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CONDITION-DEPENDENCE OF THE SEXUALLY DIMORPHIC TRANSCRIPTOME IN *DROSOPHILA MELANOGASTER*

Minyoung J. Wyman,[1,2] Aneil F. Agrawal,[1] and Locke Rowe[1]

[1]*Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, Ontario, M5S 3B2, Canada*

[2]*E-mail: minyoung.yi@utoronto.ca*

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Sexually dimorphic traits are by definition exaggerated in one sex, which may arise from a history of sex-specific selection—in males, females, or both. If this exaggeration comes at a cost, exaggeration is expected to be greater in higher condition individuals (condition-dependent). Although studies using small numbers of morphological traits are generally supportive, this prediction has not been examined at a larger scale. We test this prediction across the transcriptome by determining the condition-dependence of sex-biased (dimorphic) gene expression. We find that high-condition populations are more sexually dimorphic in transcription than low-condition populations. High-condition populations have more male-biased genes and more female-biased genes, and a greater degree of sexually dimorphic expression in these genes. Also, condition-dependence in male-biased genes was greater than in a set of unbiased genes. Interestingly, male-biased genes expressed in the testes were not more condition-dependent than those in the soma. By contrast, increased female-biased expression under high condition may have occurred because of the greater contribution of the ovary-specific transcripts to the entire mRNA pool. We did not find any genomic signatures distinguishing the condition-dependent sex-biased genes. The degree of condition-dependent sexual dimorphism (*CDSD*) did not differ between the autosomes and the X chromosome. There was only weak evidence that rates of evolution correlated with *CDSD*. We suggest that the sensitivity of both female-biased genes and male-biased genes to condition may be akin to the overall heightened sensitivity to condition that life-history and sexually selected traits tend to exhibit. Our results demonstrate that through condition-dependence, early life experience has dramatic effects on sexual dimorphism in the adult transcriptome.

KEY WORDS: **Condition-dependence, evolutionary genomics, life-history evolution, sexual selection.**

Sexual dimorphism is ubiquitous in sexually reproducing organisms (Darwin 1871; Andersson 1994). Dimorphism in morphological and behavioral traits has long been apparent, yet only recently has the widespread extent of dimorphism in transcription been appreciated (Jin et al. 2001). In *Drosophila melanogaster*, 15–70% of known genes have sexually dimorphic expression (Jin et al. 2001; Parisi et al. 2003; Ranz et al. 2003; Gibson et al. 2004). Sexual dimorphism in gene expression also is taxonomically widespread, occurring in flies, worms, mammals, and birds (Jiang et al. 2001; Jin et al. 2001; Yang et al. 2006; Ellegren et al. 2007; Ellegren and Parsch 2007). At least for classic phenotypic traits, it is well known that the extent of sexual dimorphism can vary dramatically within a single population (Darwin 1871; Andersson 1994), that is, some males are phenotypically similar to females whereas other males are much different. Quantifying and explaining this variation in sexual dimorphism in the transcriptome (Meiklejohn et al. 2003; Baker et al. 2007), as well as in classic phenotypic traits (Fairbairn et al. 2007), remains a major challenge.

Sexual dimorphism evolves as a response to sex-specific selection—in males, females, or both. Females are more fit if they limit the expression of any costly traits that primarily function

in males to increase mating success (e.g., exaggerated display traits). Likewise, males are more fit if they limit the expression of any costly traits that function in females to enhance fecundity. Thus, much selection for dimorphism may occur because traits that benefit one sex carry pleiotropic costs that affect both sexes. The same pleiotropic costs that favor sexual dimorphism can also lead to condition-dependent expression of these traits (Rowe and Houle 1996; Bonduriansky and Rowe 2005; Bonduriansky 2007). Under a variety of assumptions, males in higher condition are expected to express these costly traits to a greater extent than males in lower condition. Although this process has been discussed most often in the context of exaggerated display traits, it applies to any traits with a history of sex-biased selection. Likewise, exaggeration of life history traits (e.g., female fecundity) may carry pleiotropic costs and may therefore evolve condition-dependent expression (Houle 1998). As a consequence of condition-dependence in sexually selected traits, and other dimorphic traits, the overall degree of sexual dimorphism is itself expected to be condition-dependent. Some studies of morphological characters have verified this prediction by finding higher levels of dimorphism when individuals are in higher condition (Bonduriansky and Rowe 2005; Bonduriansky 2007). This relationship between condition and the degree of dimorphism should hold broadly, applying to any costly trait that primarily benefits one sex.

Sex-biased gene expression represents a novel character set in which to test the hypothesis of condition-dependent sexual dimorphism. Several lines of indirect evidence suggest that sex-specific selection was the driving force behind the evolution of sex-biased gene expression (Meiklejohn et al. 2003; Connallon and Knowles 2005; Reinius et al. 2008). Perhaps as a consequence of this sex-specific selection, sex-biased genes bear distinct evolutionary signatures, which they share in common with other classic sexually dimorphic traits. In *Drosophila*, male-biased genes possess greater lineage-specific divergence than unbiased or female-biased genes with respect to coding-sequence (Zhang et al. 2004; Zhang and Parsch 2005; Proeschel et al. 2006; Haerty et al. 2007; but see Metta et al. 2006) and expression state (Meiklejohn et al. 2003; Ranz et al. 2003; Zhang et al. 2007). By contrast, female-biased genes demonstrate stronger conservation than unbiased or male-biased genes. These patterns mirror the phenotypic patterns showing that male traits often diversify whereas female traits appear more consistent across closely related taxa (Darwin 1871; Andersson 1994). If similar forms of sex-specific selection have shaped these parallels, then sex-biased gene expression may share in common other similarities with classic sexually dimorphic traits. In particular, sex-biased gene expression might respond to variation in condition.

We used microarrays to assess the condition-dependence of sexually dimorphic transcription. We reared larvae on diluted and concentrated sugar-yeast medium to produce "low" and "high" condition adult flies. We predicted that high-condition individuals should be more sexually dimorphic in their expression patterns than low-condition individuals. In particular, we expect male-biased gene expression, female-biased gene expression, and the total amount of sex-biased gene expression to be greater among high than low-condition individuals. Only a handful of morphological studies have verified the condition-dependence of sexual dimorphism, and all of these studies have focused upon a small number of nonrandomly selected traits (David et al. 2000; Cotton et al. 2004a,b; Bonduriansky and Rowe 2005; Bonduriansky 2007; Boughman 2007; Punzalan et al. 2008). Testing condition-dependence in transcriptional sexual dimorphism across the entire genome reduces the potential for discovery bias while also extrapolating the prediction to the molecular level for the first time.

Methods

MICROARRAYS

We used the two-channel Oligo 14kv1 microarrays printed by the Canadian Drosophila Microarray Center (CDMC) in Mississauga, Ontario. Arrays were synthesized with CMT-UltraGAPS slides using a SpotArray 72 microarrayer and the 65–69mer probes were based on release 4.1 of the *D. melanogaster* genome from April 2005 (GEO accession # GPL3603). The array had 13,880 unique spots, representing *D. melanogaster* sequences (13,319 unique genes), blanks, buffer spots, and *Arabidopsis* controls; each spot was printed twice consecutively on the array. The CDMC handled all aspects of reverse-transcription, sample labeling, array hybridization, and slide scanning (see www.flyarrays.com for protocols). Our data are MIAME compliant and are available in the Gene Expression Omnibus repository.

MICROARRAY EXPERIMENTAL DESIGN

In the basic experimental block, condition was manipulated at two food levels (high or low) for both sexes (male or female). Each sex-by-diet combination was replicated twice within an experimental block for a total of eight biological samples. The within-block replication enabled each sex-by-diet combination to be labeled once with Alex647 and once with Alexa555 in a loop fashion, allowing us to account for dye-introduced variance. We used two genotypes; each genotype had three replicates of the basic experimental block. This resulted in 48 biological samples (2 sexes $\times$ 2 diets $\times$ 2 dyes $\times$ 2 genotypes $\times$ 3 experimental blocks) hybridized to 24 arrays.

CONDITION MANIPULATION

In the high-condition treatment, flies were reared on standard sugar-yeast medium. In the low-condition treatment, flies were

reared on medium at 25% of the standard sugar-yeast concentration. Size is a good proxy for condition in many insects including *D. melanogaster* because it correlates positively to the total energy reserves available at eclosion—corresponding to one aspect of "condition." Our weight measurements confirmed that sex and larval diet treatments produced differences in adult size (Sex: $F_{1,116} = 491$, $P < 0.0001$; Diet: $F_{1,116} = 169$, $P < 0.0001$). Females were larger than males; high-condition flies were larger than low-condition flies. We observed sex × condition interactions on size ($F_{1,116} = 6.96$, $P = 0.009$). High-condition females were 36% larger than low-condition females; high-condition males were 43% larger than low-condition males. In other studies, we have found that reductions in larval nutrition have perceptible effects on adult sexual dimorphism, reducing fecundity in females and mating success in males (Sharp and Agrawal 2009).

EXPERIMENTAL TREATMENTS AND REARING

Each genotype was the F1 hybrid offspring of two inbred lines founded from wild populations in North Carolina (courtesy of G. Gibson). The first genotype was the progeny of We61 × We29 (female × male); the second genotype was the progeny of We32 × We107. Each cross was performed only in one direction (no reciprocal crosses). We used these replicable genotypes for two reasons. First, the low larval diet treatment causes greater preeclosion mortality than the high larval diet treatment. Using individuals with the same genotype ensures that the survivors have the same genotype as the nonsurvivors. Thus, expression differences between the high- and low-condition individuals result directly from diet manipulation, rather than indirectly through differential selection. Second, inbred lines typically become homozygous for many loci but independent lines carry different alleles. Crossing two lines creates heterozygous individuals that are more representative of field-caught individuals (except for X-linked loci in males). Finally, we used two genotypes to expand the breadth of our results so that they are not confined to a single, perhaps unusual, genotype.

Virgin females from the inbred lines were mated to their respective males en masse in population cages containing grape-agar plates. Groups of 40 first instar larvae were picked from the grape-agar plates into 8-dram vials with 7.5 mL of 25% or 100% medium and a small pellet of yeast. Virgin adult flies from high and low treatments were collected within 8 h of eclosion and held in fresh vials with live yeast for 2 days. Adult flies from both larval diet treatments had access to food ad libitum. This ensured that any changes in adult gene expression could be attributed to larval, rather than adult diet. On the second day, 100 flies of the same sex and same diet treatment were placed in food bottles and allowed to mate with 100 control-mates from a separate outbred stock (Dahomey population collected from West Africa in 1970s) reared on 100% food. This "mating" bottle allowed the experimental flies to recognize and court members of the opposite sex—events that are integral to adult maturation and that significantly alter gene expression (Lawniczak and Begun 2004; McGraw et al. 2004; Mack et al. 2006; McGraw et al. 2008). We visually confirmed that flies from both diet treatments and of both sexes mated; we also confirmed that the newly mated females laid viable eggs. There were four bottle-level replicates of each sex-by-diet treatment. After 24 h, the experimental flies were separated out and their mates were discarded. RNA was extracted from each group of ~100 experimental flies using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer directions.

STATISTICAL ANALYSIS

To reduce noise, the intensity data were not background-corrected (Gibson and Wolfinger 2004). We performed a series of normalizations on the log base 2 intensity measurements to adjust for local, global, and array-specific effects (Quackenbush 2002) using the *limma* package (Smyth and Speed 2003) for R v. 2.7.1 (R Development Core Team 2008). We loess-normalized the intensities by print-tip-group (span = 0.4) and within arrays (span = 0.4) and across arrays with the quantile method. The normalized data were analyzed with PROC MIXED in SAS v. 9 with gene-specific analyses of variance (ANOVAs) of the form:

$$\begin{aligned} Y_{ijklmn} = {} & \mu + \text{Array(Block(Genotype))}_{l(m(n))} \\ & + \text{Block(Genotype)}_{m(n)} + \text{Genotype}_n + \text{Sex}_j + \text{Diet}_k \\ & + (\text{Sex} \times \text{Condition})_{jk} + \text{Dye}_i + \varepsilon_{ijklmn}, \end{aligned}$$

where Y is the normalized expression for a gene labeled with dye i for sex j from condition k for array l, nested in block m, which is nested within genotype n, with residual error ε. Array, block, and genotype were random effects. Sex, diet, and dye were fixed effects. This analysis allows us to assign an expression value to each gene for each treatment cell using the LSMEANS option in PROC MIXED (Gibson et al. 2004; Gibson and Wolfinger 2004; McGraw et al. 2008). The LSMEANS statement extracts the least-squares means, which we used to assess the significance of the sex and diet treatments by using the DIFFS option in PROC MIXED. A gene was considered sexually dimorphic in expression if the least-squares mean difference between males and females was statistically different from zero. This amounts to a t-test and is one method of expressing the extent of sexual dimorphism (Lovich and Gibbons 1992). To take into account multiple testing issues, we applied a false discovery rate (FDR) correction (Storey and Tibshirani 2003). We used a q-value cutoff of 0.01, which means that on average 1% of the genes reported as significant are truly null.

TECHNICAL CONSIDERATIONS

Sexual dimorphism can occur in two ways. First, one sex may express a trait not found in the other sex. Second, each sex may express the same trait but in a different manner. Ideally, we would like to be able to distinguish between these two forms of dimorphism. However, this is difficult due to technical limitations of microarray data, where it is not possible to distinguish zero expression from very low-level expression. We considered genes showing a very large difference in expression between the sexes to be likely to be sex-limited; specifically, we imposed a 10-fold cutoff to the difference of the least-squares means between the sexes (*Female–Male*) to distinguish sex-biased genes (i.e., expressed in both sexes) from sex-limited genes (i.e., expressed in one sex). As sexual dimorphism is usually measured in shared traits with an index requiring male and female measurements (Lovich and Gibbons 1992), we excluded sex-limited genes and analyzed only sex-biased genes. This excluded 192 genes from our final analysis. Although using a high cutoff is a practical way to identify genes that are potentially sex-limited, it is important to recognize that the 10-fold cutoff is an arbitrary distinction. This arbitrariness is evident in the observation that even after applying the cutoff, three genes, which were classified as "sex-biased" under one condition, were classified as were "sex-limited" in the other condition. Nonetheless, the use of such a cutoff at the very least provides a rough distinction between sex-limited versus sex-biased genes. More importantly, our main results remain unchanged whether we exclude genes identified as sex-limited from our analyses, or analyze all genes.

GONAD-SPECIFIC CONDITION-DEPENDENCE

The gonads harbor the majority of the sex-biased genes in the entire body (Parisi et al. 2003, 2004). It is therefore possible that any observed increase in sexual dimorphism under high condition is entirely driven by changes in gonadal gene expression or in the relative contribution of the gonad to the whole body transcript pool. To assess this possibility, we compared the condition-dependence of genes expressed in the gonads to genes expressed only outside of the gonads. We used previously published datasets (Parisi et al. 2003, 2004) to assign genes to one of four tissue types: ovaries, testes, female-soma minus the ovaries ("female-soma"), and male-soma minus the testes ("male-soma"). We used hybridizations that directly compared ovaries to gonadectomized females (GEO accessions: GSM16554, GSM16555, GSM16542, and GSM16550), or testes to gonadectomized males (GSM16569 and GSM16556).

A gene in the Parisi et al. (2004) dataset was considered specific to the gonad or soma if the mean expression difference across arrays met a particular expression cutoff. We used four cutoffs, twofold, fourfold, eightfold, or 16-fold (Figs. 3, 4, S1, and S2). This enabled us to test how the results relied upon the cutoffs employed. We cross-referenced these tissue-assigned genes to the sexually dimorphic genes identified by our study. Based upon the expression values from our study, we then calculated the 95% confidence intervals for genes in each of the expression-by-tissue categories. After tissue-assignment and cross-referencing, some categories did not have any genes remaining; for instance, at the 16-fold cutoff there were no unbiased genes remaining in the testes. This effect occurs because we considered only genes whose expression was exclusive to each tissue type within a sex; there are still unbiased genes expressed in the testes but these are shared between the testes and the male-soma. To maximize the number of genes included in the analysis, we focused on the twofold cutoff, although the three more stringent cutoffs are presented in the Supporting information.

CHARACTERISTICS OF CONDITION-DEPENDENT SEX-BIASED GENES

We looked at whether the extent of condition-dependent sexual dimorphism varied according to genomic location, rate of evolution, and functional categories for the genes identified as sex-biased under either of the two conditions. To test for differences in these features, we constructed an index of condition-dependent sexual dimorphism ($CDSD = |Female_{High} - Male_{High}| - |Female_{Low} - Male_{Low}|$). For positive values, sexual dimorphism is greater under high condition; for negative values, sexual dimorphism is greater under low condition. For $CDSD = 0$, sexual dimorphism does not differ between the conditions. However, it is theoretically possible for a gene to reverse sex-biased expression (e.g., female-biased gene becomes male-biased), thereby making the interpretation of *CDSD* problematic. For instance, equal but opposite reversals in sex-biased expression would also produce $CDSD = 0$. However, no genes statistically identified as sex-biased showed such a reversal between conditions, precluding this issue. We chose *CDSD* to quantify the change in dimorphism over using the estimate of the interaction coefficient from the linear model. This is because the biological interpretation of the interaction coefficient depends on the values of the main effects. By contrast, *CDSD* has a simple sign with a simple interpretation that does not rely upon the main effects.

For genomic location, we calculated the mean *CDSD* according to chromosomal location and male- or female-biased expression. For rates of evolution, we regressed *CDSD* against $\ln(d_N/d_S)$. Chromosomal locations and the pairwise d_N/d_S values (for *D. melanogaster–D. simulans*) were obtained from the Sebida database (Gnad and Parsch 2006). For functional categories, we compared sex-biased genes in the top 25% of our index *CDSD* to sex-biased genes in the bottom 25% of *CDSD* for differences in the Gene Ontology (GO) Biological Processes category. GO Biological Process includes the most obvious terms relevant to sexual selection (e.g., mating and reproduction related functions).

Table 1. Number of sex-biased genes under low and high condition. Gene expression status could remain the same or change between the condition treatments. Values in parentheses indicate the percent of the total number of genes on the array. Sex-limited genes (as defined by our study) are not shown.

	Status under low condition	Status under high condition	Number of genes
Unchanged	Unbiased	Unbiased	7023 (52.7)
	Female-biased	Female-biased	2655 (20.4)
	Male-biased	Male-biased	2219 (17.6)
Lost sex-bias	Female-biased	Unbiased	203 (1.5)
	Male-biased	Unbiased	138 (1)
Gained sex-bias	Unbiased	Female-biased	482 (3.6)
	Unbiased	Male-biased	407 (3.1)

The two-tailed Fisher's exact test provided through FatiGO, Babelomics 2008 (Al-Shahrour et al. 2006) analyzes over- or under-representation in functional terms between any two gene lists using 2×2 contingency tests (FDR-corrected).

Results

CHANGES IN THE NUMBER OF SEX-BIASED GENES

We found strong condition effects on the total number of genes identified as sexually dimorphic in expression. Low-condition flies had fewer genes that were sexually dimorphic than high-condition flies when analyzing the difference of the least-squares means (Table 1). In high-condition flies, 5763 genes show significant sex-biases ($Female_{High}$—$Male_{High}$): 2626 genes with male-biased expression and 3137 genes with female-biased expression. In low-condition flies, only 5215 genes show significant sex-biases ($Female_{Low}$–$Male_{Low}$): 2357 genes with male-biased expression and 2858 genes with female-biased expression. According to this metric, there was a ∼10% increase in the number of genes ($n = 548$) with sex-biased expression in high-condition flies, and this difference was significant ($X^2_{\mathrm{df}=1} = 46.84$, $P < 0.0001$). Although some genes that were identified as sex-biased in low-condition flies lost their bias in high-condition flies, more than twice as many genes acquired sex-biased expression under high condition than lost it ($X^2_{\mathrm{df}=1} = 244.15$, $P < 0.0001$). Yet, diet manipulation did not affect the relative number of male- and female-biased genes in low versus high-condition flies; the ratio of male-biased to female-biased genes did not depend upon treatment ($X^2_{\mathrm{df}=1} = 0.1366$, $P = 0.71$).

The above analysis comparing the number of sexually dimorphic genes in high- and low-condition flies is sensitive to statistical power; a gene was only classified as sexually dimorphic if it had a significant q-value. However, if we had extremely large sample sizes, we would expect that almost all genes would be classified as sexually dimorphic in both conditions because very few genes might be expressed to exactly the same level by both sexes (e.g., increased sample sizes decrease the standard error about the estimate). Thus, we sought to confirm the pattern of more sexually dimorphic genes in high- than low-condition flies without using a statistical definition of sex-biased expression. Rather, we classified genes as sex-biased by setting a minimum threshold expression difference between the sexes. This minimum difference was gradually increased from zero (e.g., all genes were classified as sexually dimorphic) to 3.32 (e.g., only genes with at least a 10-fold difference between the sexes were classified as sexually dimorphic). We then asked whether high- and low-condition flies differed in the number of sexually dimorphic genes at a given threshold value (Fig. 1). Across this range, sex-biased gene number was greater in high- than low-condition flies (except at zero when the numbers are exactly equal).

CHANGES IN THE EXTENT OF SEX-BIASED GENE EXPRESSION

In addition to asking whether condition affects the number of dimorphic genes, we also asked whether condition affects the extent of dimorphism. Using only those genes classified by their q-value as dimorphic in at least one treatment, we performed a one-way ANOVA with the two-level factor condition as the independent variable and sexual dimorphism ($|Female_{High} - Male_{High}|$ or $|Female_{Low} - Male_{Low}|$) as the dependent variable. These analyses included all genes identified as sex-biased in at least one treatment (high or low condition). The average extent of sex-biased expression was greater by ∼10% in high- than low-condition flies ($F_{1,12234} = 72.44$, $P < 0.0001$). This increase occurred independently of the increase in sex-biased gene number; using only those genes that were sex-biased in both treatments we still find that the average extent of sex-bias is greater in high-condition than low-condition flies ($F_{1,9774} = 60.34$, $P < 0.0001$).

Although the absolute female-to-male difference in expression increased among the sex-biased genes, it was unclear if this occurred through expression changes in males, females, or both. We used a two-way ANOVA to quantify the concurrent effects of condition and sex on sex-biased expression. We performed

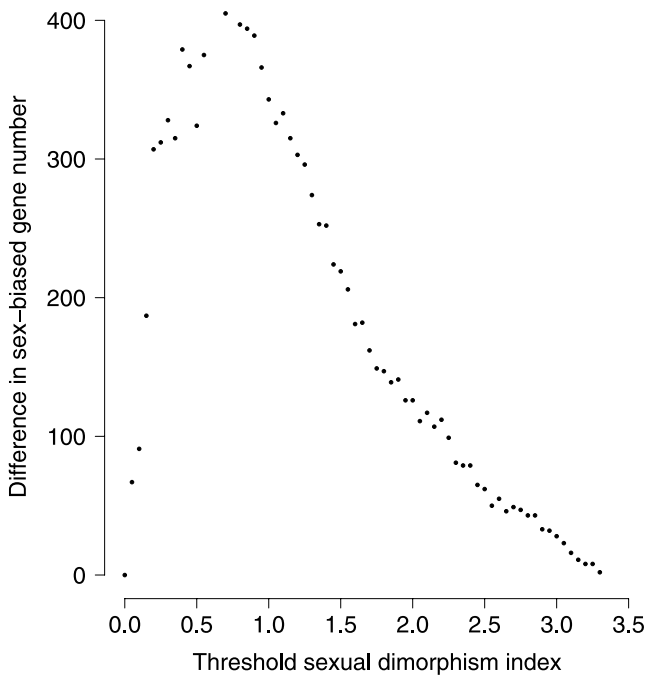

Figure 1. Difference in the number of sexually dimorphic genes in high versus low condition. We calculated the index of sexual dimorphism $D = |Female - Male|$ from the least-squares means for all genes on the microarray, under high and low condition separately. Within each treatment, we calculated $N[x]$ as the number of genes for which the level of dimorphism is greater than x (i.e., $D > x$). This plot shows the difference between treatments in the number of genes meeting a specified threshold level of dimorphism (i.e., $N_{high}[x] - N_{low}[x]$). Across the range of threshold levels (x), the difference is positive. Thus, the number of sexually dimorphic genes is greater under high condition regardless of the threshold level used to classify a gene as dimorphic.

this test separately for male- and female-biased genes. In the first analysis, we used genes identified as biased under either high or low condition. Within male-biased genes, there was a significant condition effect ($F_{1,11052} = 7.0435$, $P = 0.008$) and a significant sex × condition interaction ($F_{1,11052} = 5.3646$, $P = 0.021$; Fig. 2A). Condition increased the expression of male-biased genes more in males than in females, accounting for the significant interaction term. For female-biased genes (Fig. 2C), there is no significant condition effect ($F_{1,13356} = 0.0624$, $P = 0.8026$). However, there was a significant sex × condition interaction ($F_{1,13356} = 4.1467$, $P = 0.0417$); this interaction occurs because of the slight (but nonsignificant) upregulation of female-biased gene expression in females and slight (but nonsignificant) downregulation in males.

Confining our analysis only to those genes with sex-bias under both conditions, we found similar results. There was a significant condition effect ($F_{1,8872} = 7.7983$, $P = 0.0052$) and sex × condition interaction ($F_{1,8872} = 4.2805$, $P = 0.0386$) for the male-biased genes (Fig. 2B); there was no condition effect ($F_{1,10616} = 0.0019$, $P = 0.9657$) or interaction ($F_{1,10616} = 3.3844$, $P = 0.0658$) for the female-biased genes (Fig. 2D).

GONAD-SPECIFIC CONDITION-DEPENDENCE

At the twofold cutoff, we find that male-biased genes expressed in the testes and the soma were more condition-dependent than unbiased genes (i.e., 95% confidence intervals do not overlap). Moreover, male-biased genes expressed in the male-soma responded similarly to diet as those in the testes; unbiased genes also responded similarly between the soma and testes (Fig. 3). These patterns persist at the fourfold cutoff (Fig. S1). At the eightfold cutoff, the male-soma and the testes are still not distinct from each other for their average level of condition-dependence; however, the male-biased genes are no longer distinct from the unbiased genes for condition-dependence (although there is a trend). At 16-fold, the male-soma and testes are again not different with regard condition-dependent male-biased gene expression (Fig. S1).

In contrast to male-biased genes, female-biased genes show some evidence that the average level of condition-dependence differs between the female-soma and the ovaries. At the twofold cutoff, female-biased genes in the ovaries appear to be more condition-dependent than female-biased genes in the female-soma, but this pattern breaks down at the fourfold cutoff (Figs. 4 and S2). And in contrast to the male-biased genes, female-biased genes do not show greater condition-dependence than the unbiased genes on average for a given tissue, regardless of the cutoffs employed (Fig. 4).

In sum, these results suggest that the condition-dependent changes in expression for male biased genes are not entirely due to changes in gonad size. Male-biased genes show increased expression at high condition regardless of whether those genes are expressed mostly in the testes or in the soma. This does not appear to be the case for female-biased genes. Female-biased genes in the ovaries appear to show increased expression under high condition but genes expressed mostly outside the ovaries do not. However, we have less power to make this comparison for female-biased genes than male-biased genes because there are fewer female-biased genes that meet our selection criteria.

SENSITIVITY TO CONDITION

The degree of sex-biased gene expression (for genes with sex-bias under either of the diet treatments) correlated with the degree of condition-dependence (i.e., $CD_{Female} = Female_{High} - Female_{Low}$ and $CD_{male} = Male_{High} - Male_{Low}$). However, the direction and strength of the correlation depended upon the sex in which male-biased (Fig. 5A,C) or female-biased expression (Fig. 5B,D) was measured.

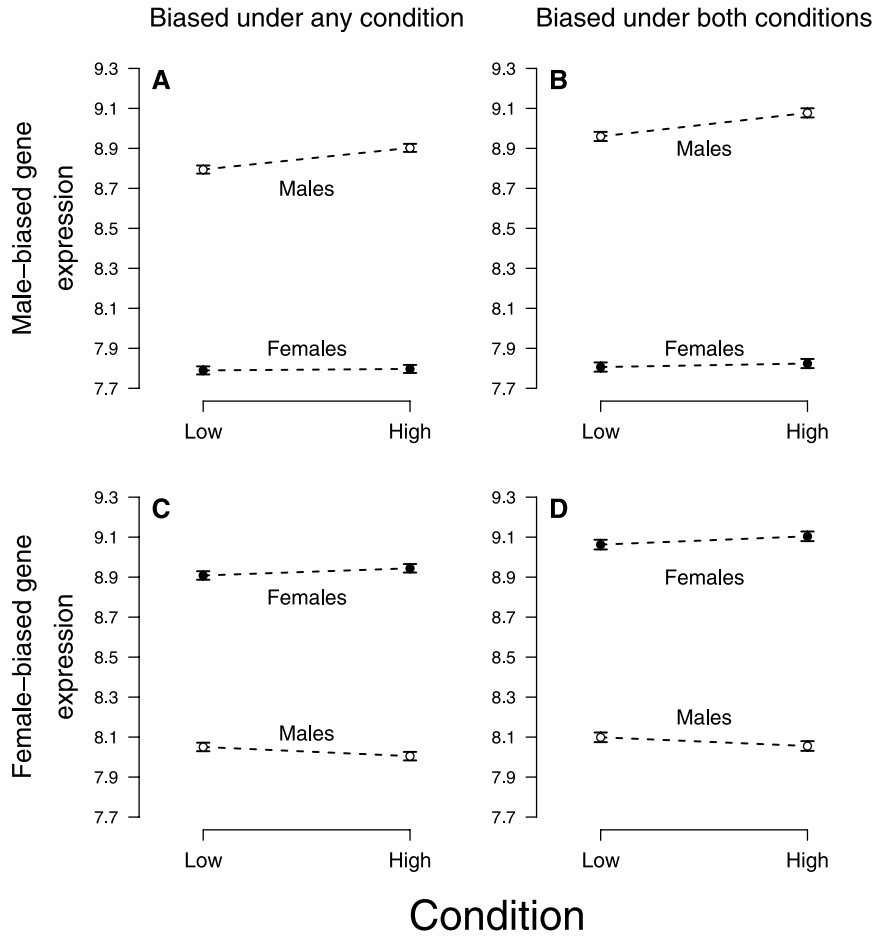

Figure 2. **Condition effects on sex-biased gene expression (log base 2). Sex-biased genes were pooled to assess condition and sex effects on expression. Genes were grouped according to male-biased (panels A and B) and female-biased (panels C and D) expression. Genes were also pooled according to whether they demonstrated sex-biased expression in at least one of the two condition treatments (A, C) or in both treatments (B, D). For both pools of male-biased genes (A, B) there were significant condition and sex × condition effects, resulting from an increase in expression of these genes at high condition in males that was greater in males than females. By contrast, there was no main effect of condition on the expression of female-biased genes in either pool of genes. When considering genes that were female-biased in at least one treatment, we observed a significant sex × condition interaction (C); this interaction occurred because of slight male downregulation and slight female upregulation of female-biased genes. When considering only those genes that were female-biased in both treatments, the interaction was no longer significant.**

The correlation was positive for male-biased genes expressed in males (Fig. 5A; $n = 2764$; $slope = 0.085$, $r^2 = 0.08$, $P < 0.0001$). The correlation was also positive for male-biased genes expressed in females (Fig. 5C; $n = 2764$; $slope = 0.044$, $r^2 = 0.03$, $P < 0.0001$). However, the slope and percent variance explained is greater in males than in females, showing that male-biased genes expressed in males responded more strongly to condition. Our nonparametric analyses (not shown) for these correlations were also significant. Condition-dependence of female-biased genes expressed in females was also an increasing function of sexual dimorphism (Fig. 5D; $n = 3340$; $slope = 0.054$, $r^2 = 0.02$, $P < 0.0001$). By contrast, female-biased genes expressed in males decreased with increasing sexual dimorphism (Fig. 5B; $n = 3340$; $slope = -0.0402$, $r^2 = 0.02$, $P < 0.0001$). Thus, although condition explains the same amount of variance in the extent of female-biased expression for both sexes, the sign of the correlation differs.

For each of regressions listed above, a component of the X variable is present in the Y variable (e.g., in Fig. 5A both X and Y variables contain the value $Male_{High}$ to calculate the difference in the least-squares means). It is possible that such reiterations can result in spurious significant correlations (Jackson and Somers 1991). To test this possibility, we performed a randomization test (for details, see Supporting Information). We find that the observed slopes in Figure 5 always fall well outside the distribution of the permuted slopes (Fig. S3). We conclude that these significant regressions (Fig. 5) are not spurious.

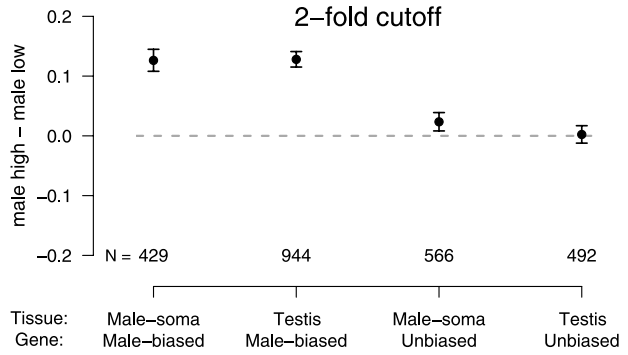

Figure 3. Extent of condition-dependence in the testes and male-soma. Condition-dependence (mean ± 95% confidence intervals) is defined as $Male_{High} - Male_{Low}$. Values differ if 95% CI do not overlap among groups. A gene from the Parisi et al. (2004) dataset was assigned to either the testes or the male-soma if it showed at least a twofold difference in expression between the two tissues (see text for details). Male-biased genes expressed in the testes and the male-soma share a similar degree of condition-dependence. This was true when the threshold specificity was increased (see Fig. S1).

Overall, these results show that sex-biased gene expression's sensitivity to condition depended upon the degree of sexual dimorphism as well as upon the sex in which they were expressed. Finally, male-biased genes were more sensitive to condition than female-biased genes regardless of the sex in which they were expressed.

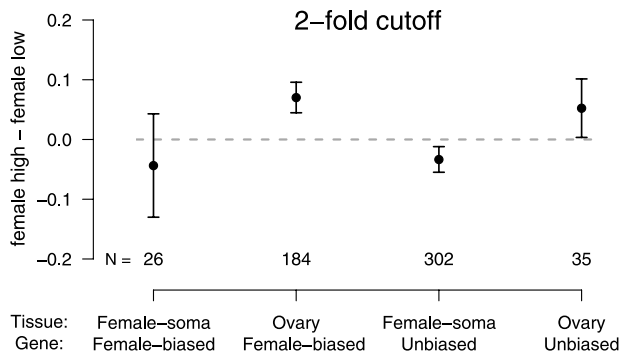

Figure 4. Extent of condition-dependence in the ovaries and female-soma. Condition-dependence (mean ± 95% confidence intervals) is defined as $Female_{High} - Female_{Low}$. Values differ if 95% CI do not overlap among groups. A gene from the Parisi et al. (2004) dataset was assigned to either the ovaries or the female-soma if it showed at least a twofold difference in expression between the two tissues (see text for details). At the twofold cutoff, the ovaries and female-soma seem to differ slightly in their degree of condition-dependence. However, this relationship breaks down at higher thresholds (see Fig. S2). Female-biased genes do not seem more condition-dependent than the unbiased genes within a particular tissue type or within a given fold cutoff.

CHARACTERISTICS OF CONDITION-DEPENDENT SEX-BIASED GENES

The autosomes and X chromosome differ slightly in their average extent of male-biased and female-biased gene expression (Parisi et al. 2003). We tested whether this difference in the extent of sex-bias was related to a difference in the degree of *CDSD*. At a twofold cutoff to distinguish soma-specific versus gonad-specific genes, we did not find differences in *CDSD* between chromosome types (i.e., 95% confidence intervals did not overlap). This lack of a chromosome effect occurred whether we took into account tissue of expression (Fig. 6). Interestingly, female-biased genes expressed in the female-soma show a lower degree of *CDSD* than sex-biased genes in the other categories.

Condition-dependent sexual dimorphism may facilitate or hamper the rate of adaptive evolution. To test these alternatives, we regressed $\ln(d_N/d_S.)$ against *CDSD*. We removed genes whose $d_N/d_S > 2$ to correct for saturation effects. The correlation was negative in female-biased genes ($n = 2832$; $slope = -0.44$; $r^2 = 0.004$, $P = 0.0005$) but positive in male-biased genes ($n = 2375$; $slope = 0.45$; $r^2 = 0.0049$, $P = 0.0004$). We removed 293 female-biased genes and 140 male-biased genes with extremely small d_N/d_S values. However, the correlations were significant even when including these outliers. Nonparametric rank tests also showed significant correlations for male- and female-biased genes. However, these correlations are weak and the percent variance in $\ln(d_N/d_S)$ explained by the index of condition-dependence is small.

Because sex-biased genes varied in their degree of *CDSD*, we tested for differences in functional enrichment between sex-biased genes in the top versus bottom quartile of *CDSD*. We found GO Biological Process terms within the male-biased genes that were enriched in the bottom quartile of *CDSD*; no terms directly related to male reproduction were enriched in the top quartile of *CDSD*. In the female-biased genes, we found that genes in the top quartile of *CDSD* were enriched for terms related to sexual reproduction and gametogenesis (Table 2).

Discussion

The degree of sex-biased expression can evolve within populations and species (Meiklejohn et al. 2003; Connallon and Knowles 2005; Baker et al. 2007; Zhang et al. 2007) and may correlate to the intensity of sex-specific selection (Reinius et al. 2008). Here we show that variation in condition can induce substantial variation in sexually dimorphic patterns of gene expression. Individuals reared under high condition had more male-biased gene expression, female-biased gene expression, and total sex-biased gene expression than individuals reared under low condition. These results corroborate the morphological data demonstrating

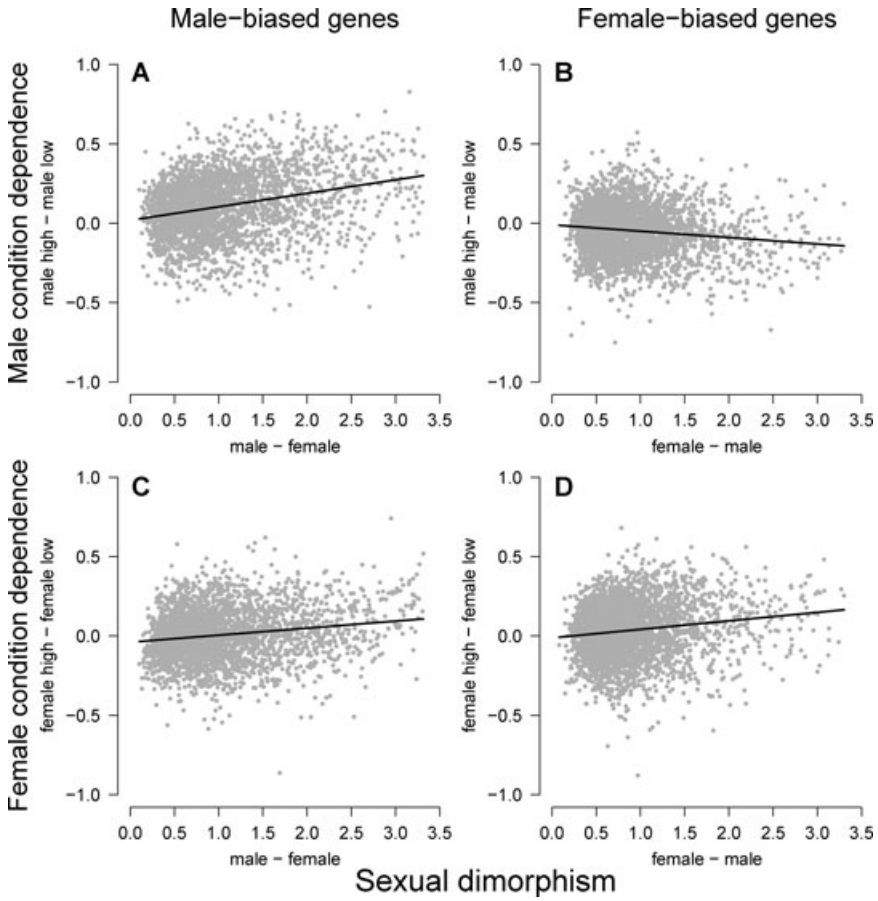

Figure 5. Extent of sex-biased expression and condition-dependence within each sex. The extent of sex-biased expression was correlated to the extent of condition-dependence. The direction and strength of the correlation relied upon the sex in which it was measured. Male-biased genes expressed in males (A) and in females (C) had a strong positive correlation. Female-biased genes expressed in females had a positive correlation (D). However, female-biased gene expressed in males had a negative correlation (B).

that condition can modulate the degree of sexual dimorphism (Bonduriansky and Rowe 2005; Bonduriansky 2007).

CONDITION-DEPENDENT MALE-BIASED GENE EXPRESSION

Condition-dependent male-biased gene expression assumed two forms in this study. First, condition affected the total number of detectable male-biased genes. Second, high-condition males increased expression of genes that were already male-biased under low condition (Figs. 2 and 3), resulting in a significant condition and sex × condition interaction. Even though there is no direct evidence that male-biased genes are generally under sexual selection, there is evidence that sex-specific selection can shape sex-biased gene expression (Zhang et al. 2004; Reinius et al. 2008). Male-biased genes bear the hallmarks of traditional sexually selected traits (Darwin 1871; Andersson 1994), evolving rapidly and showing strong divergence between closely related lineages (Meiklejohn et al. 2003; Ranz et al. 2003; Zhang et al. 2004, 2007). Insofar as their total number and extent of bias are condition-dependent, male-biased genes appear similar to other classic sexually selected traits in yet another respect.

As expected, male-biased genes were more condition-dependent than unbiased genes in the subset analyzed in the testes and male-soma (Fig. 3). Moreover, the correlation between degree of male-biased expression and degree of condition-dependence was significantly positive in males (Fig. 5A); genes with greater male-bias were more sensitive to condition. However, at present it is unclear if these patterns result because extremely male-biased genes impose greater costs (Rowe and Houle 1996), or for some unrelated reason. Curiously, these same male-biased genes expressed in females also showed a highly significant positive correlation between degree of male-biased expression and female condition-dependence (Fig. 5C), but the correlation was not as strong. Such a correlation may indicate unresolved intralocus conflict. Connallon and Knowles (2005) have shown that *D. melanogaster* male-biased genes are significantly over-represented for genes that are upregulated in both

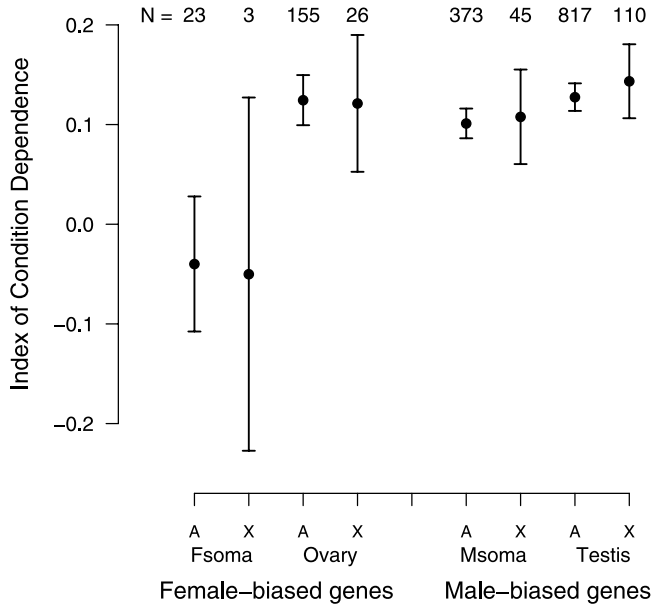

Figure 6. Condition-dependence of sexual dimorphism (*CDSD*) on the X and autosomal chromosomes. *CDSD* is defined as $|Female_{High} - Male_{High}| - |Female_{Low} - Male_{Low}|$. Male- and female-biased genes are defined as those that were identified as biased in either of the condition treatments (see Table 1). Tissue location was based upon a twofold cutoff between the gonad and soma within each sex. Means are significantly different if 95% confidence intervals do not overlap. Among female-biased genes, there was no difference in *CDSD* among the chromosome types regardless of their expression in the female-soma or ovaries. There was no difference in *CDSD* among male-biased genes regardless of their chromosomal location or tissue as well (Fsoma = female-soma; Msoma = male-soma).

males and females relative to unbiased genes. When male-biased genes evolve increased expression in males, increased expression also occurs in females. So condition-dependence may itself have a high intersexual correlation (Bonduriansky and Rowe 2005), causing the response in females.

Our observation of greater expression of male-biased genes under high condition could simply reflect the relatively larger contribution of the testes to whole-body sex-biased gene expression under high condition. Overall, male-biased gene expression is known to be greater in the testes than in the rest of the male-soma (Parisi et al. 2004). Moreover, testes size has a positive allometry with body size in *Drosophila* (Pitnick 1996; Bangham et al. 2002). However, we found that male-biased genes expressed mostly outside of the testes show the same elevated level of condition-dependence as genes expressed mostly in the testes (Fig. 3). Although the testes may make a larger contribution to the total expression pool under high condition, this effect alone cannot explain the heightened expression of male-biased genes.

CONDITION-DEPENDENT FEMALE-BIASED GENE EXPRESSION

There was evidence that female-biased genes were condition-dependent. High-condition females had more female-biased genes than low-condition females. However, unlike for the male-biased genes, the extent of female-biased gene expression was only weakly affected by condition. For instance, although female-biased genes had a significant sex × condition interaction (Fig. 2C), there was no significant diet effect. High-condition females do not significantly increase expression of female-biased genes; rather, slight female upregulation and slight male downregulation of female-biased genes accounts for the interaction. Furthermore, the interaction disappears when we consider only genes with female-biased expression under both condition treatments (Fig. 2D). This suggests that overall, female-biased gene expression in females shows greater resilience to variation in condition.

The positive correlation between the extent of female-bias and condition-dependence in females while significant (Fig. 5D) was weaker than the correlation between the extent of male-bias and condition-dependence in males (Fig. 5A). This positive correlation nonetheless suggests that genes with greater female-bias are more sensitive to condition. By contrast, the correlation for female-biased genes expressed in males was significantly negative (Fig. 5B). Relative to low-condition males, high-condition males appear to downregulate the most strongly female-biased genes. High-condition males appear to be more masculine in male-biased gene number and expression while also being less feminine in female-biased gene expression, consistent with previous morphological work (Bonduriansky and Rowe 2005; Bonduriansky 2007). Finally, unlike for the male-biased genes, we were not able to rule out the effect of positive allometry on increases in female-biased gene expression. At the twofold cutoff, the female-biased genes in the ovaries appear to be more condition-dependent than the female-biased genes in the female-soma (Fig. 4). Unbiased genes in the ovaries are also more condition-dependent than unbiased genes in the female-soma; in fact, unbiased genes in the female-soma show decreased expression under high condition. Altogether, this suggests that the overall increase in female biased gene expression at high condition may be due to a larger contribution of the ovaries to the total expression pool. However, at the fourfold cutoff, these patterns disappear and both female-biased and unbiased genes expressed in either the ovaries or female-soma are not condition-dependent (Fig. S2). Thus, the positive allometry interpretation for increased female-biased gene expression under high condition has some support but it remains inconclusive.

Female-biased genes may serve as the genomic analogues of life history traits. Phenotypic life history traits rely heavily upon condition and can experience strong directional selection

Table 2. Gene Ontology Biological Process. We compared sex-biased genes in the top 25% (T) of our index *CDSD* to sex-biased genes in the bottom 25% (B). This test looks for over- and under-representation in functional categories between gene lists. The sign indicates the list that is enriched for terms relative to the other list.

Gene Ontology (GO) Biological Process		Enriched gene list	Adjusted *P*-value
Female-biased genes			
Level 3			
GO:0048869	Cellular developmental process	T>B	0.0021
GO:0019953	Sexual reproduction	T>B	0.0069
Level 4			
GO:0030154	Cell differentiation	T>B	0.0019
GO:0007276	Gametogenesis	T>B	0.0434
Level 5			
GO:0048468	Cell development	T>B	0.0112
Level 6			
GO:0022607	Cellular component assembly	T>B	0.0266
Male-biased genes			
Level 3			
GO:0050789	Regulation of biological process	T<B	0.0158
GO:0048869	Cellular developmental process	T<B	0.0469
Level 4			
GO:0009653	Anatomical structure morphogenesis	T<B	0.0417
GO:0009790	Embryonic development	T<B	0.0417
GO:0030154	Cell differentiation	T<B	0.0417
GO:0046903	Secretion	T<B	0.0417

without appearing to evolve, for example, breeding date in birds (Price et al. 1988). In a corresponding manner, condition can affect the extent of female-biased gene expression in females (Figs. 4 and 5D) and exhibits signatures of evolutionary conservation compared to male-biased genes (Zhang et al. 2004). In support of this interpretation, we found that female-biased genes in the top quartile of *CDSD* are relatively enriched for genes involved in reproduction and gametogenesis (Table 2).

CONDITION-DEPENDENT SEXUAL DIMORPHISM

Our results from the transcriptome demonstrate that sexual dimorphism itself is condition-dependent, corroborating earlier more limited studies of condition-dependence in phenotypic sexual dimorphism. In contrast to prior studies, which were confined to a few selected morphological phenotypes (David et al. 2000; Cotton et al. 2004a,b; Bonduriansky and Rowe 2005; Bonduriansky 2007; Boughman 2007; Punzalan et al. 2008), our study is both much greater in scope and less biased in its selection of traits. High condition increased the total number of sex-biased genes by several hundred genes. And although genes could lose sex-biased expression under high condition, overall, there were many more gains than losses of sex-biased expression. High condition also increased the overall expression difference between the sexes by ~10%.

This work emphasizes that sex-biased gene expression is plastic. Perhaps the lack of selective constraint on sex-biased genes has facilitated both sex-biased gene expression and its sensitivity to condition. Yet, if condition-dependence were simply an outcome of the general flexibility in expression of sex-biased genes, the greater sexual dimorphism in high condition is not expected. Rather one might expect variance in condition to introduce random variance in sex-biased gene expression (e.g., as many genes being dimorphic under high condition as low condition). Furthermore, we found only very weak evidence that condition-dependent sex-biased genes are either free from selective constraint, or experience evolutionary constraint, as measured by d_N/d_S. Finally, *CDSD* did not demonstrate any unique genomic patterns across chromosomes (Fig. 6). There is no evidence that X-linked *CDSD* is stronger or weaker than autosomal *CDSD*. However, female-biased genes expressed in the female-soma have the lowest degree of *CDSD* compared to the other gene categories. The reasons for this pattern are unclear.

There have been several previous attempts to connect changes in gene expression to diet manipulation in *D. melanogaster* (Pletcher et al. 2002; Zinke et al. 2002; Carsten et al. 2005; Harbison et al. 2005; McGraw et al. 2007); two pertain to our study. Harbison et al. (2005) found that differences in current adult nutrition affected 12% of all sexually dimorphic genes—confirming that when organisms are tested at the same developmental stage as the diet treatment, gene expression changes are common (Endo et al. 2002; Pletcher et al. 2002; Zinke et al. 2002; Carsten et al. 2005). Our study differs from Harbison et al.'s

(2005) because we manipulated larval diet and measured changes in adult gene expression (keeping adults fed ad libitum). Our approach corresponds more closely to the life history definition of condition (Rowe and Houle 1996), and is not confounded by short-term responses to feeding. Like our study, McGraw et al.'s (2007) reared larvae on different nutrient levels and tested the condition-dependence of adult gene expression. Their study focused on nine male-limited accessory gland genes (i.e., proteins present in seminal fluids), of which only one had detectable condition-dependent expression in adult males. By applying a cutoff to remove potentially sex-limited genes, our study shows that condition also more broadly affects the sex-biased genes.

Conclusions

It remains a challenge to decipher the relative importance of natural and sexual selection on sex-biased genes. There is much underlying variation that requires further study. Why are genes with greater sex-bias more condition-dependent? Theory predicts that the optimal level of condition-dependence will depend on the relationship between expression level and its costs and benefits (Rowe and Houle 1996; Houle 1998). Does the observed variation in condition-dependence reflect variation in these relationships or is it simply due to gene-specific constraints on sensitivity to condition? Addressing these questions will require a more detailed understanding of the function of these genes and the extent of selection and constraint on their expression.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Additional thresholds to define tissue specificity in male gene expression.

Figure S2. Additional thresholds to define tissue specificity in female gene expression.

Figure S3. Permutation analyses of regression slopes to test for bias.

Supporting Information may be found in the online version of this article.

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