Sex-Biased Gene Expression and Sexual Conflict throughout Development

Fiona C. Ingleby, Ilona Flis, and Edward H. Morrow

Evolution, Behaviour and Environment Group, School of Life Sciences, University of Sussex, John Maynard Smith Building, Falmer, Brighton BN1 9QG, United Kingdom

Correspondence: f.ingleby@sussex.ac.uk

Sex-biased gene expression is likely to account for most sexually dimorphic traits because males and females share much of their genome. When fitness optima differ between sexes for a shared trait, sexual dimorphism can allow each sex to express their optimum trait phenotype, and in this way, the evolution of sex-biased gene expression is one mechanism that could help to resolve intralocus sexual conflict. Genome-wide patterns of sex-biased gene expression have been identified in a number of studies, which we review here. However, very little is known about how sex-biased gene expression relates to sex-specific fitness and about how sex-biased gene expression and conflict vary throughout development or across different genotypes, populations, and environments. We discuss the importance of these neglected areas of research and use data from a small-scale experiment on sex-specific expression of genes throughout development to highlight potentially interesting avenues for future research.

As most of the genome is shared between males and females, differences in gene expression between the sexes are thought to underlie much of the sexual dimorphism observed in nature (Ellegren and Parsch 2007). These sex-biased genes are either expressed in one sex only (sex specific or sex limited) or expressed more strongly in one sex than the other (sex enriched). Patterns of sex-biased gene expression are widely documented and are often associated in the literature with sexual conflict (e.g., Connallon and Knowles 2006; Ellegren and Parsch 2007). The rationale behind this is based on two conceptual premises. First, sexual conflict occurs when fitness optima differ between the sexes for a shared trait, generating sexually antagonistic selection that will favor trait evo-

lution in opposite directions between the sexes. Second, sex differences in gene expression could potentially alleviate such conflict by facilitating the evolution of sexual dimorphism.

A common assumption made, however, is that sexual dimorphism can be used as an indicator or proxy of ongoing sexual conflict, and at present, this has not been extensively studied empirically. Crucially, very few empirical studies have measured both gene expression levels and sex-specific fitness, and as a result, we have a poor understanding of how gene expression might mediate conflict. Here, we review the body of research documenting genome-wide patterns of sex-biased gene expression across a range of species, and we highlight the need for more studies that measure sex-specific fitness as

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well as gene expression. We argue that only by considering both of these aspects can patterns of sex-biased gene expression be interpreted within the context of sexual conflict.

In addition, very little is known about the progression of sex-biased gene expression throughout development and how these changes might relate to sex-specific fitness at each stage (Ellegren and Parsch 2007). Sexual dimorphism generally increases throughout development, and it has been suggested that the relationship between sexual dimorphism and sexual conflict might vary throughout ontogeny as well. Theory predicts that conflict will be strongest as the sexes differentiate and the evolutionary interests of males and females diverge (Badyaev 2002; Cox and Calsbeek 2009), yet empirical studies addressing this idea are lacking. Developmental studies on model organisms have provided in-depth examination of development (e.g., Arbeitman et al. 2002) and of the sex-specific expression of genes that are involved with particular developmental pathways of interest (Rinn and Snyder 2005), but ontogenetic studies of genome-wide sex-biased gene expression are few. We therefore consider studies of genome-wide sex-biased gene expression throughout development and present the results of a small-scale experiment on this subject.

Finally, we discuss how future research on sexual conflict could benefit from studies that bring together analysis of sex-biased gene expression, changes in the transcriptome throughout development, and sex-specific fitness to give a more comprehensive understanding of the relationship between sex-biased gene expression and sexually antagonistic selection.

QUANTIFYING SEX-BIASED GENE EXPRESSION

Genome-wide sex-biased gene expression has been studied in a wide range of species, thanks in large part to the increasing availability of high-throughput transcriptomic techniques such as microarrays and RNA sequencing (Ellegren and Parsch 2007). The research summarized in Table 1 gives some indication of the

breadth of species and questions that have been studied. Whereas a lot of these studies focus on model species, it is clear that this area of research spans a diverse range of animal phyla and that high-throughput transcriptomic techniques are beginning to be applied to nonmodel species.

Perhaps unsurprisingly, there is no general consensus as to what extent the transcriptome is sex biased, and research so far has shown wide variation between species. At one end of the scale, for example, only about 2% of the transcriptome of the marine snail *Littorina saxatilis* was found to be sex biased (Martinez-Fernandez et al. 2010), whereas in *Drosophila melanogaster*, sex-biased expression for as much as 90% of transcripts has been shown (Ayroles et al. 2009; Innocenti and Morrow 2010; Wayne et al. 2012). More interestingly, the extent of sex bias can vary even between studies of the same species. There are a number of potential sources of this variation, including, but not limited to, tissue specificity of gene expression, developmental stage, intraspecific genetic and environmental variation, and the experimental design and analytical techniques specific to each study. Table 1 summarizes some of the studies that have examined each of these sources of variation.

First, tissue-specific sex-biased expression has been examined in several species. For example, in mice, patterns of sex-biased gene expression vary between different tissues (Rinn et al. 2004; Yang et al. 2006). In zebrafish, *Danio rerio*, sex bias in the transcriptome of the brain tissue was found to be quite low (~2%) (Santos et al. 2008), but a different study identified sex biases across 38% of the transcriptome that were attributed to differences in gene expression in male and female reproductive tissues (Small et al. 2009). Similarly, in the silkworm *Bombyx mori*, sex-biased gene expression varied between 2% and 30%, depending on the tissue examined (Xia et al. 2007). A number of studies have only looked at a particular tissue of interest (e.g., Malone et al. 2006; Scholz et al. 2006; Grosse-Wilde et al. 2011; Naurin et al. 2011; Pitts et al. 2011), and so to what extent the sex-biased gene expression identified in these studies is repre-

Table 1. Examples of studies with original data on sex-biased patterns of genome-wide gene expression

Species	Developmental stage(s)	Experimental design	Criteria for sex bias	Evidence for sex-biased gene expression	References
Mammals					
<i>Mus musculus</i>	Embryos	Microarray (5m + 5f)	$P < 0.05$ -fold change > 1.1	$< 1\%$ of transcripts showed sex-biased gene expression in brain tissue of embryos before gonadal formation	Dewing et al. 2003
<i>M. musculus</i>	Adults	Microarray (3m + 3f)	$P < 0.001$ -fold change > 3	Sex-biased gene expression in kidney, liver, and reproductive tissues	Rinn et al. 2004
<i>M. musculus</i>	Adults	Microarray (165m + 169f)	$P < 0.01$	13%–70% of transcripts were sex biased, depending on the tissue	Yang et al. 2006
<i>M. musculus</i>	Adults	(114m + 93f)	$P < 0.05$	31% of eQTLs showed sex-biased expression	Bhasin et al. 2008
Primates ^a	Adults	RNA-seq (3m + 3f per species)	$P < 0.05$	Sex-specific gene expression and splicing and enrichment of sex-biased genes with RNA splicing functions	Blekman et al. 2010
Fish					
<i>Danio rerio</i>	Adults	Microarray (3m + 3f)	Fold change	$< 1\%$ of transcripts showed female-biased expression in both germline and somatic tissue	Wen et al. 2005
<i>D. rerio</i>	Adults	Microarray (6m + 5f)	$P < 0.05$ -fold change > 1.2	$\sim 2\%$ of transcripts in the adult brain tissue were sex biased	Santos et al. 2008
<i>D. rerio</i>	Adults	Microarray (3m + 3f)	$P < 0.05$	38% of transcripts were sex biased (all evidence of sex bias was attributable to differences between reproductive tissues)	Small et al. 2009
<i>Gasterosteus aculeatus</i>	Adults	Microarray (27m + 8f)	$P < 0.05$	$\sim 10\%$ of transcripts were sex biased, concentrated on sex chromosomes	Leder et al. 2010
<i>Poecilia reticulata</i>	Juveniles	RNA-seq (3m + 3f)	$P < 0.05$	Sex-biased gene expression in juvenile brain and whole-body samples	Fraser et al. 2011
Birds					
<i>Gallus gallus</i>	Embryos	Microarray (12m + 12f)	$P < 0.0005$	Sex-biased gene expression (mostly male biased) in embryo brain tissue; sex biases present before gonad differentiation	Scholz et al. 2006

Continued

Table 1. Continued

Species	Developmental stage(s)	Experimental design	Criteria for sex bias	Evidence for sex-biased gene expression	References
<i>G. gallus</i>	Early embryo, late embryo, and adults	Microarray (3m + 3f per stage)	$P < 0.05$ -fold change > 2	Sex-biased gene expression in the gonads increased throughout development from ~10% to ~50% of transcripts	Mank et al. 2010
Passerines ^b	Adults	Microarray (6–12m + 6–12f per species)	$P < 0.03$	~2% sex-biased gene expression in the brain tissue of each species, mostly male biased	Naurin et al. 2011
Amphibians					
<i>Xenopus</i> spp. ^c	Adults	Microarray (~4m + 4f per species)	$P < 0.05$	Extensive male-biased expression in gonad tissue of both species, with ~70% of the transcriptome showing sex bias	Malone et al. 2006
<i>Drosophila</i> spp.					
<i>Drosophila</i> spp. ^d	Adults	Microarray (3m + 3f per species)	$P < 0.03$	Many genes with divergent expression between species were also sex biased	Ranz et al. 2003
<i>D. spp.</i> ^e	Adults	Microarray (min. 4m + 4f per species)	$P < 0.01$	Patterns of sex-biased (especially male biased) gene expression have diverged between closely related <i>Drosophila</i> species	Zhang et al. 2007
<i>D. spp.</i> ^f	Adults	Microarray (3m + 3f)	$P < 0.01$	~80% of the transcriptome was sex biased in each species, and $> 80\%$ of expression divergence between species was because of sex-specific expression changes	Jiang and Machado 2009
<i>D. melanogaster</i>	Adults (two age classes)	Microarray (12m + 12f across two genotypes)	$P < 0.0001$	Variation in gene expression between sexes, genotypes, and sex x genotype interaction (7% of genes were sex biased)	Jin et al. 2001
<i>D. melanogaster</i>	Larvae, pupae, and adults	Microarray (2m + 2f)	$P < 0.01$ -fold change > 2	A small number of somatic transcripts showed sex-biased expression	Arbeitman et al. 2002
<i>D. melanogaster</i>	Adults	Microarray (2m + 2f)	Fold change > 2	Extensive sex-biased gene expression, with underrepresentation of male-biased genes on the X chromosome	Parisi et al. 2003
<i>D. melanogaster</i>	Adults	Microarray (32m + 32f across eight lines)	$P < 0.05$	~50% of transcripts showed sex-biased expression, and there was evidence of sex-specific splicing patterns	McIntyre et al. 2006

Continued

Table 1. Continued

Species	Developmental stage(s)	Experimental design	Criteria for sex bias	Evidence for sex-biased gene expression	References
<i>D. melanogaster</i>	Adults	Microarray (80m + 80f across 40 lines)	$P < 0.001$	~88% of the transcriptome was sex biased, and there was sex-specific variation in multiple fitness-related traits	Ayroles et al. 2009
<i>D. melanogaster</i>	Adults (two female age classes)	Microarray (8m + 8f per time point)	Fold change > 2	~50% of transcripts showed sex-biased expression (mostly male biased), which varied throughout female oogenesis	Baker and Russell 2009
<i>D. melanogaster</i>	Adults	RNA-seq (2m + 2f)	Fold change > 2	~56% of transcripts were sex biased in wild-type flies, and evidence for sex-specific splicing and chromatin structure in adult gonad tissue	Gan et al. 2010
<i>D. melanogaster</i>	Adults	Microarray (60m + 60f across 15 lines)	$P < 0.001$	~90% of transcripts showed sex-biased expression, whereas 8% of the transcriptome was under sexually antagonistic selection	Innocenti and Morrow 2010
<i>D. melanogaster</i>	Adults	Microarray (24m + 24f across two genotypes)	$P < 0.01$	Evidence for sex-biased gene expression was stronger in adults from a high-quality diet than in adults from a low-quality diet	Wyman et al. 2010
<i>D. melanogaster</i>	Adults	RNA-seq (6m + 6f)	$P < 0.05$	~17% of transcripts showed sex-biased expression, and there was evidence of sex-specific splicing	Chang et al. 2011
<i>D. melanogaster</i>	Adults (pooled-sex larval and pupal samples)	RNA-seq and microarray (3m + 3f)	$P < 0.001$	~30% of transcripts were sex-biased across the adult transcriptome, with more male- than female-biased expression	Graveley et al. 2011
<i>D. melanogaster</i>	Adults	RNA-seq (4m + 4f)	$P < 0.05$	Low levels of sex-biased gene expression in the brain tissue; X chromosome was enriched with male-biased genes	Catalan et al. 2012
<i>D. melanogaster</i>	Adults	Microarray (2m + 2f per genotype)	$P < 0.001$	~90% of the transcriptome showed sex-biased expression, and there were sex-specific patterns of inheritance of gene expression	Wayne et al. 2012
<i>D. melanogaster</i>	Larvae and prepupae	RNA-seq (3m + 3f)	$P < 0.05$ -fold change > 2	$> 50\%$ of transcripts in larvae and prepupae showed sex-biased expression in the pregonad tissue	Perry et al. 2014
Other insects					
<i>Anopheles gambiae</i>	Adults	Microarray (3m + 3f)	$P < 0.001$	22% of transcripts were sex biased and changes in gene expression were also observed after females had a bloodmeal	Marinotti et al. 2006

Continued

Table 1. Continued

Species	Developmental stage(s)	Experimental design	Criteria for sex bias	Evidence for sex-biased gene expression	References
<i>A. gambiae</i>	Adults	Microarray (4m + 4f)	$P < 0.05$ -fold change > 2	54% of transcripts were sex biased, and adult gene expression was tissue specific	Baker et al. 2011
<i>A. gambiae</i>	Larvae, pupae, and adults	Microarray (3m + 3f per stage)	Fold change > 1.7	Widespread sex-biased gene expression throughout development (1%–28% sex bias from larvae to adults)	Magnusson et al. 2011
<i>A. gambiae</i>	Adults	RNA-seq (3m + 3f)	Fold change	Sex-biased gene expression and evidence for tissue specificity of sex bias in chemosensory tissue	Pitts et al. 2011
<i>Bombyx mori</i>	Larvae	Microarray (2m + 2f minimum)	$P < 0.01$	2%–30% of transcripts were sex biased, depending on the tissue, with variation between tissues in the extent of male and female bias	Xia et al. 2007
<i>B. mori</i>	Larvae and pupae	Microarray (2m + 2f minimum)	Fold change > 2	Sex-biased expression increased throughout development (from ~2%–13%), with more male bias than female bias, and enrichment of sex-biased genes on the sex chromosomes	Zhao et al. 2011
<i>Manduca sexta</i>	Adults	Microarray (4m + 4f)	$P < 0.05$	More evidence of female-biased than male-biased gene expression in antennae tissue	Grosse-Wilde et al. 2011
<i>Solenopsis</i> spp. ^g	Pupae and adults	Microarray (five replicates per caste per stage)	$P < 0.001$	~20% of genes were sex biased between castes (males, workers, and queens), with more male-biased genes than others, and variation in caste-biased expression between stages	Ometto et al. 2011
<i>Tribolium castaneum</i>	Adults	Microarray (4m + 4f)	$P < 0.01$	~20% of the transcriptome was sex biased, with enrichment of female-biased genes on the X chromosome	Prince et al. 2010
Nematodes					
<i>Brugia malayi</i>	Adults	RNA-seq (no replication)	$P < 0.01$	Enrichment of sex-biased expression in the germline, and ~20% of transcripts overall showed sex bias	Choi et al. 2011
<i>Caenorhabditis elegans</i>	Multiple larval stages and adults	Microarray (4m + 4h)	$P < 0.05$ -fold change > 1.5	Differential gene expression between males and hermaphrodites throughout development in ~10% of the transcripts studied	Jiang et al. 2001
<i>C. elegans</i>	Multiple larval stages and adults	Microarray (4m + 4h per stage)	$P < 0.01$ -fold change > 2	~1% of transcripts differed in expression between males and hermaphrodites throughout development in the somatic and germline tissue	Reinke et al. 2004

Continued

Table 1. Continued

Species	Developmental stage(s)	Experimental design	Criteria for sex bias	Evidence for sex-biased gene expression	References
<i>C. elegans</i>	Multiple larval stages	Microarray (4m + 4h per stage)	$P < 0.01$ -fold change > 2	More male-biased than hermaphrodite-biased gene expression, and an increase in differential gene expression throughout larval development	Thoenike et al. 2005
Platyhelminthes					
<i>Schistosoma mansoni</i>	Adults	Microarray (5m + 5f)	Fold change	Evidence for low levels of sex-biased gene expression in adults	Fitzpatrick et al. 2005
Crustaceans					
<i>Daphnia pulex</i>	Adults	Microarray (8m + 8f)	$P < 0.05$ -fold change > 2	50% of assayed transcripts showed sex bias between adult males and females	Eads et al. 2007
Molluscs					
<i>Littorina saxatilis</i>	Adults	cDNA-AFLP (6m + 6f)	$P < 0.05$	1.8% of transcripts showed sex-biased gene expression, along with low levels of phenotypic sexual dimorphism	Martinez-Fernandez et al. 2010

This summary excludes studies which reanalyze previous data or focus only on a subset of genes. Because of the different terminology used to refer to sex-biased gene expression, this list is unlikely to be exhaustive, but it gives a good indication of the range of species studied, the widespread evidence for sex-biased gene expression, and the increase in research in this area in recent years. Studies that have examined males and females from more than one developmental stage are highlighted in dark gray, and studies that have provided data giving some indication of how gene expression relates to fitness in males and females are highlighted in light gray. “Experimental design” gives detail on the method used to quantitate gene expression and the number of male (m), female (f), and, where relevant, hermaphrodite (h) biological replicates. “Criteria for sex bias” indicates, where relevant, (i) the statistical level of significance for differential expression between the sexes and (ii) the cut-off level of fold change in expression between the sexes.

^a*Homo sapiens*, *Macaca mulatta*, and *Pan troglodytes*.

^b*Sylvia communis* and *Taeniopygia guttata*.

^c*Xenopus laevis* and *Xenopus muelleri*.

^d*D. melanogaster* and *Drosophila simulans*.

^e*Drosophila. ananassae*, *D. melanogaster*, *Drosophila mojavensis*, *Drosophila pseudoobscura*, *D. simulans*, *Drosophila virilis*, and *Drosophila yakuba*.

^f*Drosophila persimilis*, *D. pseudoobscura*, and *Drosophila pseudoobscura bogotana*.

^g*Solenopsis invicta* and *Solenopsis richteri*.

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sentative of the whole animal is yet to be determined.

Research has also found intraspecific genetic variation for patterns of sex-biased gene expression through a combination of quantitative genetics and microarray experiments in *D. melanogaster* (Jin et al. 2001; Ayroles et al. 2009; Innocenti and Morrow 2010). An environmental component of variation in sex-biased gene expression has also been shown in *D. melanogaster*, where there was more evidence of sex-biased gene expression in populations reared on a high-quality diet than in populations on a poor diet (Wyman et al. 2010). This genetic and environmental variation implies that the potential for sex-biased gene expression to evolve could vary between different populations and environments. It will be useful to examine how this variation relates to sexual conflict, and if it affects the potential for sex-biased expression to resolve conflict. Indeed, a few studies have found interactions between sexually antagonistic selection and environmental variation (e.g., Kwan et al. 2008; Long et al. 2012), and so whether or not sex-biased gene expression underlies such interactions will be an interesting direction for future research.

Finally, in terms of the experimental design and analysis, it is apparent from the summary in Table 1 that sample size and analytical criteria for identifying sex-biased expression can significantly impact on the results obtained. Clearly, sample size will affect the power of the experiment to detect significant sex-biased expression. In addition, studies have used a wide variety of analytical criteria for identifying sex-biased transcripts, involving some combination of statistical criteria (*P* values adjusted for false discovery rate) and relative expression levels between sexes (fold-change ratios). Usefully, Yang et al. (2006) present their data analyzed with different criteria for identifying sex bias. They examined gene expression in four mouse tissues and showed that whereas statistical criteria alone identified 72% of transcripts as sex biased in liver tissue, imposing just a 1.2 minimum fold-change threshold meant that only 13.5% of transcripts were identified as sex biased, and a fold-change threshold of 3 reduced this

to only 0.5%. This trend was similar in the other tissues analyzed (Yang et al. 2006), calling into question the biological significance of fold-change criteria, and also highlighting the difficulty in comparing results between studies with different analytical methods.

To illustrate this point further, consider two *D. melanogaster* studies, Ayroles et al. (2009) and Innocenti and Morrow (2010), in which large data sets of a comparable sample size were produced, and each analysis used the same statistical criteria for identifying sex-biased transcripts. As such, the results can be compared more reliably, and both studies came to the conclusion that ~90% of the *D. melanogaster* transcriptome exhibited sex-biased expression. In fact, the correlation between levels of sex-biased gene expression identified in each study was exceptionally high ($r^2 = 0.96$) (see Innocenti 2011). The implementation of a widely accepted standard criterion for identifying sex-biased gene expression would clearly facilitate comparisons between studies (Ellegren and Parsch 2007).

SEX-BIASED GENE REGULATION AND EVOLUTION

Studies documenting patterns of sex-biased gene expression also shed some light on the potential mechanisms that might regulate sex biases. For example, sex-biased gene expression could result from alternative splicing of gene transcripts. In *D. melanogaster*, 95% of known splicing regulators were found to exhibit significant sex-biased expression (McIntyre et al. 2006; Telonis-Scott et al. 2009). Furthermore, increased use of RNA-sequencing technology in recent years has enabled the direct quantification of splicing variants, and as a result, sex-specific splicing patterns have been shown in *D. melanogaster* (Gan et al. 2010; Chang et al. 2011) and in a group of primate species (Blekhusman et al. 2010). In the primate study, they also showed the enrichment of sex-biased genes with genes with known RNA splicing functions, providing further empirical support for the importance of sex-specific splicing in controlling sex-biased gene expression.

Another mechanism that might regulate sex-biased gene expression involves short (~22 nucleotides) RNA molecules known as microRNAs (miRNAs) (Morgan and Bale 2012). These miRNAs target mRNAs with complementary sequences and bind to them to regulate their expression, potentially by tagging the mRNA for destruction, or by preventing their translation (Pasquinelli 2002). Relatively little is known about sex-biased expression of miRNAs, although RNA-sequencing in the zebra finch, *Taeniopygia guttata*, has found genome-wide sex-biased expression of some miRNAs (Luo et al. 2012), and patterns of sex-biased miRNA expression have also been found in halibut (Bizuyehu et al. 2012), silkworm (Liu et al. 2010), and schistosomes (Marco et al. 2013). Taken together with the evidence for sex-biased miRNA expression in *D. melanogaster* from our unpublished data described below, it seems that further research on the sex-specific functions of miRNAs could shed some light on the mechanisms that control sex-biased gene expression. At present, very little is known about miRNA functions, and even less is known about sex-biased expression patterns of miRNAs. These small RNAs could be extremely important, however, and are thought to have a role in the regulation of gene expression in sex-biased diseases (Sharma and Eghbali 2014), but far more data are needed before this can be discussed in detail.

Sex-biased expression could also be facilitated by translocation of genes from autosomes to sex chromosomes (or alternatively, gene translocation from autosomes to sex chromosomes could promote sex-biased gene expression). In species with chromosomal (XY or ZW) sex determination, one sex is hemizygous for the X or Z chromosome, and so genes on these chromosomes will be subject to different dynamics of sex-specific selection than genes on autosomes. As a result, it is often predicted that sex chromosomes will harbor more sexually antagonistic alleles than autosomes (Rice 1984; Ellegren and Parsch 2007; Wyman et al. 2012; but see Fry 2010). Some empirical research has approached this idea by testing for enrichment of the sex chromosomes with sex-biased genes, but it may be the case that the model predictions,

which concern the relative fitness of different alleles, do not directly translate to empirical work that examines sex-biased gene expression. Indeed, the data on sex-biased gene expression on the sex chromosomes are mixed. For example, the X chromosome in flour beetles is enriched with female-biased genes (Prince et al. 2010), and the X chromosome of *Caenorhabditis elegans* is almost completely lacking any genes involved in sperm production (Reinke et al. 2000). In *Drosophila*, the X chromosome of both *D. melanogaster* and *D. simulans* is depleted for male-biased genes but enriched for female-biased genes (Parisi et al. 2003; Ranz et al. 2003). This pattern is apparently not limited to protein-coding genes, as the X chromosome is also enriched with male-biased miRNAs (Zhang et al. 2010). In the silkworm *B. mori*, where females are the heterogametic sex with ZW sex chromosomes, the Z chromosome is enriched with male-biased genes (Zhao et al. 2011). However, these patterns are not entirely consistent. In *Drosophila*, there is some evidence that the trend might be tissue dependent, as Catalan et al. (2012) found that the X chromosome of *D. melanogaster* was enriched for genes with male-biased expression in brain tissue. In mice, although one study found the X chromosome to be enriched with female-biased genes (Khil et al. 2004), another found a substantial number of testis-specific genes on the X (Wang et al. 2001).

If genes under sexual conflict are translocated to the sex chromosomes this might facilitate the evolution of sex-biased gene expression and sexual dimorphism and allow a release from conflict. The evidence that sex chromosomes are enriched with sex-biased genes is therefore compelling, but to directly address the theory, the chromosomal distribution of sex-biased genes needs to be associated with sexually antagonistic selection on these genes. In a quantitative genetic framework, studies have found that the X chromosome in *D. melanogaster* harbors a lot of sexually antagonistic genetic variation (Gibson et al. 2002; Pischedda and Chipindale 2006). However, to our knowledge, only two studies have explicitly addressed the relationship between sex-specific fitness and sex-specific gene expression phenotypes (Innocenti

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and Morrow 2010; Abbott et al. 2013). Because direct measurement of how these are linked is key to understanding the adaptive significance of sex-biased gene expression, more research in this area will be useful.

Several studies have considered the molecular evolution of sex-biased genes as a means to determine their adaptive significance. In both *Drosophila* species and the crustacean *Daphnia pulex*, male-biased genes tend to evolve more quickly than female-biased genes (Meiklejohn et al. 2003; Eads et al. 2007), suggesting that male-biased genes are under stronger selection and might contribute more to adaptive evolution. Consistent with this, there is also evidence in *Drosophila* that female-biased genes exhibit higher levels of pleiotropy than male-biased genes (Assis et al. 2012), which could limit the evolutionary potential of such genes. Indeed, it has also been shown that the majority of sex-biased expression in *Drosophila* seems to have evolved through adaptive changes in the male transcriptome (Connallon and Knowles 2006). The adaptive significance of sex-biased genes is further highlighted by studies of species divergence between *Drosophila* species. For example, 83% of the transcriptome changes between *D. simulans* and *D. melanogaster* are accounted for by sex-biased gene expression (Ranz et al. 2003), and male-biased genes have a strong and consistent signal of positive selection, indicating adaptive evolution, which is weaker in female-biased genes and lacking in non-sex-biased genes (Pröschel et al. 2006). Similarly, a large study of seven species of *Drosophila* found that male-biased genes showed the greatest divergence between species, in terms of both expression and sequence (Zhang et al. 2007), and another study between three recently diverged species in the *D. pseudoobscura* clade showed that sex-specific changes in expression explained more than 80% of transcriptome variation between species (Jiang and Machado 2009). Together, this research suggests that sex-biased gene expression, and particularly male-biased genes, play an important role in species divergence, which is interesting given the potential for sexually antagonistic selection to fuel species divergence (Parker and Partridge

1998). However, as pointed out by Rice et al. (2005), this molecular evidence is not enough by itself to conclusively show a role of sexual conflict in species divergence, and so future research will need to examine selection on sex-biased genes and how this could drive species divergence.

IDENTIFYING SEXUALLY ANTAGONISTIC GENES

It is tempting to use sex-biased gene expression (or indeed any sexual dimorphism) to make inferences about sexual conflict. However, sexual dimorphism alone does not necessarily mean a trait is under sexual conflict. To draw conclusions about sexual conflict, data on both dimorphism and sex-specific selection are needed (Cox and Calsbeek 2009). For instance, if the extent of dimorphic gene expression matches the difference between the sex-specific fitness optima, then sex-biased gene expression may signal a resolved conflict, but if not, then it is likely that there is ongoing sexual conflict despite the sex differences in gene expression. It is also important to recognize that in some cases, sexual dimorphism may have arisen without sexual conflict. For example, in butterflies, sexually dimorphic wing patterns are known to have evolved through traits that have initially arisen in one sex only (Oliver and Monteiro 2011).

Similarly, sexually monomorphic traits do not necessarily indicate an absence of sexual conflict—if fitness optima differ between the sexes for traits that do not exhibit dimorphism, then sexual conflict will exist. As such, identifying sex-biased gene expression is not enough on its own to discriminate between resolved or ongoing sexual conflict (Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009; Pennell and Morrow 2013).

Although the body of research documenting sex-biased gene expression is growing rapidly, very few of these studies have also looked at sex-specific fitness. In fact, this seems to have only been directly considered by two *D. melanogaster* studies. Ayroles et al. (2009) measured sex-biased gene expression and related this to sex-specific phenotypic variation in fitness-

related traits such as lifespan, locomotor response, and mating speed. They identified modules of genes that were associated with the phenotypic variation and that were therefore likely to be under sexual conflict. Innocenti and Morrow (2010) also reported widespread sex bias in the *D. melanogaster* transcriptome, and tested how this was associated with male and female fitness using line-specific values of reproductive success. They identified about 90% of transcripts as being sex biased, whereas only 8% were subject to sexually antagonistic selection (the X chromosome being enriched for them). This indicates that almost a tenth of the transcriptome harbored sexually antagonistic genetic variation, but it also reveals a majority of genes with sex-biased expression that are not currently subject to sexually antagonistic selection during the adult stage. It is worth noting that because only adults were studied, these results could have underestimated the full extent of sexual conflict throughout an individual's life cycle. Possibly, some transcripts might only experience sexual conflict at earlier life stages, which will have been overlooked in this study. We discuss this possibility in greater depth in the next section.

In the sexual conflict literature more generally, sexually antagonistic genetic variation has been identified in a number of studies. For example, a quantitative genetic study of *D. melanogaster* showed a negative genetic correlation between sex-specific adult fitness, suggesting ongoing sexual conflict (Chippindale et al. 2001). Experimental evolution in which selection on females was suppressed for several generations resulted in masculinized populations with high male fitness and low female fitness, again indicating extensive sexually antagonistic genetic variation (Prasad et al. 2007; Abbott et al. 2010). However, similar to the studies looking at transcriptome variation, there is no clear relationship between dimorphism and conflict. Although sexually antagonistic selection on highly dimorphic traits has been found in some studies (e.g., Pischedda and Chippindale 2006; Long and Rice 2007; Bedhomme et al. 2008; Cox and Calsbeek 2009), in other studies, there is no evidence of sexually antagonistic se-

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lection on dimorphic traits (Cox and Calsbeek 2009; Bedhomme et al. 2011). These studies highlight further that sexual dimorphism cannot be equated to sexual conflict without additional information on sex-specific patterns of selection.

SEXUAL CONFLICT AND SEX-BIASED GENE EXPRESSION THROUGHOUT DEVELOPMENT

The relationship between sexual conflict and sexual dimorphism is, in fact, likely to vary throughout development. As stated briefly in the introduction, a general prediction has been made that whereas sexual dimorphism usually increases throughout development, as the sexes differentiate in morphology and behavior (Badyaev 2002; Cox and Calsbeek 2009), sexually antagonistic selection is likely to be strongest at points of development when distinct male and female traits are specified and produced. However, in terms of empirical work to support this idea, very few studies have examined the progression of sexual dimorphism and sexual conflict throughout development. Although for particular genetic pathways (e.g., sexual differentiation), sex-specific gene expression throughout development has been studied in great detail (Rinn and Snyder 2005), a genome-wide approach will be important for studies of sexual conflict, as highlighted above. Table 1 shows that many genome-wide studies of sex-biased gene expression focus on only one developmental stage. This is perhaps not surprising, because quantifying the entire transcriptome for both sexes in multiple developmental stages is, of course, no small feat, in terms of both logistics and resources. However, there are some studies that have achieved this level of detail to some extent in *D. melanogaster* (Arbeitman et al. 2002; Perry et al. 2014), the mosquito *Anopheles gambiae* (Magnusson et al. 2011), *B. mori* (Zhao et al. 2011), fire ants (Ometto et al. 2011), and *C. elegans* (Reinke et al. 2000, 2004; Jiang et al. 2001; Thoenke et al. 2005). Sex-biased gene expression appears to be dynamic throughout development in a number of species, and so focusing on the ex-

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pression profile for a single developmental stage clearly risks overlooking important variation.

Furthermore, unpublished preliminary data from our laboratory clearly shows patterns of developmental changes in sex-biased expression of both mRNA and miRNA in *D. melanogaster*. We processed two biological replicates of each sex from each of three developmental stages (third-instar larvae, pupae, and adults) on (1) GeneChip *Drosophila* Genome 2.0 Affymetrix microarrays, and (2) GeneChip miRNA 3.0 Affymetrix microarrays (deposited in the GEO database, accession number GSE57322). Both mRNA and miRNA expression data were analyzed in R v.2.15.2 (www.R-project.org), using several packages on the BioConductor platform (www.bioconductor.org) (Gentleman et al. 2004), and sex-biased gene expression was identified using statistical criteria ($P < 0.01$ after correction for false discovery rate [Benjamini and Hochberg 1995]). Full details of the analysis can be provided upon request to the corresponding author (F.C.I.). We identified extensive sex-biased mRNA expression: 2638 transcripts out of the total 3754 (70%) were sex-biased during at least one developmental stage, although when comparing this to larger-scale experiments, it is likely to be an underestimate (Ayroles et al. 2009; Innocenti and Morrow 2010). Furthermore, at each developmental stage, most (in excess of 80%) of the sex-biased mRNA transcripts were male biased, consistent with previous work on *D. melanogaster* adults (Graveley et al. 2011). We also identified an increase in the overall extent of sex-biased mRNA expression throughout development, from 34% of the total transcripts being sex biased in larvae to 45% in pupae and 64% in adults. A similar trend was found in *A. gambiae* larvae, pupae, and adults (Magnusson et al. 2011).

Our preliminary data also highlight an interesting question that will have been overlooked by research that focuses on only one developmental stage: Are the same genes under sexually antagonistic selection throughout development or does selection vary? Although further research that combines measurement of gene expression and fitness throughout development will be needed to address this ques-

tion fully, our results nonetheless indicate stage specificity of sex-biased expression. Approximately two-thirds of the sex-biased mRNAs we identified were either only transiently sex biased throughout development or, in some cases, changed throughout development from being male biased to female biased (or vice versa). These patterns could be explained by differences in timing of key developmental events between males and females, or they could reflect fluctuations in sexual conflict throughout development.

We also identified significant sex-biased miRNA expression: 39 miRNAs out of 213 in total (18%) were sex biased at some point during development. Each of these sex-biased miRNAs exhibited sex-biased expression in adults, but sex-biased miRNA expression was lower in pre-adults (with 5% and 4% of the total miRNAs being sex biased in larvae and pupae, respectively). Genome-wide sex biases in miRNA expression have also been identified in zebra finches (Luo et al. 2012), silkworm (Liu et al. 2010), schistosomes (Marco et al. 2013), and halibut brain and gonad tissue (Bizuyehu et al. 2012). In halibut, patterns of sex-biased expression suggest a role of miRNAs in sexual development. However, research on miRNA expression and function is a relatively new area. Although previous research has supposed that miRNAs suppress translation of target mRNAs (Pasquinelli 2002), it is also known that they can up-regulate gene expression (e.g., Carroll et al. 2012) and that networks of interacting miRNAs and mRNAs might exist. This suggests that the relationship between miRNA and mRNA could be more complex than previously assumed (Salmena et al. 2011). The small sample size of this experiment prevents us from looking at this in more detail, but clearly, sex-specific interactions between miRNAs and mRNAs could be a valuable area for future research.

Together, these studies reveal potentially interesting patterns of sex-biased gene expression fluctuating throughout development not only in protein-coding mRNA, but also in miRNAs with potential regulatory roles. More research is needed to fully understand the interactions between mRNAs and miRNAs, and how these

relate to patterns of selection, thus, it will be interesting to examine sex-specific fitness throughout development, allowing interpretation of these patterns of gene expression in terms of sexual conflict.

At present, we know even less about how sexual conflict progresses throughout development than we do about patterns of sex-biased gene expression. Studies have examined sex-specific patterns of selection throughout development in some species (e.g., water striders [Preziosi and Fairbairn 2000], sticklebacks [Reimchen and Nosil 2002], and lizards [Le Galliard and Ferriere 2008]), and selection seems to vary a lot between juveniles and adults. However, a meta-analysis focusing on whether levels of sexually antagonistic selection differ between life stages produced mixed results overall (Cox and Calsbeek 2009). In addition, Chippindale et al. (2001) found a positive genetic correlation between male and female fitness in juvenile *D. melanogaster*, suggesting no conflict in pre-adults, whereas at the adult stage, a negative intersexual genetic correlation for fitness revealed sexual conflict. On the other hand, Svensson et al. (2009) showed that sexual conflict in lizards was consistent across juvenile and adult stages.

In a recent study of sex-biased gene expression in *D. melanogaster* larvae and pre-pupae, Perry et al. (2014) used the direction of selection (DoS) statistic to suggest that their results showed sex-specific selection patterns in pre-adult stages. However, although DoS has proven extremely useful in molecular evolution studies covering major evolutionary timescales (Stoletzki and Eyre-Walker 2011), it is unclear how relevant this metric is when applied to questions of current patterns of selection, and research examining sexually antagonistic selection might be better to measure relative male and female fitness within an experimental generation to directly associate patterns of sex-biased gene expression with sex-specific selection.

Theoretically, sex-biased gene expression and sexual conflict should become more prevalent as development progresses, as the sexes differentiate and the evolutionary interests of the sexes diverge after individuals have reached

reproductive age (Badyaev 2002; Cox and Calsbeek 2009). This prediction might partly explain the apparent focus on adults in research on sex-biased gene expression and sexual conflict, but the little data we have across developmental stages do not provide conclusive support for the prediction. First, it is difficult to come to any general conclusion from the handful of sexual conflict studies cited above because of the conflicting results. Second, in terms of sex-biased gene expression, there are only a few studies that suggest that the prevalence of sex-biased gene expression increases throughout development (in chickens [Mank et al. 2010] and *B. mori* [Zhao et al. 2011]). Conversely, there is also a lot of evidence for widespread sex biases in the transcriptome in juvenile stages of development (e.g., Fraser et al. 2011; Magnusson et al. 2011; see below), and even before sex differentiation in embryo brain tissue of mice (Dewing et al. 2003) and chickens (Scholz et al. 2006).

It is also possible that the ontogenetic relationship between sexual dimorphism and sexual conflict will vary, depending on factors such as the timing of sex differentiation during development or the genes involved in the sex differentiation pathway. For instance, in a holometabolous animal like a fruit fly, male and female larvae are similar in terms of morphology and behavior, and their evolutionary interests more or less aligned. As such, we might expect male and female larvae to be under similar patterns of selection, and thus, there will be limited potential for sexual conflict (e.g., Chippindale et al. 2001). At the pupal stage, however, individuals undergo drastic metamorphosis as the body is completely restructured into an adult male or female. In the example of fruit flies, there is extensive sexual dimorphism in adults, and many of these differences are laid down in the pupal stage. As such, perhaps sexual conflict will be at its most intense in pupae, and the body of research largely focused on conflict in adults might provide an underestimate of the overall influence of sexual conflict. On the other hand, in species that do not develop through these distinct stages (e.g., hemimetabolous insects, birds, and mammals), sexual dimorphism

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generally develops more gradually as sex-specific traits are acquired. In this case, a more constant buildup of sexual conflict throughout the entirety of development may be expected. Another possibility is that in all species, sexual conflict is most prevalent in reproductive individuals (i.e., adults). A further consideration will be traits that are laid down by earlier patterns of sex-biased gene expression, but not subject to selection at the phenotypic level until later in life. In this sense, studies will need to consider how gene expression data and fitness data are coupled together. If gene expression is measured as a snapshot of a given developmental stage, should fitness data be measured in a similarly stage-specific manner or be taken as an overall lifetime fitness measurement? Given an evolutionary definition of fitness as the lifetime reproductive success of an individual relative to others in the population, stage-specific fitness measures might be difficult to define. Either way, further research will need to focus on determining how sex-biased gene expression throughout development might relate to levels of sexual conflict. Without data relating sex-specific selection and gene expression throughout development, we cannot differentiate between the predictions discussed here. Sexual conflict at any stage of development could potentially affect the expression of shared traits and overall individual fitness, and this is clearly a neglected area of research.

CONCLUDING REMARKS

Whereas it is clear that sex-biased gene expression is a widespread phenomenon, the evolutionary significance of these patterns of gene expression is less clear because of a paucity of studies that examine fitness or selection in conjunction with the molecular data. The assumption that sex-biased gene expression could be used as an indicator of sexually antagonistic selection is evidently not reliable. Where the association between sex-specific fitness and sex-biased gene expression has been directly tested, it has been shown that sex-biased genes are not necessarily subject to sexually antagonistic selection. Moreover, distinguishing be-

tween ongoing and resolved sexual conflict requires the quantification of both the extent of dimorphism in gene expression and the discrepancy in sex-specific fitness optima. Arguably, it is genes with monomorphic patterns of expression that may experience the greatest degree of sexually antagonism. As such, future research will benefit greatly from more studies that bring together measures of sex-biased gene expression and sex-specific selection. Only by doing so can sex-biased gene expression be interpreted in the context of sexual conflict, or in an evolutionary framework more generally, for example, with regards to the role of sex-biased genes in speciation.

Our review of the existing literature and our small-scale experiment also highlight potentially important avenues for future research on sex-biased gene expression. First, it will be interesting to consider how sex-biased gene expression and sexual conflict vary throughout development. Studies so far show sex-biased expression to be dynamic from one developmental stage to another, and so relating these patterns to fitness and conflict will provide useful insight. Research integrating data on gene expression, fitness, and development will be demanding, but it should be increasingly feasible as technology for examining gene expression becomes more accessible. Furthermore, sex biases seem to vary not only throughout development, but across environments and genotypes as well. This is likely to have important implications for the evolution of sex-biased gene expression and could mean that the potential for sexual conflict to be resolved through sex-biased gene expression varies between environments and populations, an idea that might also benefit from further research.

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REFERENCES

- Abbott JK, Bedhomme S, Chippindale AK. 2010. Sexual conflict in wing size and shape in *Drosophila melanogaster*. *J Evol Biol* **23**: 1989–1997.
- Abbott JK, Innocenti P, Chippindale AK, Morrow EH. 2013. Epigenetics and sex-specific fitness: An experimental test using male-limited evolution in *Drosophila melanogaster*. *PLoS ONE* **8**: e70493.
- Arbeitman MN, Furlong EEM, Imam F, Johnson E, Null BH, Baker BS, Krasnow MA, Scott MP, Davis RW, White KP. 2002. Gene expression during the life cycle of *Drosophila melanogaster*. *Science* **297**: 2270–2275.
- Assis R, Zhou Q, Bachtrog D. 2012. Sex-biased transcriptome evolution in *Drosophila*. *Genome Biol Evol* **4**: 1189–1200.
- Ayroles JF, Carbone MA, Stone EA, Jordan KW, Lyman RF, Magwire MM, Rollmann SM, Duncan LH, Lawrence E, Anholt RRH, et al. 2009. System genetics of complex traits in *Drosophila melanogaster*. *Nat Rev Genet* **41**: 299–307.
- Badyaev AV. 2002. Growing apart: An ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol Evol* **17**: 369–378.
- Baker DA, Russell S. 2009. Gene expression during *Drosophila melanogaster* egg development before and after reproductive diapause. *BMC Genomics* **10**: 242.
- Baker DA, Nolan T, Fischer B, Pinder A, Crisanti A, Russell S. 2011. A comprehensive gene expression atlas of sex- and tissue-specificity in the malaria vector, *Anopheles gambiae*. *BMC Genomics* **12**: 296.
- Bedhomme S, Prasad NG, Jiang PP, Chippindale AK. 2008. Reproductive behaviour evolves rapidly when intralocus sexual conflict is removed. *PLoS ONE* **3**: e2187.
- Bedhomme S, Chippindale AK, Prasad NG, Delcourt M, Abbott JK, Mallet MA, Rundle HD. 2011. Male-limited evolution suggests no extant intralocus sexual conflict over the sexually dimorphic cuticular hydrocarbons of *Drosophila melanogaster*. *J Genetics* **90**: 443–452.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc B* **57**: 289–300.
- Bhasin JM, Chakrabarti E, Peng DQ, Kulkarni A, Chen X, Smith JD. 2008. Sex-specific gene regulation and expression QTLs in mouse macrophages from a strain intercross. *PLoS ONE* **3**: e1435.
- Bizuayehu TT, Babiak J, Norberg B, Fernandes JMO, Johansen SD, Babiak I. 2012. Sex-biased miRNA expression in Atlantic halibut (*Hippoglossus hippoglossus*) brain and gonads. *Sex Dev* **6**: 257–266.
- Blekhman R, Marion JC, Zumbo P, Stephens M, Gilad Y. 2010. Sex-specific and lineage-specific alternative splicing in primates. *Genome Res* **20**: 180–189.
- Bonduriansky R, Chenoweth SE. 2009. Intralocus sexual conflict. *Trends Ecol Evol* **24**: 280–288.
- Carroll AP, Tran N, Tooney PA, Cairns MJ. 2012. Alternative mRNA fates identified in microRNA-associated transcriptome analysis. *BMC Genomics* **13**: 561.
- Catalan A, Hutter S, Parsch J. 2012. Population and sex differences in *Drosophila melanogaster* brain gene expression. *BMC Genomics* **13**: 654.
- Chang PL, Dunham JP, Nuzhdin SV, Arbeitman MN. 2011. Somatic sex-specific transcriptome differences in *Drosophila* revealed by whole transcriptome sequencing. *BMC Genomics* **12**: 364.
- Chintapalli VR, Wang J, Dow JAT. 2007. Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. *Nat Genet* **39**: 715–720.
- Chippindale AK, Gibson JR, Rice WR. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *PNAS* **98**: 1671–1675.
- Choi YJ, Ghedin E, Berriman M, McQuillan J, Holroyd N, Mayhew GF, Christensen BM, Michalski ML. 2011. A deep sequencing approach to comparatively analyze the transcriptome of lifecycle stages of the filarial worm, *Brugia malayi*. *PLoS Negl Trop D* **5**: e1409.
- Connallon T, Clark AG. 2011. Association between sex-biased gene expression and mutations with sex-specific phenotypic consequences in *Drosophila*. *Genome Biol Evol* **3**: 151–155.
- Connallon T, Knowles LL. 2006. Intergenomic conflict revealed by patterns of sex-biased gene expression. *Trends Genet* **21**: 495–499.
- Cox RM, Calsbeek R. 2009. Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *Am Nat* **173**: 176–187.
- Dewing P, Shi T, Horvath S, Vilain E. 2003. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Mol Brain Res* **118**: 82–90.
- Eads BD, Colbourne JK, Bohuski E, Andrews J. 2007. Profiling sex-biased gene expression during parthenogenetic reproduction in *Daphnia pulex*. *BMC Genomics* **8**: 464.
- Ellegren H, Parsch J. 2007. The evolution of sex-biased genes and sex-biased gene expression. *Nat Rev Genet* **8**: 689–698.
- Fitzpatrick JM, Johnston DA, Williams GW, Williams DJ, Freeman TC, Dunne DW, Hoffmann KE. 2005. An oligonucleotide microarray for transcriptome analysis of *Schistosoma mansoni* and its application/use to investigate gender-associated gene expression. *Mol Biochem Parasitol* **141**: 1–13.
- Fraser BA, Weadick CJ, Janowitz, Rodd FH, Hughes KA. 2011. Sequencing and characterization of the guppy (*Poecilia reticulata*) transcriptome. *BMC Genomics* **12**: 202.
- Fry JD. 2010. The genomic location of sexually antagonistic variation: Some cautionary comments. *Evolution* **64**: 1510–1516.
- Gan Q, Chepelev I, Wei G, Tarayrah L, Cui K, Zhao K, Chen X. 2010. Dynamic regulation of alternative splicing and chromatin structure in *Drosophila* gonads revealed by RNA-seq. *Cell Res* **20**: 763–783.
- Gautier L, Cope L, Bolstad BM, Irizarry RA. 2004. Affy-analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* **20**: 307–315.
- Gentleman R, Carey V, Bates D, Bolstad B, Dettling M, et al. 2004. Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol* **5**: R80.

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- Gibson JR, Chippindale AK, Rice WR. 2002. The X chromosome is a hot spot for sexually antagonistic fitness variation. *Proc R Soc Lond B* **269**: 499–505.
- Graveley BR, Brooks AN, Carlson JW, Duff MO, Landolin JM, Yang L, Artieri CG, van Baren MJ, Boley N, Booth BW, et al. 2011. The developmental transcriptome of *Drosophila melanogaster*. *Nature* **471**: 473–479.
- Grosse-Wilde E, Kuebler LS, Bucks S, Vogel H, Wicher D, Hansson BS. 2011. Antennal transcriptome of *Manduca sexta*. *PNAS* **108**: 7449–7454.
- Innocenti P. 2011. Sexual conflict and gene expression: Exploring sex-specific associations between fitness and transcriptional variation. *Fly* **5**: 10–13.
- Innocenti P, Morrow EH. 2010. The sexually antagonistic genes of *Drosophila melanogaster*. *PLoS Biol* **8**: e1000335.
- Jiang ZF, Machado CA. 2009. Evolution of sex-dependent gene expression in three recently diverged species of *Drosophila*. *Genetics* **183**: 1175–1185.
- Jiang M, Ryu J, Kiraly M, Duke K, Reinke V, Kim SK. 2001. Genome-wide analysis of developmental and sex-regulated gene expression profiles in *Caenorhabditis elegans*. *PNAS* **98**: 218–233.
- Jin W, Riley RM, Wolfinger RD, White KP, Passador-Gurgel G, Gibson G. 2001. The contributions of sex, genotype and age to transcriptional variance in *Drosophila melanogaster*. *Nat Genet* **29**: 389–395.
- Khil PP, Smirnova NA, Romanienko PJ, Camerini-Otero RD. 2004. The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. *Nat Genet* **36**: 642–646.
- Kwan L, Bedhomme S, Prasad NG, Chippindale AK. 2008. Sexual conflict and environmental change: Trade-offs within and between the sexes during evolution of desiccation resistance. *J Genet* **87**: 383–394.
- Leder EH, Cano JM, Leinonen T, O'Hara RB, Nikinmaa M, Primmer CR, Merila J. 2010. Female-biased expression on the X chromosome as a key step in sex chromosome evolution in threespine sticklebacks. *Mol Biol Evol* **27**: 1495–1503.
- Le Galliard JF, Ferriere R. 2008. Evolution of maximal endurance capacity: Natural and sexual selection across age classes in a lizard. *Evol Ecol Res* **10**: 157–176.
- Liu S, Gao S, Zhang D, Yin J, Xiang Z, Xia Q. 2010. MicroRNAs show diverse and dynamic expression patterns in multiple tissues of *Bombyx mori*. *BMC Genomics* **11**: 85.
- Long TAF, Rice WR. 2007. Adult locomotory activity mediates intralocus sexual conflict in a laboratory-adapted population of *Drosophila melanogaster*. *Proc R Soc Lond B* **274**: 3105–3112.
- Long TA, Agrawal AF, Rowe L. 2012. The effect of sexual selection on offspring fitness depends on the nature of genetic variation. *Curr Biol* **22**: 204–208.
- Luo GZ, Hafner M, Shi Z, Brown M, Feng GH, Tuschl T, Wang XJ, Li X. 2012. Genome-wide annotation and analysis of zebra finch microRNA repertoire reveal sex-biased expression. *BMC Genomics* **13**: 727.
- Magnusson K, Mendes AM, Windbichler N, Papathanos PA, Nolan T, Dottorini T, Rizzi E, Christophides GK, Crisanti A. 2011. Transcription regulation of sex-biased genes during ontogeny in the malaria vector *Anopheles gambiae*. *PLoS ONE* **6**: e21572.
- Malone JH, Hawkins DL, Michalak P. 2006. Sex-biased gene expression in a ZW sex determination system. *J Mol Evol* **63**: 427–436.
- Mank JE, Nam K, Brunstrom B, Ellegren H. 2010. Ontogenetic complexity of sexual dimorphism and sex-specific selection. *Mol Biol Evol* **27**: 1570–1578.
- Marco A, Kozomara A, Hui JHL, Emery AM, Rollinson D, Griffiths-Jones S, Ronshaugen M. 2013. Sex-biased expression of microRNAs in *Schistosoma mansoni*. *PLoS Neglect Trop D* **7**: e2402.
- Marinotti O, Calvo E, Nguyen QK, Dissanayake S, Ribeiro JMC, James AA. 2006. Genome-wide analysis of gene expression in adult *Anopheles gambiae*. *Insect Mol Biol* **15**: 1–12.
- Martinez-Fernandez M, Bernatchez L, Rolan-Alvarez E, Quesada H. 2010. Semi-quantitative differences in gene transcription profiles between sexes of a marine snail by a new variant of cDNA-AFLP analysis. *Mol Ecol* **10**: 324–330.
- McIntyre LM, Bono LM, Genissel A, Westerman R, Junk D, Telonis-Scott M, Harshman L, Wayne ML, Kopp A, Nuzhdin SV. 2006. Sex-specific expression of alternative transcripts in *Drosophila*. *Genome Biol* **7**: R79.
- Meiklejohn CD, Parsch J, Ranz JM, Hartl DL. 2003. Rapid evolution of male-biased gene expression in *Drosophila*. *PNAS* **100**: 9894–9899.
- Morgan CP, Bale TL. 2012. Sex differences in microRNA regulation of gene expression: No smoke, just miRs. *Biol Sex Differences* **3**: 22.
- Naurin S, Hansson B, Hasselquist D, Kim YH, Bensch S. 2011. The sex-biased brain: Sexual dimorphism in gene expression in two species of songbirds. *BMC Genomics* **12**: 37.
- Oliver JC, Monteiro A. 2011. On the origins of sexual dimorphism in butterflies. *Proc Biol Sci* **278**: 1981–1988.
- Ometto L, Shoemaker D, Ross KG, Keller L. 2011. Evolution of gene expression in fire ants: The effects of developmental stage, caste, and species. *Mol Biol Evol* **28**: 1381–1392.
- Parisi M, Nuttall R, Naiman D, Bouffard G, Malley J, Andrews J, Eastman S, Oliver B. 2003. Paucity of genes on the *Drosophila* X chromosome showing male-biased expression. *Science* **299**: 697–700.
- Parker GA, Partridge L. 1998. Sexual conflict and speciation. *Phil Trans R Soc Lond B* **353**: 261–274.
- Pasquinelli AE. 2002. MicroRNAs: Deviants no longer. *Trends Genet* **18**: 171–173.
- Pennell TM, Morrow EH. 2013. Two sexes, one genome: The evolutionary dynamics of intralocus sexual conflict. *Ecol and Evol* **3**: 1819–1834.
- Perry JC, Harrison PW, Mank JE. 2014. The ontogeny and evolution of sex-biased gene expression in *Drosophila melanogaster*. *Mol Biol Evol* **31**: 1206–1219.
- Pischedda A, Chippindale AK. 2006. Intralocus sexual conflict diminishes the benefits of sexual selection. *PLoS Biol* **4**: e356.
- Pitts RJ, Rinker DC, Jones PL, Rokas A, Zwiebel LJ. 2011. Transcriptome profiling of chemosensory appendages in the malaria vector *Anopheles gambiae* reveals tissue-specific signatures of odor coding. *BMC Genomics* **12**: 271.

Sex-Biased Gene Expression and Sexual Conflict

- Prasad NG, Bedhomme S, Day T, Chippindale AK. 2007. An evolutionary cost of separate genders revealed by male-limited evolution. *Am Nat* **169**: 29–37.
- Preziosi RF, Fairbairn DJ. 2000. Lifetime selection on adult body size and components of body size in a waterstrider: Opposing selection and maintenance of sexual size dimorphism. *Evolution* **54**: 558–566.
- Prince EG, Kirkland D, Demuth JP. 2010. Hyperexpression of the X chromosome in both sexes results in extensive female bias of X-linked genes in the flour beetle. *Genome Biol Evol* **2**: 336–346.
- Proschel M, Zhang Z, Parsch J. 2006. Widespread adaptive evolution of *Drosophila* genes with sex-biased expression. *Genetics* **174**: 893–900.
- Ranz JM, Castillo-Davis CI, Meiklejohn CD, Hartl DL. 2003. Sex-dependent gene expression and evolution of the *Drosophila* transcriptome. *Science* **300**: 1742–1745.
- Reimchen TE, Nosil P. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. *Evolution* **56**: 2472–2483.
- Reinke V, Smith HE, Nance J, Wang J, Van Doren C, Begley R, Jones SJM, Davis EB, Scherer S, Ward S, et al. 2000. A global profile of germline gene expression in *C. elegans*. *Mol Cell* **6**: 605–616.
- Reinke V, Gil IS, Ward S, Kazmer K. 2004. Genome-wide germline-enriched and sex-biased expression profiles in *Caenorhabditis elegans*. *Development* **131**: 311–323.
- Rice WR. 1984. Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**: 735–742.
- Rice WR, Linder JE, Friberg U, Lew TA, Morrow EH, Stewart AD. 2005. Inter-locus antagonistic coevolution as an engine of speciation: Assessment with hemiclinal analysis. *PNAS* **102**: 6527–6534.
- Rinn JL, Snyder M. 2005. Sexual dimorphism in mammalian gene expression. *Trends Genet* **21**: 298–305.
- Rinn JL, Rozowsky JS, Laurenzi IJ, Peterson PH, Zou K, Zhong W, Gerstein M, Snyder M. 2004. Major molecular differences between mammalian sexes are involved in drug metabolism and renal function. *Dev Cell* **6**: 791–800.
- Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. 2011. A ceRNA hypothesis: The rosetta stone of a hidden RNA language? *Cell* **146**: 353–358.
- Santos EM, Kille P, Workman VL, Paull GC, Tyler CR. 2008. Sexually dimorphic gene expression in the brains of mature zebrafish. *Comp Biochem Phys* **149**: 314–324.
- Scholz B, Kultima K, Mattsson A, Axelsson J, Brunstrom B, Halldin K, Stigson M, Dencker L. 2006. Sex-dependent gene expression in early brain development of chicken embryos. *BMC Neuroscience* **7**: 12.
- Sharma S, Eghbali M. 2014. Influence of sex differences on microRNA gene regulation in disease. *Biol Sex Differ* **5**: 3.
- Small CM, Carney GE, Mo Q, Vannucci M, Jones AG. 2009. A microarray analysis of sex- and gonad-biased gene expression in the zebrafish: Evidence for masculinization of the transcriptome. *BMC Genomics* **10**: 579.
- Stoletzki N, Eyre-Walker A. 2011. Estimation of the neutrality index. *Mol Biol Evol* **28**: 63–70.
- Svensson EI, McAdam AG, Sinervo B. 2009. Intralocus sexual conflict over immune defense, gender load, and sex-specific signaling in a natural lizard population. *Evolution* **63**: 3124–3135.
- Telonis-Scott M, Kopp A, Wayne ML, Nuzhdin SV, McIntyre LM. 2009. Sex-specific splicing in *Drosophila*: Widespread occurrence, tissue specificity and evolutionary conservation. *Genetics* **181**: 421–434.
- Thoenke K, Yi W, Ross JM, Kim S, Reinke V, Zarkower D. 2005. Genome-wide analysis of sex-enriched gene expression during *C. elegans* larval development. *Dev Biol* **284**: 500–508.
- Wang PJ, McCarrey JR, Yang F, Page DC. 2001. An abundance of X-linked genes expressed in spermatonia. *Nat Genet* **27**: 422–426.
- Wayne ML, Telonis-Scott M, Bono LM, Harshman L, Kopp A, Nuzhdin SV, McIntyre LM. 2012. Simpler mode of inheritance of transcriptional variation in male *Drosophila melanogaster*. *PNAS* **104**: 18577–18582.
- Wen C, Zhang Z, Ma W, Xu M, Wen Z, Peng J. 2005. Genome-wide identification of female-enriched genes in zebrafish. *Dev Dynamics* **232**: 171–179.
- Wyman MJ, Agrawal AF, Rowe L. 2010. Condition-dependence of the sexually dimorphic transcriptome in *Drosophila melanogaster*. *Evolution* **64**: 1836–1848.
- Wyman MJ, Cutter AD, Rowe L. 2012. Gene duplication in the evolution of sexual dimorphism. *Evolution* **66**: 1556–1566.
- Xia Q, Cheng D, Duan J, Wang G, Cheng T, Zha X, Liu C, Zhao P, Dai F, Zhang Z, et al. 2007. Microarray-based gene expression profiles in multiple tissues of the domesticated silkworm, *Bombyx mori*. *Genome Biol* **8**: R162.
- Yang X, Schadt EE, Wang S, Wang H, Arnold AP, Ingram-Drake L, Drake TA, Lusis AJ. 2006. Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Res* **16**: 995–1004.
- Zhang Y, Sturgill D, Parisi M, Kumar S, Oliver B. 2007. Constraint and turnover in sex-biased gene expression in the genus *Drosophila*. *Nature* **450**: 233–238.
- Zhang YE, Vrbancovski MD, Krinsky BH, Long M. 2010. Age-dependent chromosomal distribution of male-biased genes in *Drosophila*. *Genome Res* **20**: 1526–1533.
- Zhao M, Zha XF, Liu J, Zhang WJ, He NJ, Cheng DJ, Dai Y, Xiang ZH, Xia QY. 2011. Global expression profile of silkworm genes from larval to pupal stages: Toward a comprehensive understanding of sexual differences. *Insect Science* **18**: 607–618.

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