a 'pre-filtering' procedure to remove genes that had very low numbers of reads
192 mapping. Pre-filtering RNA-seq data has been shown to substantially increase power in detecting

193 differential gene expression by reducing the burden of multiple testing but can also result in an
194 increase in Type I error (i.e., increased false-positive rate) (Bourgon et al. 2010). To balance
195 these competing issues, we removed any genes that had fewer than an average of 50 reads
196 mapping from all 36 samples (i.e., 18 male and 18 female genotypes). We then estimated the $\log_2$
197 fold change ($\log_2$FC) in male to female gene expression for each annotated gene and retained
198 only genes that were tested for differential exon usage. In downstream analyses, we assigned
199 each gene to bins based on either their average expression of the gene or based on the $\log_2$FC in
200 male to female gene expression.

201 For visual inspection of patterns, we assigned each gene to one of seven bins of
202 predefined ranges of $\log_2$FC in gene expression: extreme female bias ($\log_2$FC < -5), strong
203 female bias (-5 ≤ $\log_2$FC < -2), moderate female bias (-2 ≤ $\log_2$FC < -0.5), unbiased
204 expression (0.5 ≤ $\log_2$FC < 0.5), moderate male bias (0.5 ≤ $\log_2$FC < 2), strong male bias (2
205 ≤ $\log_2$FC < 5), and extreme male bias ($\log_2$FC ≥ 5). Binning was conducted separately with
206 respect to expression in heads and bodies. A small number of genes for which the standard error
207 in $\log_2$FC was high (> 0.5) were excluded. The bins were used for graphical presentation of
208 results; as described below, our main analyses treat $\log_2$FC as a continuous variable. In heads,
209 there were few genes with $\log_2$FC > |2| so only the three central bins are shown in the figures.
210 Distributions of $\log_2$FC for each tissue are shown in Fig. S1.

211

212 <u>Sex-averaged expression</u>

213 For the 18 samples for each sex, we used *featureCounts* (Liao et al. 2014) to generate read
214 counts for each sample. Using *edgeR* (Robinson et al. 2010), we applied TMM normalization
215 before obtaining RPKM measures. RPKM is a normalized measure of expression that
216 incorporates read counts per gene and gene length. Gene lengths were extracted by
217 *featureCounts* from the GFF (release 6.32; Dos Santos et al. 2015). For each gene, we averaged
218 the RPKM value across the 18 samples within a sex, then used the average of male and female
219 values as a measure of the sex-averaged expression.

220

221 Tissue-specificity in expression

222 We examined how our two measures of expression dimorphism, SDIU and SBGE, are related to
223 another important aspect of expression, tissue specificity (or, conversely, "expression
224 pleiotropy"). We used the "tissue-by-stage" estimates of tissue specificity calculated by Fraïsse
225 et al. (2019) applying the method of Yanai et al. (2005) to another expression dataset
226 (modENCODE Consortium 2010). Their measure quantifies tissue specificity jointly across four
227 larval tissues (carcass, digestive system, central nervous system, imaginal discs), two pupal
228 tissues (central nervous system, fat tissue), and three adult tissues (carcass, digestive system,
229 heads).

230

231 Intersexual genetic correlation r_{mf}

232 Following Fraïsse et al. (2019), we calculated the intersexual genetic correlation in gene
233 expression from the data of Huang et al. (2015) who measured whole body gene expression of
234 185 genotypes from the Drosophila Reference Genome Panel. Using *MCMCglmm* (Hadfield
235 2010), for each gene we fit the model $z_{ijk} = \mu + \phi_{jk} + e_{ijk}$ where z_{ijk} is the normalized
236 expression of the ith replicate of sex j of genotype k. μ is the mean expression. ϕ_{jk} is the
237 random genetic effect of sex j of genotype k and follows a bivariate Gaussian distribution
238 characterized by sex-specific variances and the intersexual covariance. e_{ijk} is the residual.
239 Models were fit using uninformative priors and run for a million iterations after a burn-in period
240 of 10,000 iterations with a thinning interval of 1000. From the resulting MCMC posterior of sex-
241 specific variances and intersexual covariances, a posterior of r_{mf} was created and the mean value
242 used as the estimate of r_{mf}. We only considered r_{mf} values for genes for which both sexes had
243 genetic variances > 0.001 and statistically significant ($p < 0.05$) in at least one sex based on
244 results of Huang et al. (2015; their Table S5). In the Supplementary Material we show another
245 version of the main figure using only genes for which the genetic variance was statistically
246 significant in both sexes; the patterns are very similar.

247

248

249 Population Genomic Data

250 We obtained whole genome sequences for 205 haploid genotypes of the DPGP3
251 *Drosophila* population which were originally collected from a wild population in Siavonga,
252 Zambia (Lack et al. 2015, 2016). FASTA files were obtained from the *Drosophila* Genome
253 Nexus (DGN; Lack et al. 2015, 2016) website (https://www.johnpool.net/genomes.html) and
254 were filtered to remove sites with evidence of high relatedness and recent admixture using Perl
255 scripts provided on the DGN website. Following Sohail et al. (2017), we removed five genotypes
256 that had extremely high or low numbers of variants. Additionally, we removed a single genotype
257 (ZI28) for which the length of the X chromosome in the FASTA file differed from the other 204
258 genotypes. We then created a VCF file using the remaining 199 sequences with *SNP-sites*
259 (v2.5.1; Page et al. 2016). Coordinates for the VCF file were converted to release 6 of the
260 *Drosophila* reference genome using *UCSC liftOver* (Kent et al. 2002).

261

262 Estimating nucleotide diversity and Tajima's *D*

263 We obtained a GFF file for the *D. melanogaster* genome from Ensembl
264 (https://useast.ensembl.org/Drosophila_melanogaster/) and scored each coding site in the
265 genome as 4-fold (i.e., synonymous sites) or 0-fold (i.e., non-synonymous sites) degenerate
266 across the *Drosophila* genome using a Python script (script available at
267 https://github.com/tvkent/Degeneracy). We then parsed our VCF containing variant and invariant
268 sites in the DGRP into two files that contained either only 4-fold sites or only 0-fold degenerate
269 sites. After excluding sites with data from fewer than 100 lines and those with more than two
270 alleles, we measured per site nucleotide diversity $\pi = 2p(1-p)$, where p is the estimated
271 frequency of the reference base. Nonsynonymous diversity π_N within a gene was measured as
272 the mean nucleotide diversity at 0-fold degenerate sites within that gene and synonymous
273 diversity π_S within a gene as the mean nucleotide diversity at 4-fold degenerate sites within each
274 gene. Our analysis focuses on a measure of *relative* nonsynonymous diversity, $\tilde{\pi}_{rN} \equiv$
275 $\pi_N/(\pi_N + \pi_S)$. We use this rather than the more familiar ratio of π_N/π_S because the latter has
276 undesirable statistical properties, being highly sensitive to sampling variation in π_S and is

277 undefined if $\pi_S = 0$. Our measure $\tilde{\pi}_{rN}$ is bounded between 0 and 1 and will be similar to π_N/π_S
278 when $\pi_S >> \pi_N$, as is expected for typical genes.

279 Tajima's *D* (Tajima 1989) was estimated using 4-fold degenerate sites, accounting for the
280 sample size and number of 4-fold sites (both invariant and variant) considered for each gene
281 (Walsh and Lynch 2020, p. 303); sites with data from a fewer than 100 lines were not
282 considered. When sites varied in sample size, we individually down sampled variant sites to the
283 level of the site with the lowest sample size, then calculated Tajima's *D*. Downsampling was
284 performed 100 times per gene and the median value of Tajima's *D* was used.

285

286 Direction of Selection (*DoS*)

287 *DoS* (Stoletzki and Eyre-Walker 2011) is a metric that compares relative nonsynonymous
288 divergence to relative nonsynonymous polymorphism; in both cases, it is relative to the sum of
289 synonymous and nonsynonymous changes. We used the *DoS* values reported by Fraïsse et al.
290 (2019). They calculated divergence with respect to *D. simulans*. They calculated polymorphism
291 from 197 haploid genomes of *D. melanogaster* genomes of the DPGP3. They excluded rare
292 variants (minor allele frequency < 5%) to reduce the influence of deleterious variants on *DoS*.

293

294 Statistical Analysis

295 For each of four evolutionary genetic properties of interest—r_{mf}, $\tilde{\pi}_{rN}$, Tajima's *D*, and *DoS*—we
296 used linear models to examine whether among-gene variation in the metric was predicted by four
297 gene expression characteristics: (i) SDIU status; (ii) SBGE measured as $\log_2$FC; (iii) sex-
298 averaged expression and (iv) tissue specificity. Because the effect of SBGE could be similar for
299 both male and female bias, we included a quadratic term for SBGE. We also included
300 interactions terms between sex-averaged expression and both the linear and quadratic SBGE
301 terms. Genes with extreme sex-bias ($|\log_2 FC| > 5$) were excluded from these analyses because
302 visual inspection revealed that these genes had distinctly different values for several of the
303 evolutionary genetic properties of interest (see Results). For the results presented in the main
304 text, SDIU status was coded as a numerical value: 2 (strong evidence of SDIU: $p_{adj} < 0.01$), 1

305 (modest evidence: $0.2 < p_{adj} \leq 0.01$), or 0 (poor evidence: $p_{adj} \geq 0.2$). Model fits are better when
306 SDIU status is coded as three rather than two values but very similar results are obtained if SDIU
307 status is coded as "significant" ($p_{adj} < 0.05$) or "non-significant" ($p_{adj} \geq 0.05$); see Supplementary
308 Material.

309 All models included an indicator variable of whether the gene was autosomal or X-
310 linked. In addition, the models for each of the three population genomic metrics—$\tilde{\pi}_{rN}$, Tajima's
311 *D*, and *DoS*—included two other terms: log(recombination rate) and log(transcript length).
312 Recombination is expected to affect population genetic patterns by mediating the effects of
313 linked selection. Transcript length is also expected to affect linked selection as a given site in a
314 long gene is expected to have more closely linked targets of selection than a site in a short gene.
315 Recombination values came from Fraïsse et al. (2019), who obtained recombination measures
316 from Chan et al. (2012).

317 The statistical support for model terms was assessed in two different ways. We obtained
318 permutation-based *p*-values using the *lmp* function from the *lmperm* package (Wheeler and
319 Torchiano, 2016). We used perm = "Prob", Ca = 0.001, maxIter = 10^6 and seq = FALSE for
320 unique rather than sequential sum of squares. Second, we used the *Boot* function from the *boot*
321 package (Canty and Ripley 2020) on each linear model, followed by *confint* to obtain 95%
322 bootstrap confidence intervals of model terms. We specified method = "case" to sample over
323 cases, which is considered a conservative approach. In almost all cases, the two methods gave
324 consistent results, i.e., when terms were significant based on permutation *p*-values < 0.05, the
325 bootstrap confidence intervals for the estimate did not overlap zero.

326

327 **Results and Discussion**

328 Prevalence of Sexually Dimorphic Exon Usage

329 We find evidence of extensive sexual dimorphism in isoform usage (SDIU or 'isoform
330 dimorphism') among multi-exon genes expressed in both head and body tissue of adult
331 *Drosophila melanogaster*. Table 1 summarizes the results of our differential exon usage analysis.
332 Of multi-exon genes tested, 5375 and 790 genes expressed in adult body and head tissue exhibit
333 statistically significant SDIU respectively which accounts for 57.5% and 10.2% of all genes

334 tested in bodies and heads respectively. Considering the union of genes expressed in either
335 tissue, we detected SDIU in at least one of the tissues for 48% of genes. These data accord well
336 with several previous studies that have found evidence of widespread SDIU both in this species
337 (e.g., McIntyre et al. 2006; Telonis-Scott et al. 2009; Chang et al. 2011) as well as other species
338 of *Drosophila* (Gibilisco et al. 2016). McIntyre et al. (2006) reported that 68% of all multi-
339 transcript genes expressed in whole body tissue exhibited significant sex-bias in the expression
340 of one or more alternative isoforms in a microarray study using *D. melanogaster*. Similarly, a
341 microarray study in this species by Telonis-Scott et al. (2009) found that ~58% of multi-exon
342 genes tested in whole body tissue exhibited SDIU.

343

344 **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 tested)
Head	8879	7772	790 (10.2%)
Body	11221	9346	5375 (57.5%)
[1]Head or Body	11769	9788	5386 (47.8%)

345 [1]These data refer to the union of genes expressed, tested and exhibiting SDIU found in either
346 head, body or both.

347 Heads and bodies differ substantially in the frequency of SDIU. Although the majority of
348 genes exhibiting SDIU in head tissue also exhibited SDIU in the body, there are many genes
349 exhibiting SDIU in body tissue where we did not detect dimorphism in head tissue. 7330 genes
350 were tested in both tissues. 4187 are SDIU in the body but only 615 (16%) of these are also
351 SDIU in the head. Of the 7330, a total of 779 genes are SDIU in the head and 87% of these are
352 SDIU in the body. These results comport well with previously published data. For example,
353 Telonis-Scott et al. (2009) reported reduced levels of SDIU in genes when expressed in head
354 tissue compared to carcass (i.e., head- and gonad-less bodies) and gonadal tissue.

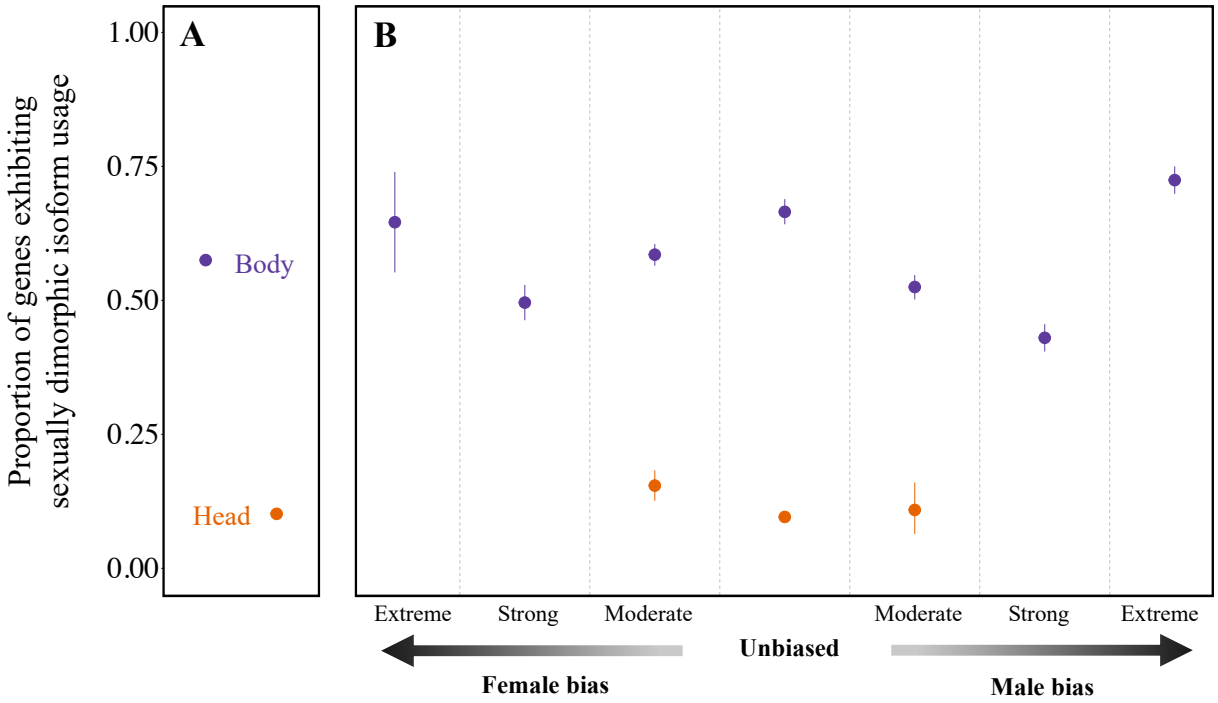
355

356

357 Coincidence of two forms of sexually dimorphism in expression

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

365



366

367 **Figure 1**. Proportion of genes exhibiting sexual dimorphic isoform usage (SDIU) (**A**) among all
368 genes tested; and (**B**) stratified with respect to sex-biased gene expression (SBGE). Points in
369 mauve and orange represent reflect characterizations based on expression in body and head
370 samples, respectively. Only three levels of SBGE are shown with respect to head because very
371 few genes fall into the other levels. Error bars represent bootstrap 95% confidence intervals. In
372 some cases, error bars are too small to be visible.

373

374 SBGE is widespread both in heads and bodies, though there is much greater variation
375 among genes in SBGE in body tissue relative to head tissue (e.g., Innocenti and Morrow 2010;

376 Baker et al. 2011; Dutoit et al. 2018). To visualize the relationship between SDIU and SBGE, we
377 divided genes into bins representing different levels of sex-bias using defined ranges of $\log_2$FC
378 in gene expression (see Methods). Within each bin, we calculated the proportion of genes
379 exhibiting SDIU. With respect to expression in the body, SDIU is more common amongst
380 unbiased genes than sex-biased genes, except for those with extreme sex-bias (Figure 1B).
381 These fly body results are in line with Rogers et al. (2020) who reported non-significant trends
382 towards a negative relationship between SDIU and SBGE in gonadal tissue of three bird species.

383 The reduction in isoform dimorphism at highly sex-biased genes expressed in the body
384 could be because SBGE and SDIU provide alternative routes to the resolution of sexual conflict
385 and thus the evolution of one reduces the selective pressure for the evolution of the other. On the
386 other hand, the pattern goes in the opposite direction with respect to expression in the head;
387 SDIU is less common among unbiased genes than sex-biased genes (at least those that are
388 female-biased). One hypothesis for this latter pattern is that the resolution of intra-locus conflict
389 may often require both forms of expression dimorphism whereas those genes experiencing
390 sexually concordant selection will evolve neither, creating a positive association between SDIU
391 and SBGE.

392 An alternative, more mundane, explanation is that the positive association of SBGE and
393 SDIU in heads exists because of how we quantify gene/isoform expression (i.e., counting reads
394 mapping to genes); we may be biased towards finding a positive association between SBGE and
395 SIDU because we are observing the same phenomenon in different ways. For example, if both
396 males and females express a single isoform of a gene at the same level but females also express
397 an additional isoform that is not expressed by males, we would detect both SBGE and SDIU at
398 this gene. However, this methodological bias must not be very strong as there are many unbiased
399 genes that exhibit SDIU (670 in head and 1007 in body). Moreover, the association between
400 SBGE and SDIU is negative in the body.

401 It is worth noting that average gene length varies across different levels of SBGE (Fig.
402 S2). SDIU genes are, on average, substantially longer than non-SDIU genes with respect to total
403 exon length (Fig. S2). To some extent this association is expected to be at least partially due the
404 greater power to detect SDIU in longer genes because there will be more reads for a given level
405 of expression of a long gene than a short one. However, the association of SDIU and SBGE does

not appear to be an artifact driven by gene length. The proportion of SDIU genes among levels of SBGE varies in a manner that is not consistent with variation in average gene length among levels of SBGE (compare Fig. 1 and Fig. S2). Moreover, the patterns in the proportion of SDIU genes among levels of SBGE remain similar even when genes are stratified by gene length (Fig. S3). Nonetheless, these associations of gene length with both SDIU and SBGE are noteworthy. Gene length is expected to affect the amount of linked selection and thereby influence population genetic properties (Larracuente et al. 2008; Fraïsse et al. 2019) that we examine later.

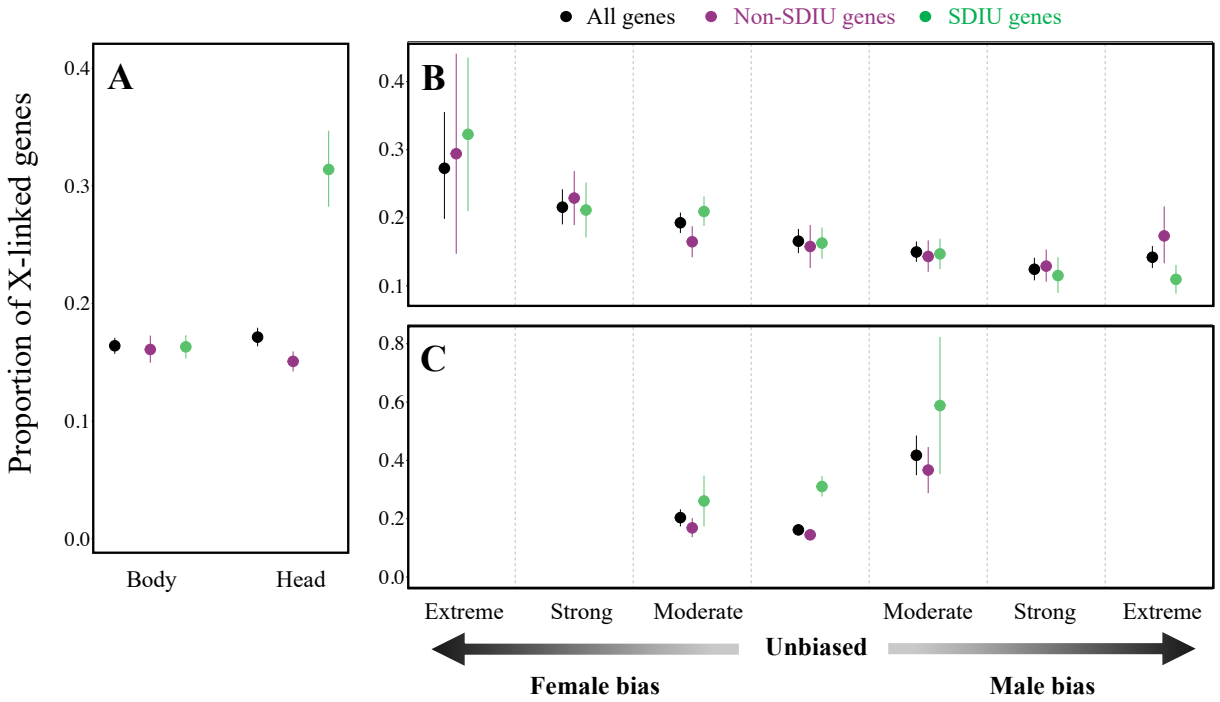


Figure 2. Proportion of X-linked genes with respect to SDIU status. (**A**) Results for body and head shown irrespective of SBGE. Results shown stratified by SBGE for (**B**) body and (**C**) head. Points in purple and green represent non-SDIU and SDIU genes, respectively; points in black are all genes irrespective of SDIU status (including genes that were not tested for SDIU). Error bars are bootstrap 95% confidence intervals. In some cases, error bars are too small to be visible.

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

425 intralocus conflict or contributing to sexual dimorphism (Rice 1987; Frank and Patten 2020).
426 This has motivated interest in examining the location of genes exhibiting expression
427 dimorphism. In the body data, there is no evidence of an enrichment of genes exhibiting SDIU
428 on the X chromosome (Figure 2). In contrast, SBGE is strongly associated with the X
429 chromosome, with female-biased genes being significantly overrepresented on the X
430 chromosome relative to unbiased genes whereas male biased genes are significantly
431 underrepresented on the X chromosome (Figure 2). This observation of a "demasculinization" of
432 the X chromosome has been well documented in previous studies of SBGE in *D. melanogaster*
433 as well as several other species (Parisi et al. 2003; Ranz et al. 2003; Khil et al. 2004; Reinke et
434 al. 2004; Sturgill et al. 2007; Meisel et al. 2012; Albritton et al. 2014).

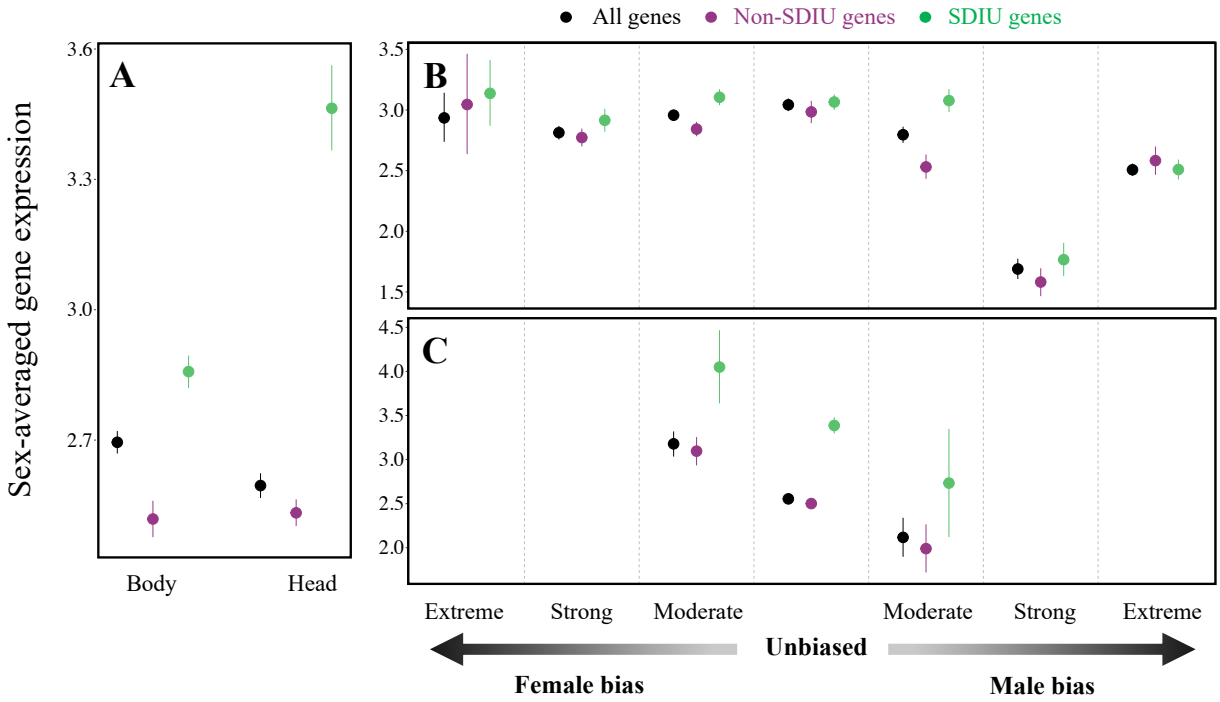
435 These patterns are quite different for the head data. In contrast to the body data, there are
436 a significantly higher proportion of X-linked genes exhibiting SDIU in the head relative to non-
437 SDIU genes (Figure 2). With respect to SBGE, both male- and female-biased genes are
438 overrepresented on the X relative to unbiased genes, with the strongest overrepresentation among
439 male-biased genes (Figure 2; see Meisel et al. (2012)). The over-representation of SDIU genes
440 on the X chromosome occurs primarily among unbiased genes rather than sex-biased genes,
441 indicating that this pattern is not driven by a positive association between SDIU and sex-biased
442 expression.

443

444 <u>Association of SDIU and SBGE with sex-averaged expression</u>

445 An important aspect of expression that we have not yet discussed is the overall level of
446 expression, measured here as the average RPKM across males and females. Both SDIU and
447 SBGE are associated with the sex-averaged expression level (Fig. 3). Expression levels are
448 higher for genes with versus without SDIU. This may simply reflect a statistical artifact as there
449 is greater power to detect SDIU in genes that have higher expression. Regardless of the
450 underlying reason, this association will be an important consideration when we later consider
451 evolutionary characteristics of genes with SDIU. In bodies, sex-averaged expression is greater
452 for unbiased than sex-biased genes, particularly the more strongly male-biased genes. In heads,
453 female-biased genes have greater average expression than unbiased genes, which in turn have
454 greater expression than male-biased genes.

455



456

457 **Figure 3.** Mean sex-averaged expression with respect to SDIU status. (**A**) Results for body and
458 head shown irrespective of SBGE. Results shown stratified by SBGE for (**B**) body and (**C**) head.
459 Points in purple and green represent non-SDIU and SDIU genes, respectively; points in black are
460 all genes irrespective of SDIU status (including genes that were not tested for SDIU). Error bars
461 are bootstrap 95% confidence intervals. In some cases, error bars are too small to be visible.

462

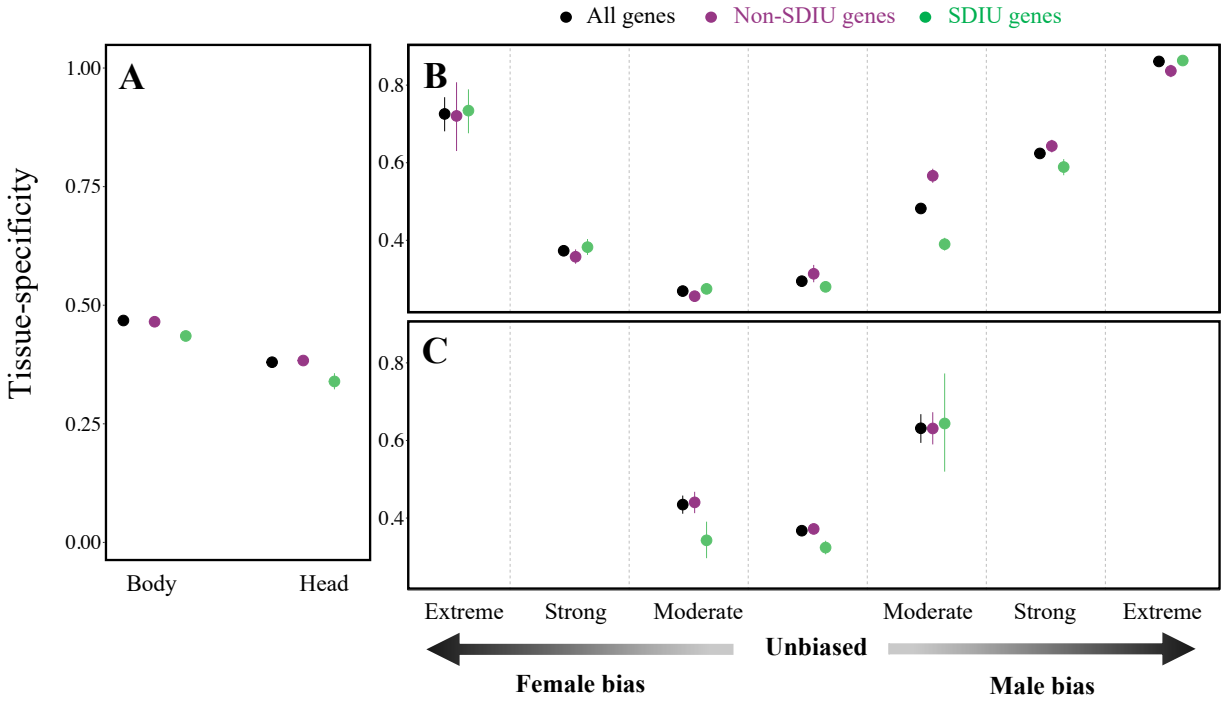
463 <u>Association of SDIU and SBGE with tissue specificity</u>

464 The final aspect of expression we consider is tissue specificity. We use the measure of tissue
465 specificity calculated by Fraïsse et al. (2019) based an independent dataset with expression
466 information from multiple tissues from several developmental stages (modENCODE Consortium
467 2010). Tissue specificity is higher for male-biased than female-biased or unbiased genes (Fig. 4).
468 Tissue specificity is lower for SDIU than non-SDIU genes. In bodies, this effect is driven largely
469 by the difference between SDIU and non-SDIU among moderately and strongly male-biased
470 genes. In heads, this effect comes largely from female-biased and unbiased genes.

471 Previous studies in *Drosophila* (Assis et al. 2012; Fraïsse et al. 2019) and other animals
472 (Mank et al. 2008; Dean and Mank 2016) have also found that sex-biased genes have higher

473 tissue specificity than unbiased genes; this has been interpreted as sexual dimorphism evolving
474 more readily when there is less pleiotropic constraint on expression. Under this premise, one
475 might expect the same for SDIU. However, in the body data, SBGE is associated with greater
476 tissue specificity (often interpreted as reduced pleiotropic constraint) whereas SDIU is associated
477 with lower tissue specificity. Similarly, Rogers et al. (2020) found that genes exhibiting SDIU
478 exhibited lower tissue specificity in gene expression than non-SDIU genes in birds.

479



480

481 **Figure 4.** Mean tissue-specificity with respect to SDIU status. (**A**) Results for body and head
482 shown irrespective of SBGE. Results shown stratified by SBGE for (**B**) body and (**C**) head.
483 Points in purple and green represent non-SDIU and SDIU genes, respectively; points in black are
484 all genes irrespective of SDIU status (including genes that were not tested for SDIU). Error bars
485 are bootstrap 95% confidence intervals. In some cases, error bars are too small to be visible.

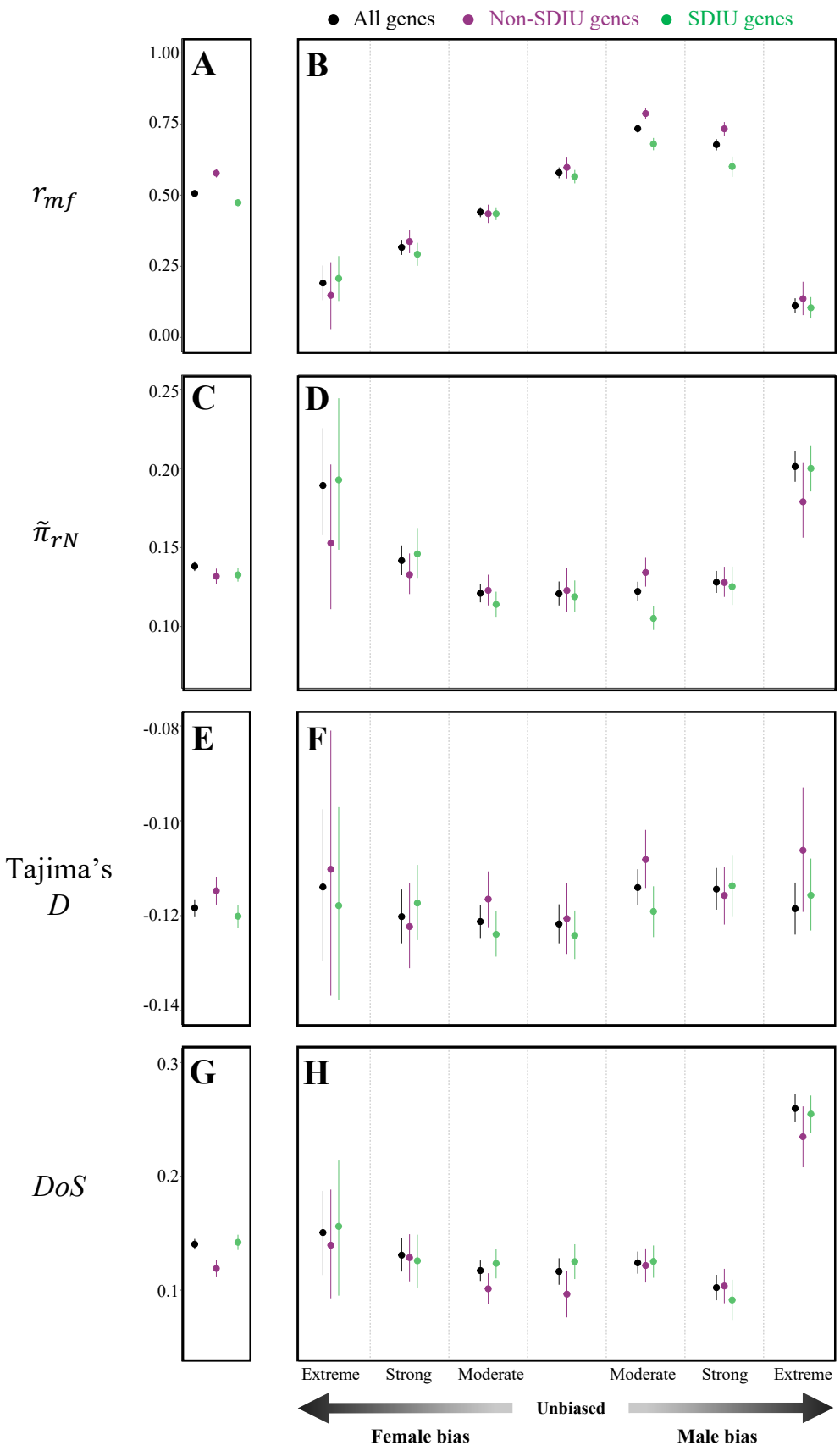
486

487 <u>Evolutionary Properties associated with SDIU and SBGE</u>

488 We next examine how each of four evolutionary properties—r_{mf}, $\tilde{\pi}_{rN}$, Tajima's *D*, and *DoS*—
489 varies across genes in relation to both SDIU and SBGE. We do this in two ways. First, we
490 examine simple associations of each evolutionary property with SDIU and SBGE, which are

491 straightforward to represent graphically. This approach ignores other factors—including sex-
492 averaged expression and tissue specificity—that could be important sources of variation in the
493 focal evolutionary property. Such factors could obscure the relationship of the focal evolutionary
494 property with SDIU or SBGE. Consequently, we also use linear models in which we examine
495 variation in an evolutionary property as a function of SDIU and SBGE as well as other relevant
496 covariates. These other covariates include sex-averaged expression and tissue specificity as well
497 as genomic location (X or autosome). For the population genetic properties—$\tilde{\pi}_{rN}$, Tajima's *D*,
498 and *DoS*—we also included two other covariates that could affect the strength of linked
499 selection: recombination rate and gene length. For simplicity, we only perform these analyses
500 with respect to expression characteristics in body samples as this is where sexual dimorphism is
501 greatest. As we shown graphically, genes with extreme sex bias (particularly extreme male-bias)
502 often differ dramatically from other genes. We exclude these genes from the linear models.

503


All genes
Non-SDIU genes
SDIU genes
A
B
C
D
E
F
G
H
r_{mf}
$\tilde{\pi}_{rN}$
Tajima's D
DoS
1.00
0.75
0.50
0.25
0.00
0.25
0.20
0.15
0.10
-0.08
-0.10
-0.12
-0.14
0.3
0.2
0.1
Extreme
Strong
Moderate
Moderate
Strong
Extreme
Unbiased
Female bias
Male bias

506

507 **Figure 5**. Four evolutionary genetic properties in relation to expression properties in the body.
508 (**A, C, E, G**) Results shown irrespective of SBGE. (**B, D, F, H**) Results shown stratified by
509 SBGE. Points in purple and green represent non-SDIU and SDIU genes, respectively; points in
510 black are all genes irrespective of SDIU status (including genes that were not tested for SDIU).
511 Error bars are bootstrap 95% confidence intervals. In some cases, error bars are too small to be
512 visible.

513

514

515

516 *Intersexual genetic correlation*, r_{mf}

517 The intersexual genetic correlation, r_{mf}, plays an important role in determining the extent to
518 which a trait can evolve independently in one sex from the other. We examine variation in the
519 intersexual genetic correlation in gene expression from the quantitative genetic study of DGRP
520 lines by Huang et al. (2015). Genes with SDIU have significantly lower r_{mf} values, on average,
521 than genes without SDIU (Fig. 5A; 95% CIs do not overlap). There is a striking relationship
522 between r_{mf} and SBGE (Fig. 5B), with female-biased genes having lower r_{mf} than unbiased
523 genes. Moderately and strongly male-biased genes have the highest average r_{mf} values. In
524 contrast, genes with extreme male bias have very low r_{mf} values on average. When we analyze
525 variation in r_{mf} using a linear model to help control for correlated variables, the patterns
526 described above are recovered (Table 2): r_{mf} is reduced for genes with SDIU; r_{mf} increases going
527 from female-biased to unbiased to male-biased genes. The model also indicates that r_{mf} is higher
528 for genes with higher tissue specificity.

529

530

531 **Table 2. Linear model results for the intersexual genetic correlation in fitness, r_{mf}**

Term	Estimate [95% bootstrap CI]	*p*-value
SDIU	-0.034 [-0.047, -0.022]	$< 10^{-6}$
SBGE	0.055 [0.037, 0.072]	$< 10^{-6}$
$SBGE^2$	-0.016 [-0.024, -0.009]	0.0005
Avg Expression	0.069 [0.057, 0.082]	$< 10^{-6}$
SBGE × Avg Expression	0.002 [-0.004, 0.007]	0.53
$SBGE^2$ × Avg Expression	0.0005 [-0.0018, 0.0028]	1.00
Tissue specificity	0.349 [0.289, 0.406]	$< 10^{-6}$
X-linkage	-0.013 [-0.027, 0.001]	0.069

532 Analysis is based on n = 3383 genes with complete information for all model terms. Genes with
533 extreme sex-bias ($\log_2$FC > |5|) are excluded from analysis. *p*-values are permutation based.

534

535

536 The relationship between r_{mf} and SBGE was first investigated by Griffin et al. (2013)
537 using microarray expression data from a smaller set of genotypes (40 vs 185 here) also from the
538 DGRP. They report a negative relationship between r_{mf} and sex-bias but they did not distinguish
539 between male- and female-biased genes. Similarly, Fraïsse et al. (2019) calculated r_{mf} using the
540 same RNAseq data set we used (Huang et al. 2015). They also noted a negative relationship
541 between r_{mf} and sex-bias but did not distinguish between male- and female-bias. In their study of
542 *D. serrata*, Allen et al. (2018) noted that r_{mf} was on average lower for female- than male-biased
543 genes, consistent with what we observe. Allen et al. (2018) also noted that the correlation
544 between r_{mf} and the magnitude of sex-bias was weaker for female- than male-biased genes; we
545 find the opposite (females: ρ = -0.20 [-0.24, -0.16]; males: ρ = -0.13 [-0.17, -0.09]; calculated
546 excluding genes in the unbiased and extreme sex-biased categories).

547

548 *Relative level of nonsynonymous diversity*, $\tilde{\pi}_{rN}$

549 A reduction in nucleotide diversity for nonsynonymous relative to synonymous variants is
550 typically interpreted as indicative of purifying selection on amino acid sequence variation, with
551 lower nonsynonymous diversity levels indicating stronger selection. A simple inspection of
552 relative levels of nonsynonymous diversity $\tilde{\pi}_{rN}$ reveals no clear difference between genes with
553 versus without SDIU (Fig. 5C, D). Genes with extreme sex-bias have elevated levels of $\tilde{\pi}_{rN}$,
554 consistent with the idea that such genes experience weaker net purifying selection because they
555 are primarily selected in only one sex (Pröschel et al. 2006; Dapper and Wade 2016, 2020).

556 Excluding the extreme sex-bias genes, we applied a linear model to variation in $\tilde{\pi}_{rN}$
557 (Table 3). This model indicated an interaction of SBGE with sex-averaged expression level. On
558 average, $\tilde{\pi}_{rN}$ is reduced in genes with high versus low average expression. This is consistent with
559 results from a variety of taxa, which is usually interpreted as indicating stronger purifying
560 selection on highly expressed genes (e.g., Carneiro et al. 2012; Paape et al. 2012; Williamson et
561 al. 2014; Ho et al. 2019). Further, our model indicates that $\tilde{\pi}_{rN}$ declines from female-biased to
562 unbiased to male-biased genes for highly and moderately expressed genes but does the opposite
563 for female-biased genes. (The interaction term indicates that $\tilde{\pi}_{rN}$ varies more with respect to sex-
564 averaged expression for female-biased genes than male-biased genes.)

565 The model also indicates that $\tilde{\pi}_{rN}$ increases with tissue specificity, consistent with the
566 idea of weaker purifying selection on genes with less pleiotropy (e.g., Osada 2007; Paape et al.
567 2013). $\tilde{\pi}_{rN}$ declines with recombination, as expected from recombination reducing selective
568 interference. However, $\tilde{\pi}_{rN}$ is elevated for X-linked genes and shorter genes. Neither of these
569 results match expectation as selection is thought to be more effective on the X than autosomes in
570 flies (Campos et al. 2018) and longer genes are expected to experience more selective
571 interference.

572

573

574 **Table 3. Linear model results for the relative level of nonsynonymous diversity, $\tilde{\pi}_{rN}$.**

Term	Estimate [95% bootstrap CI]	*p*-value
SDIU	0.0021 [0.0020, 0.0061]	1.00
SBGE	-0.011 [-0.0179, 0.0047]	$< 10^{-6}$
$SBGE^2$	0.0015 [-0.0011, 0.0039]	1.00
Avg Expression	-0.0013 [-0.0173, 0.0089]	$< 10^{-6}$
SBGE × Avg Expression	0.0031 [0.0007, 0.0054]	0.00029
$SBGE^2$ × Avg Expression	-0.000043 [-0.000935, 0.000962]	0.42
Tissue specificity	0.045 [0.024, 0.067]	$< 10^{-6}$
X-linkage	0.0069 [0.0021, 0.0119]	0.011
log(recombination)	-0.021 [-0.023, -0.018]	$< 10^{-6}$
log(gene length)	-0.025 [-0.032, -0.019]	$< 10^{-6}$

575 Analysis is based on $n = 4629$ genes with complete information for all model terms. Genes with
576 extreme sex-bias ($\log_2 FC > |5|$) are excluded from analysis. *p*-values are permutation based.

577

578 *Tajima's* D

579 Tajima's *D* is a summary statistic that reflects information about the shape of the underlying
580 genealogy, which can be affected by the strength and form of selection at linked sites. As such,
581 variation in Tajima's *D* has been used to look for indirect evidence that genes with different
582 expression properties are selected differently (Sayadi et al. 2019; Wright et al. 2019). As shown
583 in Fig. 5E, genes with SDIU have lower values of Tajima's *D* (measured using 4-fold degenerate
584 sites). Though there is no visually obvious pattern with respect to SBGE (Fig. 5F), the linear
585 model indicates strongly significant effects of SBGE involving interactions with sex-averaged
586 expression (Table 4). Tajima's *D* is reduced in genes with high sex-averaged expression.

587 Roughly speaking, the model indicates that Tajima's *D* is reduced for sex-biased genes relative
588 to unbiased biased genes, at least for genes with intermediate levels of sex-averaged expression.
589 For genes with low sex-averaged expression, the highest Tajima's *D* values are inferred to occur
590 for female-biased genes; for genes with high sex-averaged expression, the highest Tajima's *D*
591 values occur for slightly male-biased genes.

592 To the extent variation in Tajima's *D* is due to variation in selection, it could simply
593 reflect variation in the strength of purifying selection. We fit another version of the model
594 including $\tilde{\pi}_{rN}$ as a covariate in a crude attempt to control for the strength of purifying selection.
595 There was a strongly significant negative effect of $\tilde{\pi}_{rN}$, which could be interpreted as weaker
596 purifying selection causing more negative values of Tajima's *D*; a pattern expected by theory
597 over some range of selection strengths (Charlesworth et al. 1995; Cvijović et al. 2018). However,
598 the patterns of Tajima's *D* with respect to SDIU and SBGE remained qualitatively unchanged,
599 e.g., Tajima's *D* is lower for genes with SDIU. This leaves open the possibility that other
600 differences in selection contribute to these patterns. For example, genes with SDIU could be less
601 likely to experience balancing selection or more likely to experience selective sweeps than genes
602 without SDIU.

603

604

605

606 **Table 4. Linear model results for Tajima's *D*.**

Term	Estimate [95% bootstrap CI]	*p*-value
SDIU	-0.0037 [-0.0065, -0.0009]	0.0075
SBGE	-0.0066 [-0.0097, -0.0035]	$< 10^{-6}$
$SBGE^2$	-0.0018 [-0.0031, -0.0006]	$< 10^{-6}$
Avg Expression	-0.0057 [-0.0087, -0.0028]	$< 10^{-6}$
SBGE × Avg Expression	0.0022 [0.0020, 0.0033]	0.00022
$SBGE^2$ × Avg Expression	0.00067 [0.00024, 0.00110]	$< 10^{-6}$
Tissue specificity	0.037 [0.023, 0.052]	$< 10^{-6}$
X-linkage	-0.021 [-0.024, -0.017]	$< 10^{-6}$
log(recombination)	-0.0023 [-0.0036, -0.0009]	0.00031
log(gene length)	0.0067 [0.0025, 0.0110]	0.00060

607 Analysis is based on $n = 4754$ genes with complete information for all model terms. Genes with
608 extreme sex-bias ($\log_2 FC > |5|$) are excluded from analysis. *p*-values are permutation based.

609

610

611 <u>*Direction of Selection* DoS</u>

612 An excess of divergence to polymorphism for nonsynonymous relative to synonymous variants
613 can be indicative of adaptive evolution (McDonald and Kreitman 1991). Based on this idea,
614 Stoletzki and Eyre-Walker (2011) devised the *DoS* metric for which positive values are
615 indicative of adaptive evolution, with more strongly positive values reflecting more frequent
616 adaptive evolution. *DoS* is higher for genes with SDIU than those without (Fig. 5G; Table 5).
617 *DoS* is substantially elevated for genes with extreme male-bias (Fig. 5H). Excluding the genes

618 with extreme sex-bias, the linear model detects no significant effect of SBGE on *DoS*. There is
619 also a significant positive effect of sex-averaged expression, qualitatively consistent with the
620 findings of Huang (2022; but see Fraïsse et al. 2019). Consistent with other studies, the model
621 indicates more adaptive evolution for X-linked genes, genes with higher recombination rates,
622 and shorter genes (e.g., Campos et al. 2018, Fraïsse et al. 2019).

623

624

625

626 **Table 5. Linear model results for *DoS*.**

Term	Estimate [95% bootstrap CI]	*P*-value
SDIU	0.012 [0.006, 0.018]	0.00050
SBGE	-0.0058 [-0.0135, 0.0020]	0.13
$SBGE^2$	0.0012 [-0.0019, 0.0042]	1.00
Avg Expression	0.013 [0.006, 0.019]	$< 10^{-6}$
SBGE × Avg Expression	0.0013 [-0.0014, 0.0039]	0.30
$SBGE^2$ × Avg Expression	-0.00020 [-0.00123, 0.00088]	1.00
Tissue specificity	0.0076 [-0.0222, 0.0373]	1.00
X-linkage	0.027 [0.020, 0.035]	$< 10^{-6}$
log(recombination)	0.022 [0.019, .025]	$< 10^{-6}$
log(gene length)	-0.026 [-0.035, -0.017]	$< 10^{-6}$

627 Analysis is based on n = 4765 genes with complete information for all model terms. Genes with
628 extreme sex-bias ($\log_2$FC > |5|) are excluded from analysis. p-values are permutation based.

629

630 A potential concern in interpretating *DoS* is that large positive values could be primarily
631 due to low levels of nonsynonymous polymorphism, with divergence playing little role.
632 However, divergence and polymorphism components each contribute substantially to variation in
633 *DoS* in this dataset. Moreover, if we apply a linear model to just the divergence component of
634 *DoS* (i.e, $D_n/(D_n + D_s)$), we again detect a positive effect of SDIU (Table S1). In addition, the
635 divergence component of *DoS* declines from female-biased to unbiased to male-biased genes,
636 except for those genes with high sex-averaged expression (i.e., a significant negative SBGE

637 effect in combination with a significant positive interaction between SBGE and sex-averaged
638 expression).

639

640 **Conclusions**

641 SDIU and SBGE are two different ways to differentially regulate genes across the sexes and thus
642 could be mechanisms by which sexual conflict is resolved. Both SBGE and SDIU are
643 widespread in *Drosophila melanogaster*, particularly among genes expressed in body tissue.
644 Genes with SDIU have lower values of the intersexual genetic correlation in expression r_{mf}. This
645 indicates that patterns of standing genetic variation are more conducive to evolving greater sex
646 differences in a gene's total expression (i.e., stronger SBGE) for genes with SDIU versus
647 without, holding the amount of genetic variation constant. Thus, one might predict genes with
648 SDIU to have higher levels of SBGE, which stands at odds with the observed negative
649 association between these two forms of expression dimorphism in the body (Fig. 1). This could
650 be because when one of these mechanisms evolves, it may negate the selective pressure to
651 evolve the other (i.e., SDIU genes are less likely to be under antagonistic selection for total
652 expression than non-SDIU genes). A second possibility is that the genetic variants that allow for
653 a greater degree of independence in total expression—those that lower r_{mf}—have other
654 constraining pleiotropic effects (e.g., perhaps such variants are more likely to be due *trans*-
655 regulatory factors that affect many other genes) or such variants only affect the expression of
656 some isoforms and those are not the targets of antagonistic selection for expression level.

657 SDIU and SBGE are differently associated with the population genetic metrics we
658 examined, suggesting that selection acts differently on these two dimensions of expression
659 dimorphism. For example, one interpretation of the $\tilde{\pi}_{rN}$ analysis is that purifying selection tends
660 to get stronger going from female-biased to unbiased to male-biased genes (excluding genes with
661 extreme sex-bias). In contrast, purifying selection is similar on genes with versus without SDIU.
662 An interpretation of the *DoS* analysis is that SDIU genes experience more adaptive evolution
663 than genes without SDIU. Perhaps when sexes use different isoforms, it is easier for adaptation
664 to occur in each sex because there is less pleiotropic constraint imposed by the other sex. In
665 contrast, there is no clear relationship between adaptive evolution and SBGE (when genes with
666 extreme sex-bias are excluded).

667 Such interpretations should be regarded with caution for several reasons. First, population
668 genetic metrics can be influenced by a multitude of factors and the interpretations above are not
669 necessarily correct. Second, an unmeasured correlated variable could be responsible for the
670 reported associations, though we incorporated several plausibly important variables into our
671 analyses to reduce this possibility. Third, the comparison of SDIU and SBGE is complicated by
672 the nature of how they are defined and measured here. SBGE has both a direction (i.e., negative
673 to positive representing female- to male-biased) and a magnitude. SDIU, as measured here,
674 simply indicates whether the sexes differ in their distribution of isoform usage; there is no
675 direction. Magnitude only affects SDIU status via the statistical power to detect the difference,
676 which also depends on other factors (e.g., expression level, sequencing depth). (Quantifying a
677 "magnitude" for SDIU is complicated by the multitude of ways that isoform distributions can
678 differ; but see Rogers et al. 2020.)

679 Much of our analysis has focused on expression properties from whole body samples
680 because our main interest here is in dimorphism and whole bodies are much more dimorphic
681 than heads. Many related studies have also focussed on expression from whole body data (e.g.,
682 Innocenti & Morrow 2010, Griffiths et al. 2013, Cheng & Kirkpatrick 2016, Fraïsse et al. 2019).
683 On a practical level, only a subset of genes are expressed at a sufficiently high level to warrant
684 characterization of SBGE and SDIU in multiple tissue types. Nonetheless, expression properties
685 of a gene can vary across tissues, life stages, and environmental conditions. Within the first
686 section of our results, we highlighted differences between head and body including the
687 frequencies of SDIU and SBGE as well as their co-occurrence (Table 1, Fig. 1) and over/under-
688 representation on X (Fig. 2). Thus, using only one type of sample (e.g., whole body) is, at best, a
689 partial representation of a gene's expression characteristics.

690 Furthermore, whole body samples represent a collection of tissue types and expression
691 profiles differ across tissues. Thus, expression differences between sexes can be due to either
692 expression changes within tissues between sexes or the relative contribution of each tissue within
693 each sex (Montgomery & Mank 2016). We take the coarser perspective that expression in whole
694 body samples represents a set of phenotypes in which we can examine dimorphism, while
695 acknowledging the limitations on interpreting the biological source of dimorphism. Despite the
696 caveats of using whole body expression, these measures of SBGE and SDIU are associated with

several key evolutionary properties. These patterns are intriguing though a bigger challenge will be to gain a clearer understanding of why they occur.

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