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The Shared Genome Is a Pervasive Constraint on the Evolution of Sex-Biased Gene Expression

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Abstract

Males and females share most of their genomes, and differences between the sexes can therefore not evolve through sequence divergence in protein coding genes. Sexual dimorphism is instead restricted to occur through sex-specific expression and splicing of gene products. Evolution of sexual dimorphism through these mechanisms should, however, also be constrained when the sexes share the genetic architecture for regulation of gene expression. Despite these obstacles, sexual dimorphism is prevalent in the animal kingdom and commonly evolves rapidly. Here, we ask whether the genetic architecture of gene expression is plastic and easily molded by sex-specific selection, or if sexual dimorphism evolves rapidly despite pervasive genetic constraint. To address this question, we explore the relationship between the intersexual genetic correlation for gene expression (r_{MF}), which captures how independently genes are regulated in the sexes, and the evolution of sex-biased gene expression. Using transcriptome data from *Drosophila melanogaster*, we find that most genes have a high r_{MF} and that genes currently exposed to sexually antagonistic selection have a higher average r_{MF} than other genes. We further show that genes with a high r_{MF} have less pronounced sex-biased gene expression than genes with a low r_{MF} within *D. melanogaster* and that the strength of the r_{MF} in *D. melanogaster* predicts the degree to which the sex bias of a gene's expression has changed between *D. melanogaster* and six other species in the *Drosophila* genus. In sum, our results show that a shared genome constrains both short- and long-term evolution of sexual dimorphism.

Key words: **sexual dimorphism, genetic constraint, sex-biased gene expression.**

Introduction

In most species, male and female fitness is optimized through different strategies, which selects for phenotypic differences between the sexes (Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009; Van Doorn 2009). Traits that show such sexual dimorphism are common in nature (Fairbairn et al. 2007), and they typically evolve rapidly (Darwin 1871; Meyer 1997; Arnqvist 1998; Civetta and Singh 1998; Omland and Lanyon 2000; Emlen et al. 2007). On the one hand, this is expected, because sexual characters are often exposed to strong sex-specific selection (Badyaev and Martin 2000; Hoekstra et al. 2001; Kingsolver et al. 2001). On the other hand, it is a paradox, because males and females share the same genome, apart from a few genes found on the Y and W chromosomes. With most genes shared between the sexes, the evolution of sexual dimorphism should be constrained, because selection on one sex should result in a correlated response in the other. Theory confirms this verbal argument and shows that evolution of sexual dimorphism proceeds exceedingly slowly when the genetic architecture is very similar in the sexes (Lande 1980, 1987; Reeve and Fairbairn 2001).

As males and females have largely the same genes, sexual dimorphism often cannot evolve through sequence differences between the sexes. Instead, the evolution of sexual dimorphism is restricted to sex-specific expression (Rinn and Snyder 2005; Connallon and Knowles 2005; Ellegren and Parsch 2007) and splicing of genes (McIntyre et al. 2006; Telonis-Scott et al. 2009). Genomic studies of the transcriptome have revealed that a large fraction of genes in model organisms have evolved sex-biased expression (Jin et al. 2001; Rinn and Snyder 2005; Yang et al. 2006; Ellegren and Parsch 2007; Mank, Hultin-Rosenberg, Webster, et al. 2008; Reinius et al. 2008; Jiang and Machado 2009) and that sex-biased genes, particularly those with male-biased expression, undergo rapid expression evolution (Ranz et al. 2003; Meiklejohn et al. 2003; Khaitovich et al. 2005; Voolstra et al. 2007; Zhang et al. 2007; Grath et al. 2009; Jiang and Machado 2009, Parsch and Ellegren 2013). Given the rapid evolution of sexual dimorphism on all levels of phenotypic organization, does this take place despite strong constraints, or is the genetic architecture in males and females free to evolve independently?

The intersexual genetic correlation (r_{MF}) is a scaled measure of the extent to which genetic variation covaries between the sexes and ranges from −1 to 1. An r_{MF} of one means that the genetic variation for a trait has exactly the same genetic basis in males and females, whereas an r_{MF} of zero indicates that it has a sexually independent genetic foundation. If the evolution of sexual dimorphism is constrained by a shared genetic architecture, a negative association between the

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degree of dimorphism and the strength of the r_{MF} is predicted (Lande 1980; Bonduriansky and Rowe 2005; Fairbairn and Roff 2006). Such a relationship can arise in two different ways, either because traits that initially have a low r_{MF} respond faster to novel sex-specific selection or because sex-specific selection causes mutations with sex-specific effects to accumulate over time, reducing the r_{MF} (Fairbairn et al. 2007). Several mechanisms have been proposed, which should allow for evolution of sex-specific genetic variance. These include gene duplications, where each sex sequesters one of the paralogous genes (Rice and Chippindale 2001; Stewart et al. 2010; Connallon and Clark 2011; Gallach and Betran 2011a, 2011b; Hosken 2011; Wyman et al. 2012), recruitment of sex-specific transcription factor binding sites (reviewed in Williams and Carroll 2009), sex linkage (Rice 1984), and genomic imprinting (Day and Bonduriansky 2004). However, it is noteworthy that rapid fixation of alleles with sex-specific effects could mitigate the build-up of a negative association between the r_{MF} and the degree of sexual dimorphism (Meagher 1992; Reeve and Fairbairn 1996). In this scenario, sexual dimorphism evolves but leaves no lasting signature on the r_{MF}.

Empirical studies testing for an association between sexual dimorphism and the r_{MF} using traits at a high level of phenotypic organization (i.e., morphological, behavioral, and physiological) have given mixed results at the within-population level. A negative correlation has been documented in waltzing flies (Bonduriansky and Rowe 2005), water striders (Preziosi and Roff 1998; Fairbairn et al. 2007), a moss (McDaniel 2005), and a dioecious plant (Delph et al. 2004, 2010), whereas no such associations have been documented in fruit flies (Cowley et al. 1986; Cowley and Atchley 1988; association reported in Fairbarin and Roff 2006) and sticklebacks (Leinonen et al. 2011). A meta-analysis of plant species also failed to find a negative association (Ashman and Majetic 2006). However, a more extensive meta-analysis, compiling data from both animals and plants, did find a marginally significant negative correlation (Poissant et al. 2010).

Little is known about the extent to which the genetic architecture at the lowest level of phenotypic organization, gene expression, constrains the evolution of sexual dimorphism. To address this question, we used gene expression data from *Drosophila melanogaster* and contrasted it to gene expression in *D. simulans*, *D. yakuba*, *D. ananassae*, *D. pseudoobscura*, *D. virilis*, and *D. mojavensis*. We show that the r_{MF} for gene expression in general is high and that genes currently exposed to divergent selection on gene expression in the sexes have a higher r_{MF} than other genes. We further show a negative association between the r_{MF} and the degree of sex-biased gene expression within *D. melanogaster* and that the r_{MF} of a gene in *D. melanogaster* predicts the extent to which sex bias has evolved between *D. melanogaster* and other *Drosophila* species. In sum, our results provide several lines of independent evidence that the shared genome represents a pervasive constraint on the evolution of sex-biased gene expression.

Results

Estimates of the Intersexual Genetic Correlation

Across all genes in the *D. melanogaster* genome, the median r_{MF} was only 0.295 (95% confidence interval [CI] = 0.287–0.302, fig. 1, black bars). However, genetic correlations are determined by how tightly the genetic variances of two traits are associated (in this case male and female gene expression). When genotypic values are estimated with poor precision, this will, on average, reduce the association between traits and bias the estimate of the genetic correlations toward zero. We used the data from the Drosophila Genetic Reference Panel (DGRP) study of Ayroles et al. (2009) to calculate the r_{MF} across the genome of *D. melanogaster*. This data set is unique with respect to its extensive sampling of genome-wide gene expression across 40 genotypes from one population but limited in that it consists of "only" two samples per sex and genotype. Low sampling combined with potentially high levels of noise, typically associated with gene expression estimated through microarrays, thus suggest that estimates of the r_{MF} from this data set, on average, will be biased downward (given that the r_{MF} of most genes is positive).

In an attempt to reduce this problem, we applied two statistical approaches to filter out genes with high levels of sampling variation and genes without a genetic component associated with the variation (the r_{MF} is not defined for genes that lack genetic expression variation). After normalizing expression variation for each gene in each sex ($\bar{X} = 0$, $\sigma = 1$), we fitted a linear mixed effects model to each gene with the fixed factor Sex and the factors Genotype and Sex × Genotype defined as random effects. In our first approach, we classified genes according to the percentage ($\geq$20%, $\geq$40%, $\geq$60%, and $\geq$80%) of the sum of random effects variation and residual variation ("total") that had a genetic component (captured by the random effects). Our second approach used the same model as defined earlier and employed log-likelihood ratio testing to generate P value estimates for both random effects. Genes were retained if either or both of the random effects were significant where $P < 0.01$. These genes are herein referred to as having significant genetic variation (n = 8,997). The unfiltered set consisted of 12,572 genes.

Gradually removing genes, from those for which the genetic variance was a small component of the total variance, resulted in a steady increase in the r_{MF} (fig. 1, shaded bars). When we retained only the genes for which the genetic variance explained 80% or more of the total variance, the median r_{MF} was 0.724 (95% CI = 0.712–0.734, fig. 1, white bars), including only genes with significant genetic variation resulted in a median r_{MF} of 0.427 (95% CI = 0.419–0.435).

r_{MF} and Sexually Antagonistic Selection

Sexual antagonism is resolved through the evolution of sexual dimorphism. Genes whose expression levels are currently under sexually antagonistic selection should be moving toward greater sex bias. If the r_{MF} is high, then the evolution

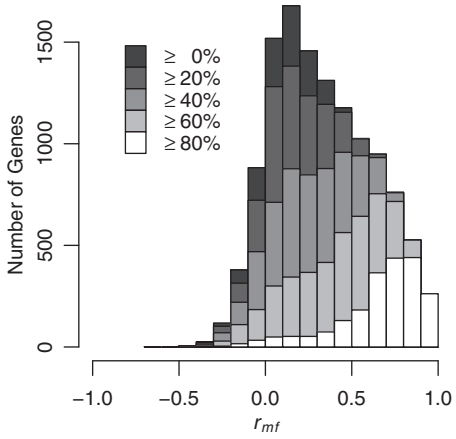

Fig. 1. Distributions of the r_{MF} value estimates. The black bars represent the full set of genes in the *Drosophila melanogaster* genome, whereas the shaded and white bars represent genes filtered according to the percentage of the total variation explained by genetic variation.

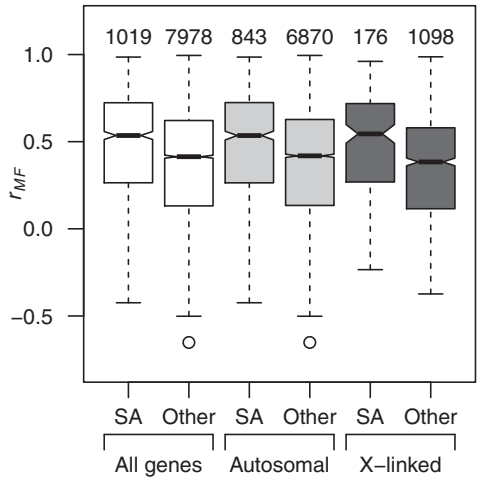

Fig. 2. r_{MF} for genes with expression under sexually antagonistic selection (SA) and genes under no or another form of selection (Other). Only the genes with significant genetic variation are included. Notches on the boxes represent approximate 95% CIs. Numbers above the boxes show how many genes each box represents.

of sex bias will proceed more slowly. Accordingly, we expect that sexual antagonism will persist for longer and genes presently experiencing sexually antagonistic selection should have a higher r_{MF} than other genes. To test this, we first gathered information on a gene's selective regime from the study of Innocenti and Morrow (2010) and r_{MF} values based on calculations from the data of Ayroles et al. (2009). Genes currently exposed to sexually antagonistic selection (SA genes) had a higher r_{MF} than other genes when only genes having significant genetic variation were analyzed (estimated coefficient for selective regime [csr] = 0.096, $P < 0.0001$; fig. 2). The same pattern was observed when genes were broken down on the X-chromosome and the autosomes (X-linked genes: csr = 0.115, $P < 0.0001$; autosomal genes: csr = 0.093, $P < 0.0001$; fig. 2). Similar results were found when the analysis included all genes (including all chromosomes: csr = 0.118, $P < 0.0001$; X-linked genes: csr = 0.139, $P < 0.0001$ autosomal genes: csr = 0.115, $P < 0.0001$).

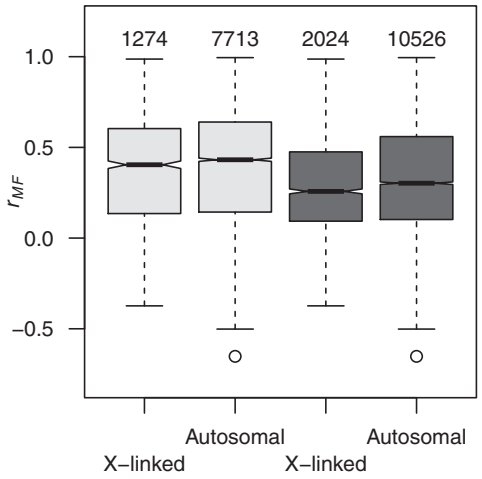

Fig. 3. r_{MF} for X-linked and autosomal genes. Light gray boxes include only significant genes, and dark gray boxes include all genes. Notches on the boxes represent approximate 95% CIs. Numbers above the boxes show how many genes each box represents.

r_{MF} and Sex Linkage

Theory predicts that sexual dimorphism should evolve more easily through genes located on the X-chromosome (Rice 1984; but see Connallon and Clark 2010). Following from this theory, it has been suggested that the X-chromosome should host more sex-specific genetic variation than the autosomes (Fairbairn and Roff 2006; Husby et al. 2013). We therefore tested whether X-linked genes have a lower r_{MF} compared with autosomal genes. X-linked genes had a small and marginally significant reduction in the r_{MF} compared with autosomal genes when only genes having significant genetic variation were included (estimated coefficient for chromosome type [cct] = 0.020, $P = 0.025$; fig. 3). Similar results were found when all genes were included (cct = 0.032, $P < 0.0001$; fig. 3).

r_{MF} and Evolution of Sex-Biased Gene Expression

The presence of genetic constraint for evolution of sex-biased gene expression should result in a negative association between the r_{MF} and the degree of sex bias. Sex-biased gene expression was indeed negatively associated to the r_{MF} for genes in *D. melanogaster* when only significant genes were included (estimated coefficient for sex-biased expression [csb] = −0.125, $P < 0.0001$; fig. 4), as well as when all genes were included (csb = −0. 100, $P < 0.0001$).

We also tested for an association between the r_{MF} in *D. melanogaster* and the degree to which genes have changed their sex-biased expression between *D. melanogaster* and six other *Drosophila* species, to test whether the genetic architecture in *D. melanogaster* is informative of the extent to which genes can change in their sex bias. In all cases, we found a negative association between the r_{MF} and the degree of change in sex-biased expression, both when only significant genes were included (estimated coefficient for $\Delta_{D.\ melanogaster-D.\ simulans}$ [$c\Delta_{D.\ simulans}$] = −0.074, $P = 0.015$; $c\Delta_{D.\ yakuba} = -0.192$, $P < 0.0001$; $c\Delta_{D.\ ananassae} = -0.066$,

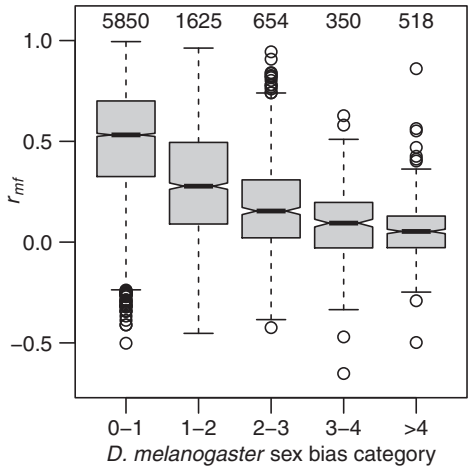

Fig. 4. r_{MF} and the degree of sex-biased genes expression within *Drosophila melanogaster*, for genes with significant variation. Notches on the boxes represent approximate 95% CIs. Numbers above the boxes show how many genes each box represents.

$P = 0.0005$; $c\Delta_{D.\ pseudoobscura} = -0.156$, $P < 0.0001$; $c\Delta_{D.\ virilis} = -0.128$, $P < 0.0001$; and $c\Delta_{D.\ mojavensis} = -0.100$, $P < 0.0001$; fig. 5), as well as when all genes were included ($c\Delta_{D.simulans} = -0.148$, $P < 0.0001$; $c\Delta_{D.\ yakuba} = -0.212$, $P < 0.0001$; $c\Delta_{D.\ ananassae} = -0.092$, $P < 0.0001$; $c\Delta_{D.\ pseudoobscura} = -0.162$, $P < 0.0001$; $c\Delta_{D.\ virilis} = -0.143$, $P < 0.0001$; and $c\Delta_{D.\ mojavensis} = -0.115$, $P < 0.0001$).

Discussion

Although theory predicts that a shared genome should pose a severe constraint on the evolution of sexual dimorphism (Lande 1980, 1987), empirical studies have given mixed support for this prediction (Delph et al. 2004, 2010; Bonduriansky and Rowe 2005; McDaniel 2005; Ashman and Majetic 2006; Fairbairn and Roff 2006; Fairbairn et al. 2007; Poissant et al. 2010; Leinonen et al. 2011). A possible explanation for this discrepancy is that previous studies have suffered from low power as they have either dealt with a limited number of traits or compiled data from many different studies and taxa. Here, we take advantage of the fact that gene expression can be viewed as a phenotypic trait and that thousands of phenotypes can be studied simultaneously through whole genome transcriptome analysis. By analyzing variation in gene expression in a population of *D. melanogaster*, and comparing it with several species in the *Drosophila* genus, we provide strong and manifold evidence that a shared genetic architecture causes a severe constraint on the evolution of sexual dimorphism.

A high r_{MF} should constrain the evolution of sexual dimorphism, and the fact that traits at a high phenotypic organizational level (morphological, physiological, behavioral, and life-history traits) have a median r_{MF} of approximately 0.8 (reviewed in Poissant et al. 2010) indicates that most traits should be constrained. As traits at a high level of phenotypic organization are composed of phenotypes at a lower level, logic suggests that the r_{MF} should be of similar magnitude also for these traits. In our study, this, at first glance, does not seem to be the case as the estimated median r_{MF} for gene expression level across the *D. melanogaster* genome is only approximately 0.3. However, when we gradually filtered out genes for which the r_{MF} was estimated with poor precision, a different pattern emerged, which suggests that the true r_{MF} for gene expression probably approaches the high value found for typical phenotypic traits.

If a shared genetic architecture poses a constraint for sex-specific evolution, intralocus sexual conflict over expression level, caused by sexually antagonistic selection, should remain unresolved longer for genes with a high r_{MF}. From this, it follows that genes with expression levels currently exposed to sexually antagonistic selection should have a higher r_{MF} than other genes. Our analyses give strong support for this prediction, despite that the r_{MF} estimates of each gene came from one population (Raleigh, North America) and assignment of selective regimes for the same genes came from another population (Modesto, North America). The fact that our predicted relationship holds between these two populations, that have been separated by more than 500 generations (Rice et al. 2005), indicates that the shared genetic architecture continues to constrain evolution for at least hundreds of generations, and that it is not rapidly broken down by sexually antagonistic selection (but see Delph et al. [2011] for an example where artificial selection for a reduced r_{MF} was successful over just a few generations).

A further prediction, with respect to the genetic architecture and its role in constraining the evolution of sexual dimorphism, is that there should be a negative association between the degree of sexual dimorphism and the strength of the r_{MF} (Lande 1980; Bonduriansky and Rowe 2005; Fairbairn and Roff 2006). This is expected to result if only traits with an initially low r_{MF} can respond to sex-specific selection or if genes with long-term exposure to sexually antagonistic selection evolve a reduced r_{MF}. This prediction has received mixed support in previous analyses on traits at a higher phenotypic organization (Delph et al. 2004, 2010; Bonduriansky and Rowe 2005; McDaniel 2005; Ashman and Majetic 2006; Fairbairn and Roff 2006; Fairbairn et al. 2007; Poissant et al. 2010; Leinonen et al. 2011). In this analysis, at the level of gene expression variation, we find substantial support for this prediction, as genes with a high degree of sex-biased expression, in general, show a substantially lower r_{MF} than genes with a more similar expression in the sexes.

A negative association between the r_{MF} and the degree of sex-biased expression can possibly also arise through genomic imprinting. Males and females that have successfully reached the mating stage will probably have a phenotype that suits their sex better than the average male and female phenotype in the population. Sons would therefore benefit from expressing their father's phenotype, and daughters from their mother's, rather than the average phenotype of their parents. Day and Bonduriansky (2004) have suggested that this problem can be solved through genomic imprinting, where sons primarily express the haploid genome they inherit from their father and daughters the haploid genome they inherit from their mother. If this were the case, imprinted genes would display a higher level of sexual dimorphism and a reduced

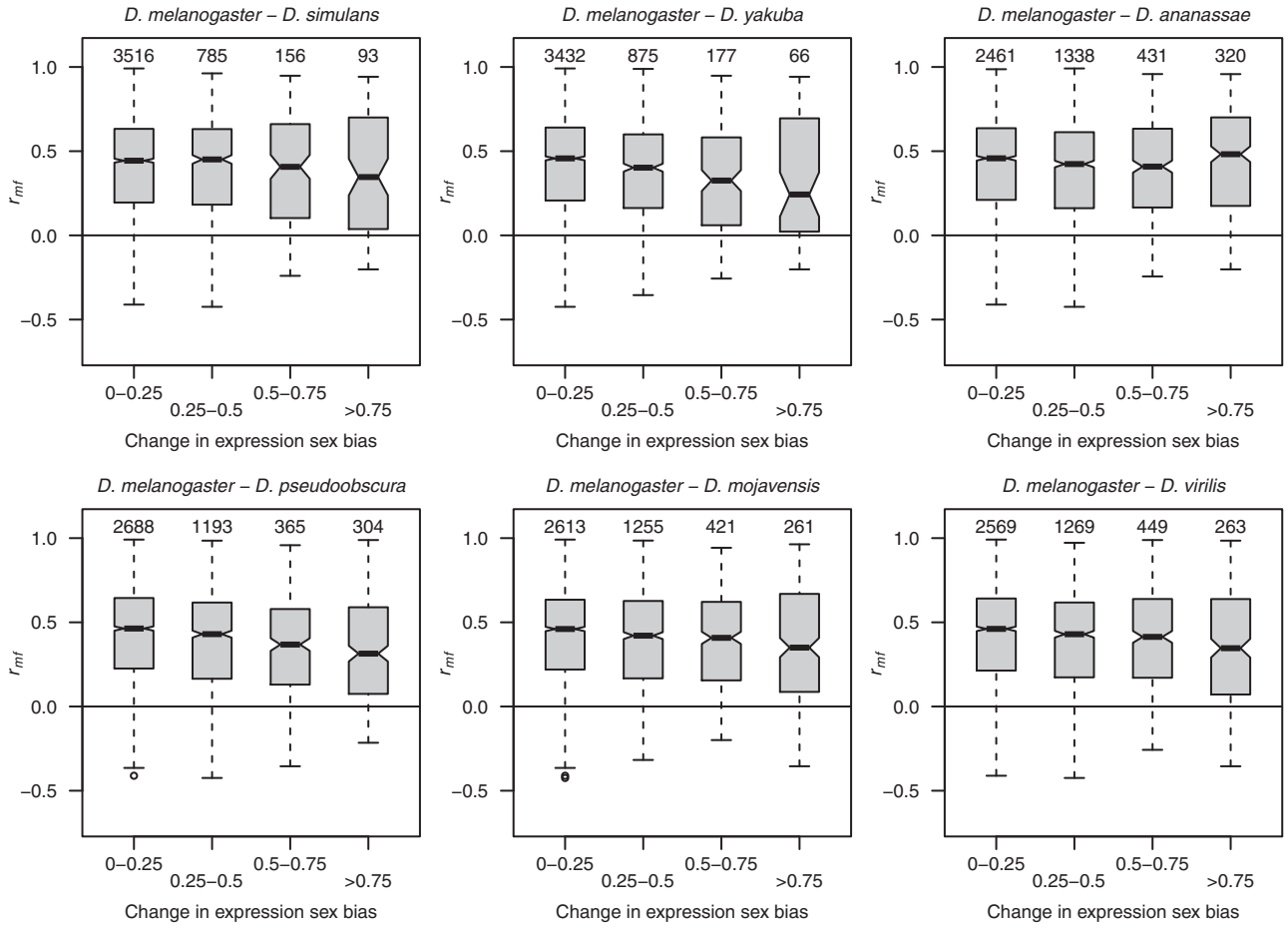

Fig. 5. r_{MF} and the degree of change in gene expression sex bias between *Drosophila melanogaster* and six other species in the *Drosophila* genus, for genes with significant genetic variation that were present in all six species. Notches on the boxes represent approximate 95% CIs. Numbers above the boxes show how many genes each box represents.

realized r_{MF}, compared with nonimprinted genes. This process could thus give rise to a negative association between the r_{MF} and the degree of sex-biased gene expression. However, although this is a plausible scenario, we do not think it applies to the negative association we document here for two reasons. First, there is very little evidence for genomic imprinting in *Drosophila* (Coolon et al. 2012). Second, in this study, we use gene expression data from inbred individuals. Males and females from the same inbred line thus had a mother and a father of the exact same genotype. It is therefore not possible for sons to express different alleles than daughters, even if sons would only express genes inherited from their father and daughters only from their mother.

The evidence we present for how a shared genetic architecture constrains the evolution of sexual dimorphism is based on both within- and between-population comparisons. However, if a shared genetic architecture is a true obstacle for the evolution of sex-specific phenotypes, constraints should remain over long periods of time. We find support for this hypothesis as r_{MF} values in *D. melanogaster* predict the extent to which evolutionary change in sex bias has occurred between *D. melanogaster* and its closest relative, *D. simulans*, as well as the more distantly related species in the *Drosophila* genus we tested here. There is no obvious trend in terms of how the strength of the negative association between the r_{MF} and the degree of change in sex-biased expression change with phylogenetic distance. We nevertheless suggest that a plausible scenario is that the change in sex bias between closely related populations is often very small, since drift and novel selection has not had the time to move traits far from their values at time of divergence. The negative association between the r_{MF} and change in degree of sex bias would then probably increase with time and reach a minimum at some point, after which it should revert back toward zero as the predictive value of the genetic architecture of a distantly related relative becomes less informative. These data presented here do not corroborate such a U-shaped relationship. The lack of support for this hypothesis may be because none of the species we studied have had enough time to completely dissociate their genetic architecture from *D. melanogaster*, although *D. melanogaster* and *D. virilis*/*D. mojavensis* are estimated to have separated approximately 60 Ma (Tamura et al. 2004). Alternatively, sex bias evolving by drift with constant mutation rates and stabilizing selection would cause the relationship between the change in sex bias and r_{MF} to remain more stable over large phylogenetic

distances (Bedford and Hartl 2009). Our results nevertheless provide strong evidence that a shared genetic architecture can constitute a long-term constraint on the evolution of sex-biased expression.

Theory predicts that sexual dimorphism should more easily evolve on the X-chromosome (Rice 1984). However, empirical studies that have tested this hypothesis for traits at a higher organizational level have been inconclusive (Reinhold 1998; Fitzpatrick 2004; Chenoweth et al. 2008; Mank 2009; Husby et al. 2013). In the case of the genomic distribution of sex-biased genes, the X-chromosome plays a special role, but usually it is only overrepresented with genes biased in either the female or the male direction and not in both (reviewed in Ellegren and Parsch 2007). A corollary to the above prediction is that the sex chromosomes should host more sex-specific genetic variation than the autosomes (Chenoweth et al. 2008; Fairbairn and Roff 2006) and thus that X-linked genes should have a reduced r_{MF} compared with autosomal genes. We find some support for this prediction, but the effect is rather small. These results hence appear not to offer support of a strong role for the X-chromosome with respect to sex-specific genetic variation. A potential caveat with this conclusion is that our r_{MF} values are estimated from variation among inbred lines. When the r_{MF} for X-linked genes is estimated from inbred genotypes in *D. melanogaster*, males and females essentially have the same genotype, because, dosage compensation makes males produce as much gene product as females from their single X. When genetic correlations are estimated from outbred genotypes this may not be the case, as females are heterozygous, whereas males are effectively homozygous for X-linked loci. This contrasts to the autosomes where both sexes will have the same levels of heterozygosity and, as such there is more potential for X-linked than autosomal sex-specific variation. Similarly though, despite substantial inbreeding in the DGRP lines, residual heterozygosity could also contribute to our observation of a slightly lower r_{MF} on the X-chromosome.

Collectively, our results provide strong evidence that the shared genome is a pervasive constraint on the evolution of sexual dimorphism. Previous attempts to show this have given equivocal results, which is surprising given that intralocus sexual conflict seems ubiquitous in both laboratory and wild populations (Chippindale et al. 2001; Rand et al. 2001; Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Foerster et al. 2007; Brommer et al. 2007; Cox and Calsbeek 2009; Mainguy et al. 2009). The pressing question then becomes; how are generally strong genetic constraints compatible with rapid evolution of sexual dimorphism, on both the trait (Darwin 1871; Meyer 1997; Arnqvist 1998; Civetta and Singh 1998; Omland and Lanyon 2000; Emlen et al. 2007) and gene expression level (Coulthart and Singh 1988; Civetta and Singh 1995; Meiklejohn et al. 2003; Zhang et al. 2004; Zhang and Parsch 2005)? Although strong sex-specific selection acting on genes with a moderate to high r_{MF} probably contributes to resolving this paradox to a small extent, the main explanation is probably different. Sex-specific selection primarily targets specific sets of genes and it is plausible that the r_{MF} values for a subset of these have evolved over time to become relatively low. These genes would then have the capacity to rapidly respond to shifts in sex-specific selection and could hence contribute largely to the rapid diversification of sexual traits between species. One such example could be genes affecting cuticular hydrocarbon (CHC) profiles. In *Drosophila*, the CHC profiles is sexually dimorphic (Fervuer and Cobb 2010) has a low r_{MF} (Sharma, Mitchell, et al. 2012) and respond rapidly to selection (Sharma, Hunt, et al. 2012). The subsets of genes regularly exposed to novel sex-specific selection do probably still frequently contribute to intralocus sexual conflict, at least transiently, because the r_{MF} of most of these genes is slightly positive and sex-specific optima probably change rapidly. Genes that primarily contribute to intralocus sexual conflict are, however, more likely to be found among pleiotropic genes (Mank, Hultin-Rosenberg, Zwahlen et al. 2008), and genes that, for other architectural reasons, are constrained from evolving a reduced r_{MF}.

Materials and Methods

Gene Expression Data

We used published data from three different sources in this study. To estimate the r_{MF} and the degree of sex-biased expression for each gene in *D. melanogaster*, we used data from the study by Ayroles et al. (2009). These data consist of whole body microarrays from 40 inbred genotypes, all derived from a single population. The raw data were downloaded from http://www.ebi.ac.uk/arrayexpress/experiments/E-MEXP-1594 (last accessed July 23, 2013) and normalized using RMA (Irizarry et al. 2003). To test whether genes currently exposed to sexually antagonistic selection have a higher r_{MF} than other genes, we gathered information on the selection regime that gene expression is under from a study of a different population of *D. melanogaster* (Innocenti and Morrow 2010). In this study, the authors measured fitness and genome-wide gene expression in males and females for a set of genotypes derived from one outbred population and used regression analysis to establish which genes were exposed to sexually antagonistic selection for gene expression. Data were collected from an online depository (http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.1000335#s4, last accessed July 23, 2013). To calculate the extent to which genes have changed with respect to their degree of sex bias between *D. melanogaster* and other *Drosophila* species, we used whole body microarray data from the study of Zhang et al. (2007). Data were downloaded from the Gene Expression Omnibus, GEO accession: GSE6640 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6640, last accessed July 23, 2013) and normalized using RMA (Irizarry et al. 2003).

Estimation of the Intersexual Genetic Correlation

The intersexual genetic correlation (r_{MF}) (Lynch and Walsh 1997) was estimated for each gene using the mean value of the two microarray samples for each sex (Ayroles et al. 2009).

The 95% CIs around the median of each described category were estimated by bootstrapping the data 10,000 times.

r_{MF} and Evolution of Sex-Biased Gene Expression

All analyses were conducted on two data sets: one including all genes (n = 12,572) and one including only those genes for which there was significant genetic variation (n = 8,997) (discussed earlier). In all analyses, we used linear regression to test for associations between the r_{MF} and the various variables we were interested in (gene selective regime, chromosome linkage, degree of sex-biased expression within *D. melanogaster* and degree of change in sex-bias expression between *D. melanogaster* and other *Drosophila* species). In all these analyses, we included expression level (μ) and tissues specificity (τ) as covariates, because these two variables have been shown to influence various aspects of sequence and expression evolution (Nuzhdin et al. 2004; Larracuente et al. 2008). For example, the relationship between r_{MF} and sex bias (sb) was modeled as $E[r_{MF}] = \alpha + \beta_1 \mathrm{sb} + \beta_2 \mu + \beta_3 \tau$. We defined μ as mean expression level across the sexes in the *D. melanogaster* data from the study by Ayroles et al. (2009), and τ was estimated as $= \sum \left[1 - \left(\log_2(t_i)/\log_2(t_{\max})\right)\right]/(n-1)$, where t_i is expression in tissue i and t_{max} is the expression in the tissue with the highest gene expression. Values of expression level in each tissue were taken from the FlyAtlas database (Chintapalli et al. 2007). Expression level and tissue specificity were both positively and significantly related to the r_{MF} in all analyses. Removing these covariates from the analyses did, however, have only a very small effect on the association between r_{MF} and any of the focal variables. We report only on the coefficient of interest and the corresponding P value.

Sex-biased gene expression was estimated as $|\log_2(\mathrm{M/F})|$, and the degree to which genes have changed with respect to sex-biased expression between *D. melanogaster* and *D. simulans*, *D. yakuba*, *D. ananassae*, *D. pseudoobscura*, *D. virilis*, or *D. mojavensis* was estimated by $|\log_2(\mathrm{M/F})_{D.\ melanogaster} - \log_2(\mathrm{M/F})_{D.\ x}|$. In these analyses, only genes that were present in all species (all genes n = 5,857, significant genes n = 4,550) were used in the pair wise comparisons. We took this approach to not change the power with which we tested for an association between the r_{MF} and the change in sex bias with phylogenetic distance. All figures were produced in the R software environment (R Development Core Team 2011) and all statistical analyses were conducted in S-plus.

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