Sex-biased gene expression under sexually antagonistic and sex-limited selection

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Abstract

Sex differences in gene expression are ubiquitous, evolve quickly, and are expected to underlie phenotypic sexual dimorphism. Despite long-standing interest, the impact of sex-specific selection on the transcriptome remains poorly understood. Here, we test fundamental questions on the role of constraints on gene expression evolution arising from the mode of selection and genetic architecture. We also test the relationship between sex-specific selection, sex-biased expression and sexual dimorphism (SD). We assess these using body size selection lines in the seed beetle, *Callosobruchus maculatus,* that have evolved variation in SD in response to sex-specific selection; either sex-limited (SL) or sexually antagonistic (SA). We find that the evolution of male-optimal smaller size is associated with an excess of expression changes of transcripts with male-biased expression, and female-optimal larger size with a deficit of changes at transcripts with female-biased expression. Expression changes are more constrained under SA selection, and especially in females, reflecting phenotypic evolution in these lines. Although SD in size has changed most under SA selection, we show that similar SL selection on males, but not females, has resulted in more sex-biased transcripts. Consequently, we find no clear transcriptome-wide association between sex-bias and SD. Overall, sex-biased transcripts show lower cross-sex correlations in expression changes than unbiased transcripts. In the light of these unique experimental insights into how sex-specific selection on size changes adult transcription, our findings have strong implications for inferring selection history and mode from patterns of sex-biased gene expression in natural populations.

Introduction

In most species with separate sexes, female and male fitness is optimized through different strategies, causing selection for phenotypic sex differences. The resulting sexually dimorphic phenotypes are some of the most extraordinary in nature (Fairbairn 2007), including sexual display traits and weaponry (Emlen 2008), but also many physiological and life history traits expressed by both sexes, such as body size (Wedell et al. 2006; Austad & Fischer 2016; Hämäläinen et al. 2018). Evolution of sexual dimorphism (SD) depends on the interplay between selection favouring sex differences and the constraint imposed by the shared genetic architecture of the trait (Lande 1980; Fairbairn 2007; Pennell & Morrow 2013). An important mechanism that should facilitate the evolution of SD is sex-biased or sex-limited expression of the underlying genes (Ellegren & Parsch 2007; Parsch & Ellegren 2013; Grath & Parsch 2016). Sex-limited (SL, i.e. selection only on one sex, with neutral evolution of the other) selection is expected to limit sexual dimorphism at both phenotypic and molecular levels due to correlated evolutionary response of the opposite sex. In contrast, sexually antagonistic (SA; i.e. opposing directions of selection in the two sexes) selection is expected to be a chief selective force behind sex-biased gene expression because it generates a genomic conflict between the sexes over shared genes that differential gene regulation can resolve (Ellegren & Parsch 2007; Parsch & Ellegren 2013; Grath & Parsch 2016). Many studies have therefore sought a link between overall levels of sexual dimorphism, sex-biased gene expression and either ongoing or historical SA selection. However, there have been few experimental tests of these commonly held expectations, and interpreting process from patterns observed in nature is far from trivial. Moreover, the genetic architecture of gene regulation itself can constrain the evolution of sex-biased gene expression, but the scope and consequence of such constraints on modulating gene expression evolution are not well understood (Ayroles et al. 2009; Griffin et al. 2013; Cheng & Kirkpatrick 2016; Allen et al. 2018; Houle & Cheng 2021).

While some studies have found a positive association between sexual dimorphism and sex-biased expression (Pointer et al. 2013, Harrison et al. 2015; Toubiana et al. 2021), others have not (Scharmann et al. 2021; Khila et al. 2012). The link between SD and sex-biased expression is thus not straightforward, making it difficult to infer optimal expression levels for either sex (Immonen et al. 2017; Parker at al. 2019; Huylmans et al. 2021). The evidence for a consistent effect of a history of SA selection on sex-biased genes is similarly mixed. Studies of flycatchers (Dutoit et al. 2018) and seed beetles (Sayadi et al. 2019) have found excess of non-synonymous polymorphisms in sex-biased genes. This is in line with a the

maintainenace of genetic variation due to overall balancing selection from opposing pressures on males and females from SA selection, a process expected from theory (Connallon & Clark 2012). Such patterns are consistent with a history of persistent sexual conflict, but also with any other form of antagonistic pleiotropy as well as relaxed selection (Dutoit et al. 2018; Dapper and Wade 2020). Ruzicka et al. (2019) measured SA fitness variation directly, and showed that SA genes in *Drosophila melanogaster* are in fact less often sex-biased in expression than expected. Meanwhile, in experimental evolution settings, sex-biased gene expression changes in response to altered mating systems as predicted. However, results are often contradictory, with monogamy (and thus relaxed sexual conflict) resulting in the "feminisation" of gene expression in some cases (i.e. increased expression of female-biased genes, and reduced expression of male-biased genes; Hollis et al. 2014), or "masculinisation" in others (Immonen et al. 2014, Veltsos et al. 2017).

From theory, it is not immediately clear what predictions should be. It is well established that phenotypic sexual dimorphism can change in response to sex-limited directional selection, or even sexually concordant selection, as long as there are quantitative asymmetries in genetic variation between the sexes available to selection, potentially making SA selection unnecessary (Fisher 1930; Lande 1980; Bonduriansky & Chenoweth 2009; Gosden et al. 2012; Cheng & Houle 2020; Houle & Cheng 2021; Kaufmann et al. 2021). In addition, the variable patterns observed across studies could partly be explained by differences in the evolutionary timescale used to assess the associations between sex-bias, sexual dimorphism and SA selection. If there is a time-lag between the evolution of sexual dimorphism and the resolution of sexual conflict over such dimorphism through sex-biased gene expression, then comparing very recently diverged populations or species may not yield the expected positive relationship. Finally, in comparative studies of sex-bias, it is typically not known what phenotypes selection has acted on, nor the exact form of selection, which are instead inferred or assumed from the data (Ellegren & Parsch, 2007; Grath & Parsch 2016; Mank 2017). It is therefore not clear what kind of signature SA and other forms of selection leave on gene expression, and the link with phenotypic sexual dimorphism remains poorly understood.

Here, we experimentally address how gene expression is altered in each sex in response to different modes of sex-specific selection on body size in the seed beetle *Callosobruchus maculatus*, by combining RNA-seq with replicated artificial selection (Kaufmann et al. 2021). Body size is a key life history trait and commonly sexually dimorphic across taxa (Fairbairn 2007; Wedell et al. 2006; Immonen et al. 2018). In *C.*

maculatus it is differentially associated with fitness in the sexes: fecundity selection favours larger females and sexual selection typically favours smaller and more active males (Arnqvist & Tuda 2010; Berger et al. 2014; 2016). Most of the autosomal additive genetic variance for body size is shared between the sexes both in the ancestral study population of *C. maculatus* and in the selected lines (autosomal $r_{MF} > 0.92$, Kaufmann et al. 2021, Kaufmann et al. 2023a). Sex-specific evolution of body size in this system therefore offers an excellent model to address how gene expression changes in the sexes in association with polygenic adaptation in the presence of this high cross-sex genetic correlation.

Full details of the artificial selection lines are given elsewhere (Kaufmann et al. 2021). Briefly, the modes of selection in our experiment include male-limited (towards smaller males: $SL_m\downarrow$, and larger males: $SL_m\uparrow$), female-limited (towards larger females: $SL_f\uparrow$) and SA selection (in the naturally observed direction of larger females and smaller males). We also include a control line (C) subjected to random selection but with otherwise similar demographic changes. The C line has largely maintained genetic variation for body size through the course of selection (Kaufmann et al. 2023a). A total of 10 generations of selection has produced a gradient of sexual size dimorphism (figure S1). SD changes range from from 50% and 30% increase under SA and $SL_m\downarrow$ selection on smaller males, respectively, through no change under $SL_f\uparrow$ selection (due to a correlated increase in size in both sexes), to some 30% decrease under $SL_m\uparrow$ selection (due to a greater increase in male size compared to female size; Kaufmann et al. 2021; see figure S1). Y-linked variance accounted for changes in SD during the first 2-3 generations (Kaufmann et al. 2021, 2023a). While most efficiently increasing sexual size dimorphism, SA selection maintained significantly more autosomal genetic variance (additive and dominance) compared to male-limited selection for similar male but not female size (Kaufmann et al. 2023a). These findings corroborate the theoretical expectations that SA selection can both target loci with mutually beneficial effects on both sexes (by increasing SD) but also impose strong genomic constraints on shared genetic variation.

Here, we take advantage of this known selection history, controlled demography and a range of phenotypic sexual dimorphism produced by selection (Figure S1), to address the following questions: *Does the degree of sexual size dimorphism covary with transcriptome-wide sex-bias? How does the mode of selection affect the extent and parallelism of expression change, sex-biased gene expression, and cross-sex correlations in gene expression?* We predict that sex differences in gene expression covary with the phenotypic sexual size dimorphism. We further expect that sex-biased transcripts are disproportionately affected by

sex-specific selection and should also show lower correlated changes between the sexes compared to unbiased transcripts, under the expectation that they have more independent regulatory architectures between the sexes. SA selection should lead to fewer and less correlated expression changes between the sexes compared to sex-limited directional selection where, unlike under SA selection, both direct and correlated evolution has changed the body size of both sexes (Kaufmann et al. 2021). We also expect some consistency between the phenotypic response to selection and expression, both in terms of extent of changes and the identity of transcripts. Parallelism can however be limited by the selection mode and genetic redundancy predicted for polygenic traits (Barghi et al. 2020).

Results

No clear association between sexual size dimorphism and transcriptome-wide sex-bias but more transcripts with sex-biased expression under sex-specific selection

We evaluated expression differences in a total of 19,373 transcripts. The vast majority of transcripts show similar sex-bias across all selection lines. The overlap is proportionally greater for female-biased transcripts where 3,036 transcripts are shared across all selection lines (figure S2). The C line shows the lowest number of sex-biased transcripts overall, and the greatest overlap with all other lines (5,743 out of 7,121 [80%]; figure 1, and figure S2). SA and $SL_m\downarrow$ lines, with increased size dimorphism and similar evolutionary trajectories for male phenotypes (but not female), express the highest numbers of male-biased transcripts out of all lines (5,597 and 5,298, respectively, figure 1 and figure S2). This is attributable to transcripts that are male-biased only in either SA lines (n = 484, 8.6%) or $SL_m\downarrow$ (n = 345, 6.5%) or in both (n = 207; figure S2). In contrast to male-biased transcripts, a significantly lower proportion of female-biased transcripts are specific to SA lines (n = 134, 3%), than to the $SL_m\downarrow$ (n = 391, 7.6%) (test of equal proportions: $X^2_1=87.49$, $p < 0.0001$; figure S2). Overall, the $SL_m\downarrow$ lines have the highest number of female-biased transcripts (n = 5,182, of which 391 [7.5%] are unique), while the SA lines show the second lowest number after the C (figure 1 and figure S2). Selection for large female size ($SL_f\uparrow$), with no change in size dimorphism, has relatively few unique sex-biased genes compared to the other sex-specific modes of selection modes (602, ~5% of all 10,123 sex-biased transcripts in $SL_f\uparrow$, figure S2). There are very few transcripts (n = 26) with differing sex-bias directions across selection lines (figure S2), and therefore the deviations in the number of sex-biased transcripts largely represent differences between the selection lines in the magnitude of expression.

Across all the lines, there is no evidence for a transcriptome-wide association between the number or proportion of sex-biased transcripts and the degree of sexual size dimorphism (SSD), for either male-biased (autosomes: rho = 0.7, p = 0.23, X-chromosome: rho = 0.36, p = 0.55) or female-biased transcripts (autosomes: rho = -0.2, p = 0.78, X-chromosome: rho = -0.1, p = 0.95; figure 1). However, if only considering the lines that evolved by selection (i.e. excluding control), male-biased autosomal transcripts show a trend of increasing number with increasing SD, in line with the prediction. Autosomal and X-linked transcript numbers show a similar rank order across the lines (figure 1). We could not detect any association between the degree of sex-bias (the median logFC between males and females) and SSD, for either male- (rho = -0.6, p = 0.35) or female-biased transcripts (rho = -0.1, p = 0.95; figure S3).

Fewer expression differences under SA selection than under SL_m↓ selection

We next examine the patterns of differential expression (DE) between the selection modes in each sex. We focus on comparing gene expression consequences of similar pairs of male, but not female, evolutionary trajectories for body size. For this we contrast the lines subjected to bi-directional body size selection on males: In the SL_m↓ vs. SL_m↑ contrast selection was for smaller (SL_m↓) and larger (SL_m↑) males through male-limited selection. Meanwhile in the SA vs. SL_m↑ contrast selection was also for smaller (SA) and larger (Sl_m↑) males, but included SA selection. We could thus explore consequences of similar phenotypic responses and different modes of selection on gene expression. We contrast these lines also to the control line (C) subjected to random selection on body size but with otherwise similar demographic changes.

First, the DE transcripts show significant deviations in the distributions across chromosomes from those expected from all annotated transcripts, with fewer transcripts than expected on sex-chromosomes (table S1). Second, males have more DE transcripts compared to females in the SL_m↓ vs. SL_m↑ contrast (1.6x more), but especially in the SA contrast (2.5x more; table S2). Third, splitting the transcripts by whether they are DE in the same direction in both contrasts or only in one of the contrasts, shows that, in males, male-limited selection (SL_m↓ vs. SL_m↑) has resulted in well over twice as many DE transcripts specific to this contrast (hereafter, *unique* SL_m↓, N = 2,027) compared to changes specific to the contrast including SA selection (hereafter *unique* SA, N = 771). Many transcripts overlap between the two contrasts, showing expression difference in the same direction (hereafter *parallel*, N = 748; table S3). Females show similarly greater expression difference in the SL_m↓ vs. SL_m↑ than in the SA vs. SL_m↑ contrast (table S3). We tested for functional parallelism by

comparing GO terms enriched among the *unique* SL_m↓ and *unique* SA transcript sets (table S4 – unique L1, table S4 – unique SA, and table S4 – unique L1 – unique SA overlap) and found that the overlap of terms is not greater than expected by chance (from 100 bootstrapped overlaps with a random set of genes).

Male DE genes indicate parallel divergence from the control line

PCA analysis of expression profiles shows that samples cluster by sex (1st PC axis) and selection line (2nd PC axis; figure S4 and figure S5) as expected. For *unique* SL_m↓ and *parallel* transcripts, the 2nd PC axis (clustering samples by selection line) explains ~16-20% of the variance (figure S5). The patterns indicate that the control line, which represents the ancestral phenotype (Kaufmann et al. 2021), clusters in between the lines with smaller and larger individuals, revealing bi-directional expression differentiation (figure S5). Specifically, the *unique* SL_m↓ transcripts show divergent expression in the SL_m↓ and SL_m↑ away from the C along the PC2 in both sexes, while the SA cluster closer to the large SL_m↑ (figure S5 A). The *unique* SA transcripts, in turn, show a clear bi-directional separation of the SA and SL_m↑ lines, while both control and SL_m↓ cluster in between (figure S5 C). Finally, PCA of *parallel* transcripts shows more limited divergence between the lines with small males (SL_m↓ and especially SA) and the control, while SL_m↑ is separated in its own cluster (figure S5 B), suggesting that most *parallel* transcript changes are caused by divergence due to large male selection (SL_m↑).

We further identified the *unique* and *parallel* transcripts that show evidence of differential expression from the C line at the level of individual transcripts (hereafter referred to as *strict* lists). These include 267, 49, and 148 transcripts in the Sl_m↓ contrast only (*strict unique* SL_m↓), in both SL_m↓ and SA (*strict parallel*), and in the SA only (*strict unique* SA), respectively. Thus, 16% (49/316) and 25% (49/197) show parallel expression change out of all the DE transcripts in males in the SL_m↓ and SA contrasts to the C, respectively.

Male-biased genes are enriched among male DE transcripts in selection for smaller males

Several of our results suggest that body size evolution in our lines disproportionately affects the expression of male-biased transcripts under selection for smaller males, independent of the selection mode. First, the transcripts DE in SL_m↓ vs. SL_m↑ contrast, for males, are enriched for male-biased transcripts compared to genome-wide expectations (*unique* SL_m↓: $X^2_2 = 57.2$, $p < 0.001$; *parallel* with SA: $X^2_2 = 65.2$, p-value < 0.001; table 1A). These patterns remain when considering divergence from the control (*strict* sets of

transcripts, table 1B) in the *strict parallel* set (i.e. significantly DE also in the SA males). Second, the transcripts specifically upregulated in small males, regardless of the mode of selection (i.e. both up-in-SL_m↓ and up-in-SA, table S5), are enriched with male-biased transcripts, while those upregulated in the large males (up-in-SL_m↑) are enriched with unbiased transcripts but only in the SA v. SL_m↑ contrast (table S5). Female-biased and unbiased transcripts are in turn under-represented among the male DE transcripts (table 1 and table S5).

The expression patterns of sex-biased transcripts in females of these lines are more variable, reflecting their different patterns of phenotypic divergence. Overall, distributions of sex-biased transcripts are different across the *unique* SL_m↓, *unique* SA, and *parallel* transcript categories also for females ($X^2_6 = 147.3$, p-value < 0.001, table 2). Compared to the expected distribution, transcripts changing only in the females of the SL_m↓ vs. SL_m↑ contrast in response selection for small males (*unique* SL_m↓) are enriched for male-biased but also female-biased transcripts ($X^2_2 = 117.33$, $p < 0.001$; table 2), and those specifically upregulated in the smaller SL_m↓ females are disproportionately female-biased (up-in-SL_m↓, table 2). Transcripts changing in parallel under SA selection in females have similarly more male-biased transcripts (*parallel*: $X^2_2 = 10.49$, $p < 0.001$), and correspondingly fewer female-biased transcripts than expected. Among those DE only in the SA females, there is an excess of unbiased transcripts (*unique* SA: $X^2_2 = 12.67$, $p < 0.001$; table 2). Even the transcripts upregulated in females of SA lines, where selection acted towards larger females (up-in-SA) are, if anything, lacking in female-biased transcripts (table S5). Those transcripts with higher expression in females due to selection for larger males (up-in-SL_m↑) are disproportionately male-biased and/or unbiased, depending on the contrast (table S5).

Female-biased transcripts show lower cross-sex correlations in expression changes than male-biased or unbiased transcripts, regardless of the mode of selection or the targeted sex

Although different numbers of transcripts are DE in the sexes, especially in the SA lines (see above), the expression changes are generally highly correlated between males and females in both SL_m↓ and SA contrasts to SL_m↑ (figure 2). However, the correlation coefficients tend to be significantly lower for sex-biased than for unbiased transcripts (i.e. non-overlapping confidence intervals), in line with the prediction that sex-biased genes can evolve more independently in the sexes (figure 2).

The mode of selection is also associated with the cross-sex correlation of expression changes (figure 2). While unbiased transcripts have generally high correlations (cor > 0.8),

the coefficient is significantly higher among the transcripts *unique* to SA (cor = 0.86 [0.84, 0.88]) than to the SL_m↓ contrast (cor = 0.81 [0.79, 0.83]). Female-biased transcripts, in turn, show significantly lower cross-sex correlations (based on non-overlapping confidence intervals) in the *unique* SA set (cor = 0.47 [0.32, 0.6]) compared to the *unique* SL_m↓ set (cor = 0.66 [0.6, 0.72]; figure 2). Even male-biased transcripts show this trend, although the confidence intervals are slightly overlapping (figure 2). These patterns generally hold also for the *strict* transcript classes (i.e. expression changes also from the C line), except in the case of *strict unique SA* where correlation coefficients are greater for male-biased transcripts than female-biased ones, but in all cases confidence intervals are overlapping (figure S7).

The sex under selection influences the extent of expression changes but not the patterns of sex-bias or cross-sex correlations

Our selection lines allow us to also test how a similar selection mode, but acting on a different sex, affects consistency in expression change, by comparing lines with female- (SL_f↑) and male-limited (SL_m↑) selection for larger size to the selection line for smaller (male) size (SL_m↓). In the SL_m↓ vs. SL_f↑ contrast, females had opposite phenotypic responses, but due to selection on different sexes. In the contrast SL_m↓ vs. Sl_m↑, females also had opposite phenotypic responses, but in this case entirely due to selection on males. We further compare female expression in these lines with the C line (producing the *strict unique* SL_f↑, *strict unique* SL_m↑, and *strict parallel* sets for females in these contrasts).

Selection solely limited to males (i.e. SL_m↑ vs. SL_m↓ contrast) has affected expression considerably more than when selection also targeted females (SL_m↓ vs. SL_f↑), with the greatest difference seen in male expression changes (table S6, table S7). Notably, unlike under male-limited selection where 1.5 times more transcripts were affected in males, a roughly equal number of transcripts have responded in each sex when selection also targeted females (tables S6). Female-biased transcripts are generally under-represented, with the exception (again) of those upregulated in the small females of SL_m↓ lines in both contrasts (table S8). DE transcripts upregulated due to female-limited selection (up-in-SL_f↑) are more commonly unbiased or male-biased in both males and females (table S8). There are 312 and 345 transcripts that respond in parallel in both contrasts which are 15% and 11% of all the DE transcripts in these contrasts in females and males, respectively (table S7). The overlap of parallel transcripts in females is thus increased compared to under selection targeting only males or antagonistically both sexes (10%, table S3). These overlapping transcripts constitute half of all the changes that have occurred with female-limited upward selection in either sex

(47% and 56% in females and males, respectively, table S8), but 18% and 12% of the changes under solely male-limited selection, reflecting the overall greater divergence under the latter. The transcripts *unique* to male-limited selection show an excess of both female- and male-biased transcripts (as before) (table S9 *c.f.* table 1), while there are no deviations from expectations for the *parallel* or *unique* changes in the contrast including female-limited selection (SL_m↓ vs. SL_f↑; table S9). Correlation coefficients of expression changes between males and females are lower for sex-biased transcripts than for unbiased transcripts and remain lowest for female-biased transcripts even among those that changed in parallel in the two contrasts (SL_m↓ vs. SL_f↑ and SL_m↓ vs. SL_m↑; figure S8). There are overall few transcripts that differ from the control only due to female-limited selection for larger size (n = 31; *strict unique* SL_f↑) while 303 transcripts have changed only due to male-limited selection for larger size (*strict unique* SL_m↑). Notably, 14 transcripts are DE in both contrasts (*strict parallel*, 31% and 14% out of all significant transcripts in the contrasts of SL_f↑ and SL_m↑ to the control, respectively). Out of the few SL_f↑ transcripts that differ from the control, only one is female-biased in expression, while 11 are male-biased, a significant deviation from expectations (X^2_2 = 9.87, p-value = 0.007). There are very few female-biased transcripts (n = 10) even at FDR threshold < 0.1.

Discussion

We experimentally tested how adult gene expression changes in response to different modes of sex-specific selection on body size. We took advantage of the known selection history and phenotypic evolution in our artificial selection lines, and compared the effects of sex-limited (SL) and sexually antagonistic (SA) selection that has acted in the same direction on males, but not females, as well as SL selection that has operated similarly but on opposite sexes. We also utilized a control line subjected to random selection on body size. We find that thousands of transcripts are differentially expressed between the lines in both sexes with the vast majority of changes at autosomal loci. PCA analyses indicate that all selection lines show divergent expression differences from the control line. Male- and female-biased transcripts are differently impacted by SA and SL selection, and collectively show less correlated responses between the sexes, than unbiased transcripts. Below we discuss in detail the extent to which expression changes reflect phenotypic evolution in the sexes and are modulated by the mode of selection, in the light of our initial study questions.

Sexual size dimorphism, sex-biased gene expression, and sexual conflict

Two decades of genome-wide studies of gene expression across taxa have revealed extensive gene regulatory differences between the sexes. The conceptual framework of sexual conflict assumes that such regulatory decoupling evolves under SA selection to alleviate sexual conflict. The direction of sex-bias is therefore thought to reflect the different fitness optima in each sex in gene expression (Ellegren and Parsch 2007; Ingleby et al. 2015; Pennell et al. 2024), and in phenotypes, predicting a positive covariation between the two (Pointer et al. 2013; Harrison et al. 2015; Mank 2017; Wright et al. 2018; Cheng & Houle 2020). To date, the evidence remains inconclusive (Pointer et al. 2013, Harrison et al. 2015; Toubiana et al. 2021 *vs*. e.g. Khila et al. 2012; Scharmann et al. 2021). Here, we tackled this question by studying how the evolution of sexual dimorphism in a single trait affects gene expression, as a population-level process. We followed a common approach in the field and used bulk RNA samples to a) study expression as a molecular phenotype, and b) make our study comparable (but see e.g. Price et al. 2022). The majority of analysed loci are similarly unbiased or sex-biased across the lines, but importantly, there is also variation in their number and proportion across the lines (figure 1 and S2). We found no clear evidence of a transcriptome-wide association between sex-biased expression and evolved sexual size dimorphism (figure 1 and figure S2). However, the number of autosomal male-biased transcripts show a trend of positive covariation with sexual size dimorphism in the lines subjected to selection (i.e. excluding the random selection control line). Multiple lines of evidence support a disproportionate change in male-biased transcripts in the lines evolving towards the male-optimal smaller size (SA and SL_m↓). These lines express the highest numbers of male-biased transcripts, due to hundreds of loci detected as male-biased only in either one of these lines, or both (figure S2). Male-biased transcripts are enriched and more commonly upregulated in the males of both of these lines (and even among females but in a small subset of loci). SA and SL_m↓ lines not only evolved significantly smaller males, but also increased sexual size dimorphism due to no (under SA) or slower (under SL_m↓) response in females (Kaufmann et al. 2021; see figure S1). The increased autosomal male-biased expression suggests that at least some of these loci are likely involved in maintaining sexually dimorphic differences in adult size, while others can be subject to linked selection or be pleiotropically affected by the same developmental processes that lead to sexually dimorphic phenotypes.

According to the chief assumption of positive relationship between molecular and phenotypic sexual dimorphism, we should have observed mirrored changes in female-biased transcripts, towards increased female-biased expression in lines that have been selected towards female-optimal larger size (i.e. SA and SL_f↑). Instead, we observed that female-

biased transcripts are most numerous, and most commonly differentially expressed in the small male selection line (SL_m↓), and are, if anything, under-represented among DE transcripts in large-female (SL_f↑) and SA selection lines, as well as among any significant changes detected in males. The deficit of changes in female-biased transcripts shows that evolution towards female-optimal phenotype does not feminise the transcriptome (Hollis et al., 2014; Immonen et al. 2014, Veltsos et al. 2017; Parker et al., 2019). These differences contribute to the lack of an overall transcriptome-wide association with sexual size dimorphism for female-biased loci. The patterns also clearly demonstrate that sex-limited selection is capable of modifying sex-biased expression to an even greater extent than SA selection, despite the latter increasing SSD by 20 percentage points more than SL selection for similarly small males (Kaufmann et al. 2021). Sexual dimorphism both at molecular and phenotypic levels can evolve even under sexually concordant selection when genetic variances are asymmetric in the sexes (Fisher 1930; Lande 1980; Bonduriansky & Chenoweth 2009; Gosden et al. 2012; Wyman et al. 2013; Cheng & Houle 2020; Houle & Cheng 2021). Our results thus support earlier predictions that any form of selection can be capable of affecting expression dimorphism (Houle and Cheng 2021).

Another factor that breaks the association between sex-bias and SD, is that the random selection control line shows (ancestral) intermediate sexual size dimorphism but the lowest number of sex-biased transcripts of all lines. Although the extent to which the sex-bias state in the C line reflects the ancestral state is not known (due to no data from the ancestral population), several lines of evidence suggest they are in close accordance. The control line has retained most ancestral genetic variance in body size (Kaufmann et al. 2023a), shows limited genome-wide divergence in allele frequencies from the ancestral population (Zwoinska et al. *in preparation*), and shows no body size divergence from the ancestral population in either sex (Kaufmann et al. 2021). In our expression analyses, the control line also shows the greatest relative overlap of sex-bias with all the other lines (figure S2). It is however still possible that some ancestral sex-bias may have become lost during random selection, which would imply that sex-specific body size selection is indeed important for maintaining sex-biased expression.

The multivariate nature of sexual conflict predicts that SA selection not only increases sex-differences but also in constrains evolutionary change when the sexes cannot simultaneously reach their respective optima (Ingleby et al. 2015; Kasimatis et al. 2017; Pennell et al. 2024). The gene expression changes under SA compared to sex-limited selection should thus be more constrained, but, in the loci where changes can occur, they

should also be less correlated between the sexes, to facilitate phenotypic dimorphism. Lower cross-sex correlations are expected specifically in sex-biased loci under the premise that they should have more sex-specific genetic architecture that can decouple expression responses in the sexes (Griffin et al. 2013; Allen et al. 2018; Houle & Cheng 2021). Our study system provides insights into each of these mutually non-exclusive outcomes.

First, the expression changes under SA selection are indeed far fewer than under SL_m↓ selection, and especially so in females (SA line males show over 2.5 times more significant changes than females, while SL_m↓ line males only 1.6 times more; table S1). This large sex difference reflects the phenotypic changes well (Kaufmann et al. 2021) and suggests that SA selection has indeed affected the sex-specific regulatory architecture underlying body-size in such a way that SA males were able to change relatively more than females. Under male-limited selection, male size evolved even slightly further than under SA, and female size showed a strong correlated response (Kaufmann et al. 2021), thereby contributing to greater erosion of genetic variance in body size under this selection mode compared to the SA (Kaufmann et al. 2023a). The greater number of changes observed in the SL_m↓ lines thus reflects this overall greater divergence.

Second, the expression changes in the SA females are predominantly in unbiased transcripts that also show a significantly higher cross-sex correlation in expression changes than under male-limited selection (figure 2). SA selection has thus led to more similar expression changes across the sexes at unbiased loci than SL selection. In contrast to the unbiased transcripts, the cross-sex correlation is lower for sex-biased transcripts, and especially female-biased transcripts, both in general, and in the SA lines in particular (figure 2). This indicates that SA selection is capable of restricting correlated responses thereby increasing sex differences in expression. However, the fact that female-biased transcripts were overall less affected by SA selection in our study also implies that, as a group, they may be less important for maintaining larger body size. This is further supported by their under-representation among transcripts changing in expression under female-limited selection on larger size. Whether SA selection leads to lower cross-sex correlations at sex-biased transcripts or whether SA selection more readily targets transcripts with lower correlations to begin with, remains to be established.

The finding of overall lower cross-sex correlations in sex-biased and especially in female-biased transcripts is independent of the sex under selection, as we detect it even in the comparison involving female-limited selection (figure S8). This general pattern indicates that the underlying cross-sex genetic correlation ($r_{m,f}$) in expression is relatively lower in sex-

biased loci, in accordance with several studies in *Drosophila*. The association between the $r_{m,f}$ and sex-bias is overall negative (Griffin et al. 2013; Allen et al. 2018; Houle & Cheng 2021), but male-biased genes also show a higher $r_{m,f}$ than female-biased genes (Allen et al. 2018).

Parallel changes and consistent targets of selection

The extent to which parallel gene expression changes underlie parallel phenotypic changes has been tested both in wild populations (Jeukens et al. 2010; Zhao et al. 2015; Yeaman et al. 2016) and in replicate populations in experimental evolution studies (Cooper et al. 2003; Immonen et al. 2014; Veltsos et al. 2017; Hsu et al. 2020; Veltsos et al. 2022; Thorhölladottir et al. 2023; Mishra et al. 2024; Ghalambor et al. 2015; José Rivas et al. 2018; McGirr & Martin 2018; Fischer et al. 2021; Jacobs et al. 2020, Immonen et al. 2023). These studies have found both repeatable and idiosyncratic changes. In typical experimental evolution studies, parallelism is inferred from consistent responses across similarly treated replicates, which is the foundation of detecting changes due to selection, as opposed to drift. Our differential expression analyses also rely on this consistency among the selection line replicates, but expands the question to whether it is possible to detect parallel changes when phenotypic evolution is similar but the mode of selection, or the sex under selection, is not. Our results indicate that the answer is "yes" to both. The SA and SL selection for small males affect the expression of the same transcripts in about a fifth of the cases (i.e. parallel changes in 24% and 16% of all the significant loci in SA and $SL_m\downarrow$ male contrasts to both control and $SL_m\uparrow$, respectively, i.e. *strict parallel*). When comparing similar selection modes but acting on a different sex, parallelism is increased to 31% (i.e. out of all changes from the control line due to sex-limited selection on females for larger size), although fewer transcripts are different from the control line. Lower parallelism when comparing the different selection modes can be expected due to the fact that the genomic constraint imposed by SA selection is restrictive in the changes that can occur. These should primarily involve loci with a net positive effect on fitness across both sexes, which is not required under male-limited selection.

Adult body size is a complex polygenic trait, and polygenic adaptation is expected to show low genetic parallelism and greater genetic redundancy (Barghi et al. 2020; Schlötterer 2023). The degree of parallelism can increase at the higher levels of biological organisation, from gene regulation to molecular pathways and physiological processes (Edelman et al. 2001). Here, we find no evidence that the overlap in enriched GO terms between unique SA and *unique* $SL_m\downarrow$ transcript sets is greater than expected by chance, suggesting that distinct

processes are impacted when selection has changed similarly male, but not female size. The selection that changes adult size ultimately targets genes that regulate growth in the
483 embryonic and/or juvenile stages. In our lines, the body size evolution seems to be produced solely by differences in initial embryo size and/or in growth rate during juvenile stages, as we have not detected any differences in developmental time (*unpublished data*). The gene
486 regulatory differences in adults thus reflects developmental inertia at causal loci or downstream pleiotropic effects of these changes. A key growth pathway across taxa – insulin/insulin-like signalling and target of rapamycin (IIS/TOR) – is responsible for growth
489 by regulating developmental proliferation of cell size and number (Nijhout et al. 2014; Texada et al. 2020), but also for maintaining the size/number of cells in adult organs by modulation of cell growth, division and death (Yang & Xu 2011). In accordance, we find and
492 *"Toll signalling pathway"* GO terms enriched among transcripts showing *parallel* expression differences in SA vs. SL_m↑ and SL_m↓ vs. SL_m↑ contrasts (table S4 - parallel). We also find enrichment of insulin receptor signalling GO terms among the transcripts showing *parallel*
495 expression differences from control lines in the SA and small male selection lines in males (table S4 – strict parallel). The terms associated specifically with changes due to SA selection (i.e. *unique* differences from control and large-male selection, table S4) include many that are
498 involved in cell differentiation of sensory systems (unbiased transcripts), metabolic processes (male-biased transcripts) and mitosis (female-biased transcripts), while those associated with small size due to male-limited selection (i.e. *unique* differences, table S4) include various
501 developmental process, apoptosis and regulation of fertility (unbiased transcripts), e.g. lipid translocation (male-biased transcripts), chromatin organization, regulation of metabolic processes and immune system processes (female-biased transcripts).

504

Conclusions

Our results demonstrate that the sexually dimorphic transcriptome evolves under sex-
507 specific selection. We show that the expression changes are less correlated at sex-biased than at unbiased loci, regardless of the mode of selection. The strength of this correlation is also modified by the mode of selection and whether the loci are male- or female-biased. We find
510 no evidence for transcriptome-wide correlation between sex-biased expression and sexual size-dimorphism across selection lines. However, male-biased autosomal loci are enriched among transcripts showing expression changes in the most sexually dimorphic lines. The lack
513 of clear association is an important result because it is frequently an implicit expectation in comparative studies of sex-biased genes, which have found very mixed support for it (Pointer

et al. 2013; Harrison et al. 2015; Toubiana et al. 2021; Scharmann et al. 2021; Price et al. 2022). Our results also indicate that inferring the selection history on each sex from patterns of sex-biased gene expression is highly questionable. SA selection affects sex-biased expression, but sex-limited directional selection can do so too, and even to a greater extent (female-biased transcripts in this case). Our results suggest that expression at male- and female-biased transcripts is not affected in the same way or to the same degree, indicating a strong dependence on the specific loci underlying the traits under selection, and the genetic architecture of gene expression at these loci. This is consistent with SA selection constraining gene expression changes relative to sex-limited directional selection. This further suggests that rapid expression evolution of sex-biased genes reported between populations and species in some studies may more likely imply an absence of intra-locus sexual conflict than the presence of it.

Materials and Methods

Study organism, selection lines, and sequencing

Callosobruchus maculatus seed beetles are a subtropical and tropical pest of bean seeds with a cosmopolitan distribution. It has a rapid life cycle of approximately three weeks. Females lay eggs on the surface of beans that serve as food resource and environment for the larval development which, in our standard laboratory conditions, lasts approximately 25 days (at 29C°, 50% relative humidity, and a 12h/12h light/dark cycle). The beetles are aphagous capital breeders and adults complete their reproductive cycle relying solely on the larval resources. The adult body mass acquisition is thus exclusively a larval developmental process. *C. maculatus* has 18 autosomes and XY sex chromosomes.

In this study, we assessed experimentally how different modes of sex-specific selection – sexually antagonistic (SA) or sex-limited (SL) – affect gene expression in adult beetles. The selection lines utilised here have been described in detail in Kaufmann et al. 2021. All lines originate from a single wild collected population (from Lome, Togo). The selection lines were created by applying 10 generations of artificial family-level selection either only on males, towards smaller or larger size ($SL_m\downarrow$, $SL_m\uparrow$ respectively), only on females, towards larger size ($SL_f\uparrow$), or sexually antagonistically (SA) on both sexes for larger females and smaller males (i.e. increasing the naturally occurring sexual size dimorphism). Each mode of selection was independently replicated twice. We also use here a control line (C) subjected to similar demographic changes as the selection lines but to relaxed (random) selection on size, which has retained the ancestral patterns of larval growth and adult body

size (line Cb in Kaufmann et al. 2021, 2023). Our quantitative genetic analysis (Kaufmann et al. 2023a) confirms that this control line has largely maintained the ancestral genetic variance for body size, which is also confirmed by our Pool-seq analysis (Zwoinska et al. *in prep.*)

Due to logistic limitations, it was not possible to start the gene expression experiments directly after selection was terminated. We therefore established isofemale lines from each selection line to limit the impact of relaxed selection. Eight isofemale lines per selection line (each started by a single full-sib family) were kept under standard rearing conditions for approximately a year (~15 generations). To create the samples for RNA-seq, the isofemale lines were first density controlled for two generations (by limiting oviposition window to 24h, on a surplus of beans). Only beans with a single egg (and thus no larval competition) were used to collect virgin adults for the experiment. We then chose six isofemale lines and made three inter-line crosses. From the F1 progeny of these crosses, we picked five males and five females per cross to produce pooled samples by sex. The adult beetles were snap-frozen in liquid nitrogen 24h after eclosure. Each sequenced sample thus represents an average profile of five individuals from two isofemale lines. For each selection line, we produced three such pools per sex, resulting in six samples representing each selection line. The total number of samples sequenced was 54. Total RNA was extracted from whole bodies, using Qiagen RNeasy, according to manufacturer's instructions. The RNA was checked for quality and quantity using NanoDrop, Qubit and BioAnalyzer. TruSeq stranded mRNA libraries were prepared for each sample including poly-A selection to enrich for mRNA. Paired-end sequencing for read lengths of 150bp was performed on a single NovaSeq 6000 S4 flowcell. Library preparation and sequencing was performed by the SNP & SEQ Technology Platform of SciLifeLab in Uppsala.

RNA-seq read pre-processing, and expression quantification

We used the most recent genome assembly (made from the same population as the selection lines) and the associated annotation (Kaufmann et al. 2023b). RNA-seq reads were first trimmed to identify and remove TruSeq adapters with cutadapt (v. 4.0; Martin 2011). After trimming, any reads <75bp in length were discarded. We quality checked against any putative remaining rRNA reads in the libraries, using SortMeRNA (v. 4.3.3; Kopylova et al. 2012) to identify reads that mapped to a set of Coleopteran rRNA sequences, obtained from NCBI (n = 2,646 sequences; https://www.ncbi.nlm.nih.gov/) and SILVA (n = 13,768 sequences; Pruesse et al. 2007; Quast et al. 2013). These sequences were clustered with cd-hit (v. 4.8.1; Li et al. 2006; Fu et al. 2012) by similarity of 95%. We retained all reads that did

not map to this curated database of rRNA sequences. We used Salmon (v. 1.6.0; Patro et al. 2017; Srivastava et al. 2020) in selective alignment mode to quantify the expression of all transcripts in the *C. maculatus* transcriptome. We engaged the salmon options --gcBias --seqBias, and --posBias.

For a subset of samples (from the control line), we mapped reads also to the genome assembly using STAR (v. 2.7.9a; Dobin et al. 2013), and used ExplorATE (Femenias et al. 2022; available from: https://github.com/FemeniasM/ExplorATE_shell_script; commit: ecebe32) to quantify the contribution of TE activity to the mRNA samples (see the Supplementary Materials: Supplementary Methods).

Prior to differential expression analysis we excluded transcripts on contigs shorter than 100kb (n = 5,977; 15%) to limit our analysis to the most reliably assembled and annotated portion of the genome (Kaufmann et al. 2023b). We also removed transcripts on the Y chromosome (n = 437) to avoid confounding results due to multi-mapping to autosomal homologs. We additionally performed minimal filtering of very lowly expressed transcripts by removing any transcripts with overall mean counts < 3 across all samples (n = 13,513). Following this filtering, we were left with expression estimates from 19,373 transcripts in total. We also conducted a principle component analysis (PCA) with the prcomp() function in R (v. 4.3.3; R Core Team 2024) to establish the overall patterns of gene expression across sexes and selection lines. For the PCA we additionally removed transcripts with no variance in the Transcripts Per Million (TPM) estimates across samples (n = 494), as these are uninformative in PCA.

Sex-biased transcript expression

We first tested for differences in expression between the sexes within each selection treatment. To account for the two replicate lines of each selection treatment, we included replicate as a fixed effect in the model:

$$y \sim replicate + sex + e$$

As we had a single replicate of the control (C) line, the model becomes:

$$y \sim sex + e$$

All the p-values were adjusted for multiple testing using the Benjamini-Hochberg correction and control FDR at $p_{adjusted} < 0.05$. For each selection treatment, we computed the number of sex-biased transcripts, as well as their relative proportions, across chromosome categories (autosomes, X-chromosome,). We then tested for an association between the number and proportion of sex-biased transcripts on each chromosome type and the degree of sexual size dimorphism in each selection treatment (the mean SSD across the two replicate selection lines, data from Kaufmann et al. 2021). We also tested for an association between the magnitude of sex-bias (median log(fold-change) between males and females across all sex-biased transcripts) and the degree of sexual size dimorphism in each selection treatment. We consider female-biased (log(fold-change) > 0) and male-based (log(fold-change) < 0) transcripts separately. Differential expression analysis was performed with the edgeR package (v. 3.36.0; Robinson et al. 2010) in R (v. 4.3.3; R Core Team 2024) with the default library normalization procedures and transcript filtering procedures. As a reference in comparative analyses between the selection lines, we used sex-biased transcripts called in the control line (C).

Gene expression differences across selection treatments

We next sought to identify transcripts showing expression differences associated with the selection treatments. In our analytical pipeline, we were interested in finding the changes associated with divergence in male size under *1)* male-limited selection ($SL_m\uparrow$ vs. $SL_m\downarrow$) and *2)* SA selection ($SL_m\uparrow$ vs. SA), and how these two contrasts may compare given the similarities in male but differences in female phenotypic evolution. *3)* We also tested whether the sex under selection matters by contrasting also the selection lines for large size via females ($SL_f\uparrow$ vs. $SL_m\downarrow$). To evaluate the degree of sex-specific responses to selection we conducted these tests separately for each sex, and tested how correlated the expression differences were between the sexes, using spearman rank correlation tests.

Because replicate lines were assigned to selection treatments independently, they should be treated as unique. To identify changes across the replicates in a given treatment, we constructed a contrast to test for an average difference between selection treatments, such that:

$$H_0: ((L_{ia}+L_{ib})/2) - ((L_{jc}+L_{jd})/2) = 0$$

Where *i* and *j* are different selection treatments, and *a*, *b*, *c*, and *d* are independent replicate lines. Additionally, for the transcripts of interest (see Results) we tested for differences between the selection treatment lines and the control line, C:

$$H_0: C - ((L_{ia}+L_{ib})/2)= 0$$

For all tests, we controlled the FDR at $p_{adjusted} < 0.05$. We note however that our results are qualitatively similar even with a more liberal FDR cut-off of <0.1.

We used the R package ViSEAGO (v. 1.14.0; Brionne et al. 2019) for GO term enrichment analysis, setting all transcripts as the background set and using the "Biological Process" ontology terms. We note that ~50% of annotated transcripts received GO term annotations, these enrichment analyses must be interpreted with that in mind. We tested for an overlap in enriched GO terms using a bootstrapping procedure. We performed 100 boostrap samples of *n* random transcripts, where *n* was always the equivalent number of transcripts to the set being compared.

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Data availability

Data and analysis scripts have been deposited in a zenodo archive with doi: 10.5281/zenodo.13919165. Raw reads have been deposited at NCBI with BioProject accession: PRJNA1178449

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Tables and figure legends

Figure 1. The proportion of **A)** male-biased and **B)** female-biased transcripts as a function of sexual dimorphism across selection line treatments. Inset text above each panel gives the result of a Spearman rank correlation test for the association between the proportion of transcripts and sexual dimorphism. Inset text next to each point give the absolute number of transcripts in each case. The data are split by chromosome category into autosomes – 'A', the X-chromsome – 'X'.

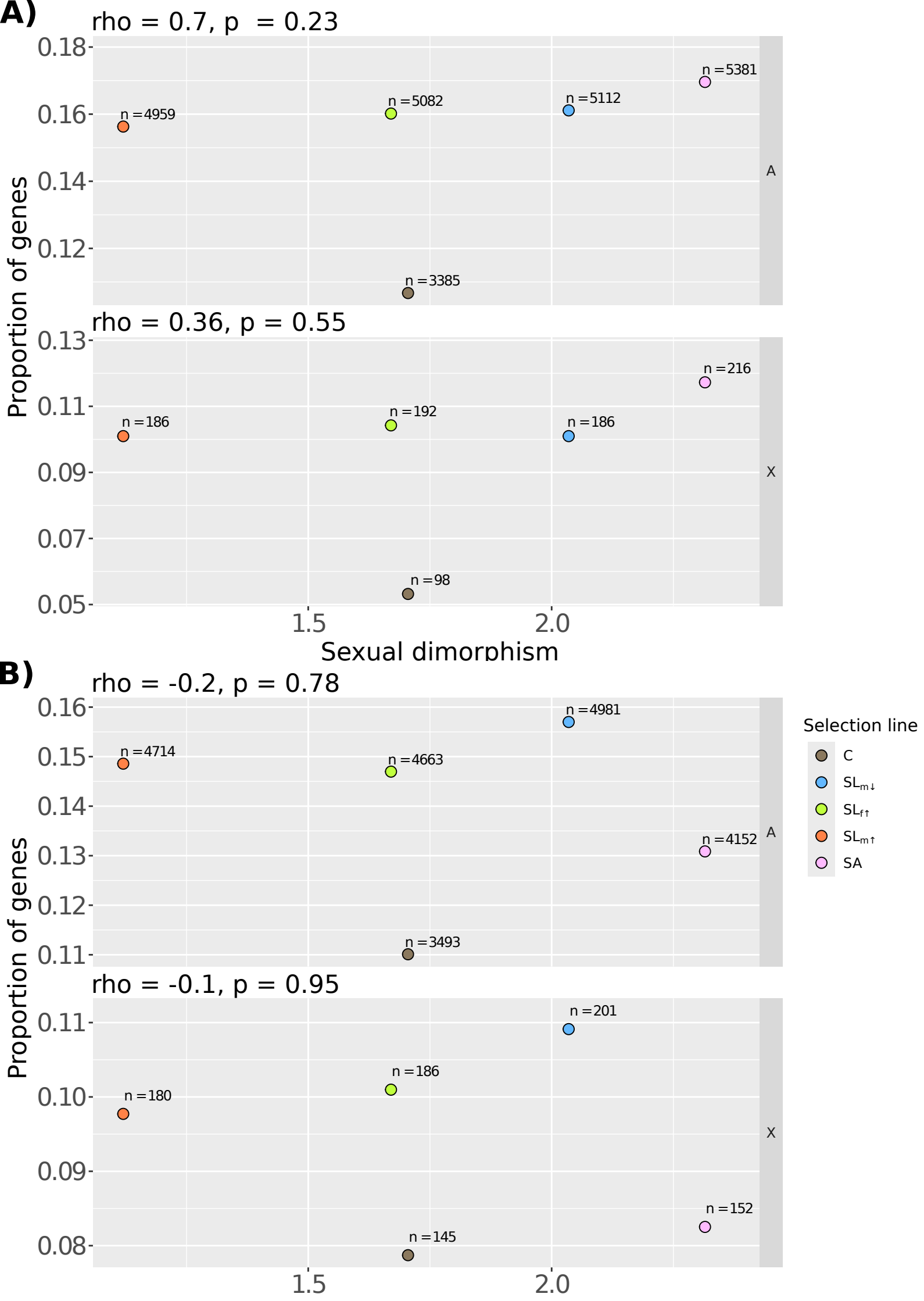
Figure 2. logFC expression between $SL_m\downarrow$ and $SL_m\uparrow$, or SA and $SL_m\uparrow$, for males and females. Data are split by whether males show a unique response in the $SL_m\downarrow$ vs. $SL_m\uparrow$ contrast, a unique response in the SA vs. $SL_m\uparrow$ contrast, or a parallel response in both, and by sex-bias status. Points are coloured by whether both of the sexes show the same significant expression difference (white) or only one of them (grey). Inset text gives results of Pearson's correlation tests as well as correlation coefficients for each panel. Note that the axis labels are different for the final column of panels. The logFC values for males and females of the parallel transcript class in the SA vs. $SL_m\uparrow$ contrasts is given in the supplementary materials (figure S6).

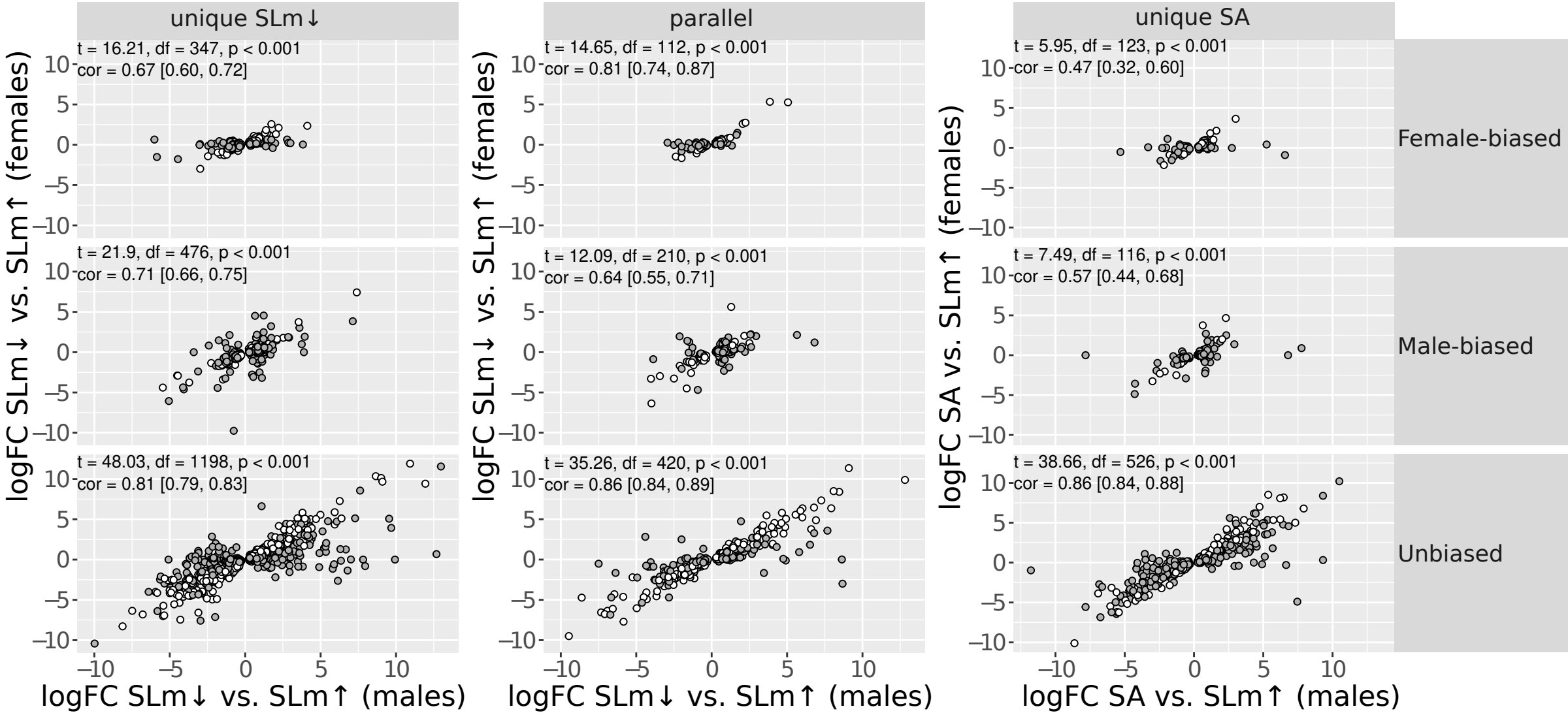
Table 1. Associations with sex-bias among differentially expressed transcripts in males (see main text for description of the categories). Counts with asterisks indicate a significantly greater proportion than expected based on genome-wide proportions of sex-biased transcripts (shown in brackets in the column headers). Proportions out of row totals are given in brackets. **A)** Counts are the number of transcript in each category. Distributions are significantly different across rows ($X^2_6 = 145.27$, $p < 0.001$). **B)** Counts are the number of transcripts in each "strict" category (i.e. differences in expression also compared to the control (C) line. Distributions are significantly different across rows ($X^2_6 = 16.56$, $p = 0.011$)

	Female-biased (0.18)	**Male-biased (0.17)**	**Unbiased (0.65)**	**total**
A)				
not-DE	2,659 (0.19)	2,200 (0.15)	9,444 (0.66)	14,303
unique SL_m↓	350 (0.17)	479* (0.24)	1,205 (0.59)	2,034
parallel	114 (0.15)	212* (0.28)	422 (0.56)	748
unique SA	126 (0.16)	119 (0.15)	533 (0.69)	778
B)				
not DE	3,429 (0.18)	3,248 (0.17)	12,228 (0.65)	18,905
strict unique SL_m↓	50 (0.19)	47 (0.18)	170 (0.64)	267
strict parallel	3 (0.06)	18* (0.37)	28 (0.57)	49
strict unique SA	28 (0.18)	21 (0.14)	103 (0.68)	152

Table 2. Associations with sex-bias among differentially expressed transcripts in females (see main text for details). Counts with asterisks indicate a significantly greater proportion than expected based on genome-wide proportions of sex-biased transcripts (shown in brackets in the column headers). Proportions out of row totals are given in brackets. Distributions are significantly different across rows ($X^2_6 = 147.3$, $p < 0.001$).

	Female-biased (0.18)	**Male-biased (0.17)**	**Unbiased (0.65)**	**total**
not-DE	2,674 (0.17)	2,610 (0.16)	10,728 (0.67)	16,012
unique SL_m↓	414* (0.27)	322* (0.21)	798 (0.52)	1,534
parallel	24 (0.10)	49* (0.21)	161 (0.69)	234
unique SA	55 (0.12)	81 (0.17)	328* (0.71)	465


A)
rho = 0.7, p = 0.23
n = 4959
n = 5082
n = 5112
n = 5381
n = 3385
A
rho = 0.36, p = 0.55
n = 186
n = 192
n = 186
n = 216
n = 98
X
Proportion of genes
Sexual dimorphism
B)
rho = -0.2, p = 0.78
n = 4714
n = 4663
n = 4981
n = 4152
n = 3493
A
rho = -0.1, p = 0.95
n = 180
n = 186
n = 201
n = 152
n = 145
X
Selection line
C
SLm↓
SLf↑
SLm↑
SA


unique SLm↓
parallel
unique SA
t = 16.21, df = 347, p < 0.001
cor = 0.67 [0.60, 0.72]
t = 14.65, df = 112, p < 0.001
cor = 0.81 [0.74, 0.87]
t = 5.95, df = 123, p < 0.001
cor = 0.47 [0.32, 0.60]
t = 21.9, df = 476, p < 0.001
cor = 0.71 [0.66, 0.75]
t = 12.09, df = 210, p < 0.001
cor = 0.64 [0.55, 0.71]
t = 7.49, df = 116, p < 0.001
cor = 0.57 [0.44, 0.68]
t = 48.03, df = 1198, p < 0.001
cor = 0.81 [0.79, 0.83]
t = 35.26, df = 420, p < 0.001
cor = 0.86 [0.84, 0.89]
t = 38.66, df = 526, p < 0.001
cor = 0.86 [0.84, 0.88]
Female-biased
Male-biased
Unbiased
logFC SLm↓ vs. SLm↑ (females)
logFC SA vs. SLm↑ (females)
logFC SLm↓ vs. SLm↑ (males)
logFC SA vs. SLm↑ (males)
10
5
0
−5
−10