E-Article

Tissue Specificity and Sex-Specific Regulatory Variation Permits the Evolution of Sex-Biased Gene Expression

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abstract: Genetic correlations between males and females are often thought to constrain the evolution of sexual dimorphism. However, sexually dimorphic traits and the underlying sexually dimorphic gene expression patterns are often rapidly evolving. We explore this apparent paradox by measuring the genetic correlation in gene expression between males and females (C_{mf}) across broad evolutionary timescales, using two RNA-sequencing data sets spanning multiple populations and multiple species. We find that unbiased genes have higher C_{mf} than sex-biased genes, consistent with intersexual genetic correlations constraining the evolution of sexual dimorphism. However, we found that highly sex-biased genes (both male and female biased) also had higher tissue specificity, and unbiased genes had greater expression breadth, suggesting that pleiotropy may constrain the breakdown of intersexual genetic correlations. Finally, we show that genes with high C_{mf} showed some degree of sex-specific changes in gene expression in males and females. Together, our results suggest that genetic correlations between males and females may be less important in constraining the evolution of sex-biased gene expression than pleiotropy. Sex-specific regulatory variation and tissue specificity may resolve the paradox of widespread sex bias within a largely shared genome.

Keywords: intersexual genetic correlation, cross-sex genetic correlations, sexual dimorphism, genetic constraint, tissue specificity, pleiotropy.

Introduction

The evolution of two sexes from the same genome (Pennell and Morrow 2013) has presented a puzzle for evolutionary biologists (Lande 1980; Fairbairn et al. 2007). Traits controlled by genes that are sex limited in their expres sion—including but not limited to genes on the sex-limited Y and W chromosomes—should be unconstrained because they are selected for in only one sex (Lande 1980). Yet very few genes are truly sex limited (Moghadam et al. 2012), and so phenotypic sexual dimorphism must largely be a product of different use in males and females of the genes that they share (Pointer et al. 2013).

Constraints to the evolution of sexual dimorphism could arise if genes present in both sexes also share the same regulatory genetic variation (Lande 1980). One way to quantify the constraints resulting from shared regulatory architecture is to measure intersexual genetic correlations (r_{mf}) for a given trait, traditionally calculated using quantitative genetic breeding designs (Lynch and Walsh 1997; Poissant et al. 2010). This approach can be extended to a multivariate **B** matrix approach to measure genetic covariances between multiple traits in males and females (Lewis et al. 2011; Stearns et al. 2012; Gosden and Chenoweth 2014). Other approaches—such as use of inbred lines (Ayroles et al. 2009; Griffin et al. 2013) and hemiclonal lines (Ingleby et al. 2014)—can also be used to tap into the genetic component and explore the relationship between intersexual genetic correlations and sexual dimorphism.

Using these approaches, a negative relationship between sexual dimorphism and intersexual genetic correlation has been identified for both phenotypic traits and gene expression (Reeve and Fairbairn 2001; Bonduriansky and Rowe 2005; McDaniel 2005; Fairbairn et al. 2007; Poissant et al. 2010; Griffin et al. 2013). This relationship has been interpreted as an intersexual genetic constraint on the evolution of sexual dimorphism and implies the presence of unresolved conflict between males and females (Cox and Calsbeek 2009; Poissant et al. 2010). However, there are two possible ways this relationship can form (Fairbairn et al. 2007). It is possible that genes or traits with low initial intersexual genetic correlation and therefore low constraint for sexual dimorphism are more likely to become sex biased under sex-specific selection. Under this explanation, the high intersexual genetic correlation indeed acts as a hindrance to the evolution of dimorphism. Alternatively, sex-specific selection might rapidly break down intersexual genetic correlation, leading to sexual dimorphism. Under this latter scenario, higher intersexual correlations for sexually

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monomorphic traits are not the result of constraint but simply because these traits have not been subjected to contrasting sex-specific selection.

Whether the shared genome imposes a constraint on the evolution of sexual dimorphism therefore depends on how easily selection can break down intersexual genetic correlations. Although we have little information on the flexibility of these genetic correlations in wild populations, a selection experiment showed that strong intersexual genetic correlations could be rapidly broken down under a novel artificial selection pressure (Delph et al. 2011), suggesting that intersexual genetic correlations may not be rigid architectures impervious to selection. Additionally, a substantial number of genome-wide association studies have revealed sex-specific regulatory architecture (Randall et al. 2013; Yao et al. 2013; Wen et al. 2014; Shungin et al. 2015), suggesting that for many complex phenotypes, regulatory networks differ substantially between males and females, thereby alleviating potential constraints in the evolution of sexual dimorphism. Furthermore, sex differences in gene regulation have been shown to vary substantially across related species (Zhang et al. 2007; Harrison et al. 2015), suggesting that if shared regulatory architecture does constrain sexual dimorphism, those constraints can be quickly broken by opposing selection in males and females.

The degree to which selection can break down intersexual genetic correlations may also depend on pleiotropy, since pleiotropic constraint is expected to impede evolvability (Yanai et al. 2005; Mank et al. 2008). Genes that have pleiotropic expression—such as genes expressed in multiple tissues—may experience a slower rate of breakdown of intersexual genetic correlations for any one function due to stabilizing selection in other functions. In contrast, genes with tissue-specific expression may be subject to less pleiotropic constraint and therefore show more rapid response to sex-specific selection pressures. The gonad hosts an abundance of sex-biased genes, and these genes may also be tissue specific in their expression. Differences in tissue specificity between sex-biased and unbiased genes may therefore play an important role in whether intersexual genetic correlations constrain the evolution of sex-biased expression (Mank et al. 2008; Meisel 2011).

The question remains, do intersexual genetic correlations constrain the evolution of sexual dimorphism such that there is widespread conflict between males and females over trait expression, or can sexual dimorphism readily evolve under sex-specific selection or tissue-specific expression? Here, we aim to explore the implications of negative correlations between sexual dimorphism and the intersexual genetic correlation (Reeve and Fairbairn 2001; Bonduriansky and Rowe 2005; McDaniel 2005; Fairbairn et al. 2007; Poissant et al. 2010; Griffin et al. 2013). We use two RNA-sequencing data sets spanning (1) short evolutionary timescales across six different populations (breeds) of chicken selected for male- or female-specific traits and (2) long evolutionary timescales (80–90 million years) across six species of birds from the Galloanserae clade that show variation in mating system and degree of morphological sexual dimorphism (Harrison et al. 2015). We measure the correlation in gene expression between males and females (C_{mf}; Dean et al. 2015) for each orthologous gene in the two data sets. We then explore how sex-biased gene expression, tissue specificity of expression (τ; a measure of pleiotropy), and expression evolution are related to C_{mf}. In accordance with previous studies (Griffin et al. 2013), we find that genes with expression differences between the sexes have lower between-sex genetic correlations for expression than genes with similar expression between the sexes across both short and long evolutionary timescales. However, we find that genetic constraint between the sexes may be more easily broken down when genes have tissue-specific expression. Furthermore, even genes with high genetic correlations can show sex-specific changes in gene expression, suggesting that rather than genetic correlations being a constraint on sexual dimorphism, sex-specific regulatory variation and lack of pleiotropic constraint may permit the evolution of sex-biased gene expression.

Methods

Short Evolutionary Timescale Expression Data Set (Population Level)

Gonad samples were dissected from six chicken breeds, representing different populations of the same species (*Gallus gallus*) that have been under artificial selection for egg laying (white Leghorn, black Minorcan), both meat and egg production (Rhode Island red), male fighting ability (Oxford Old English game) and male plumage (Yokahama) in addition to the ancestor of domestic chickens, the red jungle fowl. Four samples for each sex were collected, apart from Yokohama, for which we had three female samples. These samples comprise the population data set. Data are deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive: http://www.ncbi.nlm.nih.gov/sra/?term=PRJNA284655 (Moghadam et al. 2012*a*, 2012*b*).

Long Evolutionary Timescale Expression Data Set (Species Level)

Gonad and spleen were collected from captive-reared males and females at the start of their first breeding season for *Anas platyrhynchos* (mallard duck), *Meleagris gallopavo* (wild turkey), *Phasianus colchicus* (common pheasant), *Numida meleagris* (helmeted guinea fowl), *Pavo cristatus* (Indian peafowl), and *Anser cygnoides* (swan goose). Five samples for

each sex were collected for each species, apart from common pheasant, for which we had six males and five females. Data are deposited in the NCBI Sequence Read Archive: http://www.ncbi.nlm.nih.gov/sra/?term=PRJNA271731 (Harrison et al. 2015*a*, 2015*b*).

Data Processing and Expression Analysis

For both data sets, RNA was extracted and prepared using standard protocols (Moghadam et al. 2012; Dean et al. 2015; Harrison et al. 2015; Wright et al. 2015) and sequenced by the Wellcome Trust Centre for Human Genetics, University of Oxford. For the population data set, sequences were mapped to the chicken reference genome (WUGSC2.1/galGal3) and normalized across all samples in order to make possible comparisons across populations (Moghadam et al. 2012). Quality control, de novo assembly, and ortholog detection have been described previously (Harrison et al. 2015; Wright et al. 2015). Expression levels for species-level data were obtained by mapping reads to de novo assemblies, and in addition to standard normalization across samples within each species, a scaling factor was applied in order to make possible comparisons across species (Brawand et al. 2011; Lin et al. 2012; Harrison et al. 2015).

Sex-biased gene expression was identified using $\log_2$ fold change gene expression differences between males and females (i.e., $\log_2$ male : female expression ratios, where 1 indicates twice the expression in males compared with females and -1 indicates twice the expression in females compared with males) and *t*-tests corrected for multiple testing ($P_{adj} < .05$; Moghadam et al. 2012; Harrison et al. 2015) to identify which genes had large and significant differences in expression between the sexes. The sex-biased genes defined using our method were broadly consistent with the edgeR method, an alternative differential expression method (Robinson et al. 2010), producing an overlap in sex-biased expression of 89%–96% between both approaches (Wright et al. 2015).

For the six-species data set, genes used in all subsequent analyses were restricted to reciprocal 1-1 orthologs across all six species that were expressed in either sex. We filtered out genes with expression <2 reads per kilobase of million mappable reads in both sexes and then removed any genes that were not expressed in all six of the species (Dean et al. 2015), resulting in 2,729 autosomal genes. Similar filtering thresholds were used in the population data set, resulting in 9,698 autosomal genes.

Because of the incomplete Z chromosome dosage compensation in birds (Itoh et al. 2007) and the unique sex-specific selection pressures shaping sex chromosomes (Vicoso and Charlesworth 2006; Mank et al. 2010; Bachtrog et al. 2011), we confine our analysis here to autosomal loci. Among the autosomal genes that we examined, sex-biased expression evolves rapidly across populations and species. We have previously shown that roughly only one-third of genes show consistent patterns of sex bias across our six study species (Harrison et al. 2015), with rapid changes between male- and female-biased expression common. Across populations, we see a similar pattern, with approximately 27% of all sex-biased genes consistently sex biased in the same direction. Because of the rapid change in sex bias, we computed analyses involving transcriptional dimorphism for each population or species separately.

Measuring the Intersexual Correlation in Gene Expression

The correlation in gene expression between males and females (C_{mf}) was calculated for each orthologous autosomal gene in each of the two data sets, using Spearman's ρ correlation coefficient to measure the rank order correlation of gene expression between males and females. This measures the degree to which regulatory variation and evolution is concordant or discordant between the sexes across species or populations. Measures of C_{mf} for each gene were comprised of a correlation across six populations or species, and each data point within the correlation was the average expression across four to five replicates per sex for each population or species.

For the species-level data, it is possible to correct for phylogeny. We therefore also calculated the correlation in gene expression between males and females, using phylogenetic generalized least squares models (PGLS) in the Caper package (Orme et al. 2012; R ver. 2.15.1), using the maximum likelihood phylogeny and r^2 as a measure of the strength of the correlation (Dean et al. 2015). For the PGLS approach, sex-limited genes were removed from the analysis because this results in too little variation to conduct independent contrasts, resulting in 2,555 autosomal genes. For consistency between the two data sets, we present ρ measures for C_{mf} here and present PGLS r^2 estimates for the species-level data set in the appendix, available online.

Tissue Specificity

We used tissue specificity (τ) as a measure of pleiotropy. Tissue specificity (Yanai et al. 2005) was calculated from UniGene expressed sequence tag assemblage for chicken (http://www.ncbi.nlm.nih.gov/unigene), as previously described (Mank et al. 2008). The calculation of τ takes into account the number of tissues examined and the highest expression level detected for each gene over all tissues measured (Yanai et al. 2005; Mank et al. 2008). Tissues used in the calculation of τ included blood, cartilage, central nervous system, digestive tract, epiphyseal growth plate, eye, genitourinary tract, heart, limb, and lymphoreticular system. Values of τ range from 0 to 1, with high values of τ rep-

resenting greater tissue specific expression and low values representing even expression across the tissues examined. We had τ estimates for 7,558 genes in the population-level data set and 2,143 genes in the species-level data set.

Change in Gene Expression across Males and Females

For each pairwise comparison between species, we calculated the change in gene expression for male expression and for female expression for each gene. Gene expression is calculated on a $\log_2$ scale; therefore, a change of 1 unit represents doubled expression between the two species, and a change of -1 represents halved expression. In each data set, data were divided into genes with a high C_{mf} (top quartile) and low C_{mf} (lower quartile), and change in gene expression in males was plotted against change in expression in females. The relationship between change in gene expression in males and females was analyzed using major axis regression, using the package lmodel2 (Legendre 2014) in R (ver. 3.1.3; R Development Core Team 2015).

Results

The Relationship between Intersexual Genetic Correlation and Sex-Biased Gene Expression

We first explored the relationship between C_{mf} (Dean et al. 2015) and $\log_2$ male:female gene expression ratios ($\log_2$ m:f). Across both short (fig. 1*A*–1*F*) and long (fig. 1*G*–1*L*) evolutionary timescales, we recovered quadratic relationships between C_{mf} and $\log_2$ m:f, such that genes with higher expression in males ($\log_2$ m:f > 0) and genes with higher expression in females ($\log_2$ m:f < 0) had lower C_{mf} than genes that were unbiased in their expression ($\log_2$ m:f ~ 0). Tests for constancy of variance in C_{mf} across male:female expression ratios suggest that there is heteroscedasticity (Levene's tests, $P < .05$ for each population and species) such that variance in C_{mf} increases with magnitude of male:female expression ratios. We therefore also ran robust fitting of linear models, using rlm in the MASS package in R (Venables and Ripley 2002) to allow for heteroscedasticity of variance. In both data sets, we also see variation in the pattern of C_{mf} and sex bias such that genes with strong sex bias (deviations of $\log_2$ m:f from 0) can have high C_{mf} (fig. 1). The overall pattern is qualitatively similar (fig. A1; figs. A1–A3 available online) if C_{mf} is calculated using PGLS to control for shared ancestry in the species-level data set (Dean et al. 2015). Using absolute C_{mf} also produces similar patterns (fig. A2).

Tissue Specificity

To explore the role of tissue specificity on C_{mf}, we used a measure of tissue specificity (τ), where lower values indicate even expression distribution across tissues and larger values equate to greater levels of tissue specificity. Values of τ were derived from the chicken UniGene database (Mank et al. 2008). For each population (fig. 2*A*–2*F*) and species (fig. 2*G*–2*L*), as the magnitude of sex bias increases (deviations of $\log_2$ m:f from 0), so too does tissue specificity. The relationship between C_{mf} and τ appears slightly different between the population- and species-level data sets, and this may be due to the fact that τ estimates are based on data from chicken. Although these estimates are likely to hold up broadly across closely related species, some specific loci might vary in τ in a species-specific manner.

Change in Male and Female Gene Expression

We have previously observed that gene expression evolution is substantially greater in males than in females in birds (Harrison et al. 2015), suggesting that expression evolution is somewhat decoupled between the sexes. In order to explore this further, we selected the upper quartiles of the C_{mf} distribution in the population-level ($C_{mf} > 0.71$, $N_{genes} = 2{,}038$) and species-level ($C_{mf} < 0.66$, $N_{genes} = 669$) data sets as well as the lower quartile of the population-level ($C_{mf} < 0.09$, $N_{genes} = 2{,}372$) and species-level ($C_{mf} < 0.03$, $N_{genes} = 647$) data sets. We then measured the correlated change in gene expression for males and females for each pairwise comparison. For genes with high C_{mf}, an increase (or decrease) in gene expression in males correlates with expression change in females (figs. 3, 4, plots above diagonal). Across this gene set, the slope of the regression is <1 in each of the pairwise comparisons within the species data set, such that a large change in expression in males co-occurs with a smaller change in expression in females. For genes with low C_{mf}, there is generally no association between change in gene expression in males and females (figs. 3, 4, plots below diagonal), and a change in expression in one sex is unrelated to change in expression in the other sex.

We repeated this for genes expressed in the spleen in the species data set. In the spleen, C_{mf} is generally high for all genes (Dean et al. 2015), and change in gene expression in males and females is highly correlated for genes in both the upper (fig. A3, plots above diagonal; $C_{mf} = 1$, $N_{genes} = 699$) and lower (fig. A3, plots below diagonal; $C_{mf} < 0.83$, $N_{genes} = 408$) quartiles of genes, according to their C_{mf} score.

Discussion

We tested the relative role of intersexual genetic correlations in gene expression (C_{mf}) and τ, a measure of tissue specificity, as potential constraints in the evolution of transcriptional dimorphism.

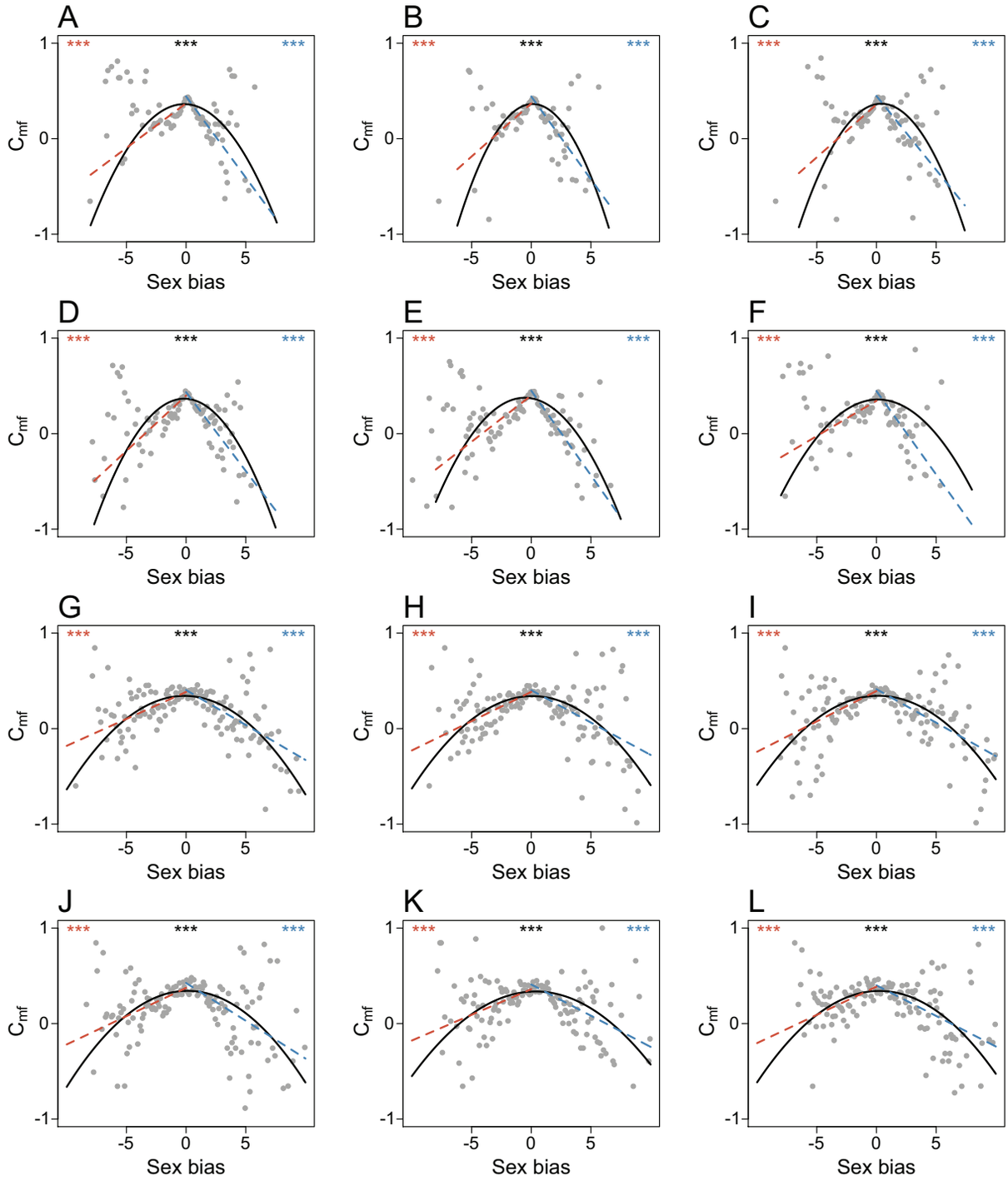

Figure 1: C_{mf} and sex bias ($\log_2$ male:female [m:f] expression ratio) across populations (*A–F*) and species (*G–L*). *A*, Black Minorcan. *B*, White Leghorn. *C*, Rhode Island red. *D*, Oxford Old English. *E*, Yokahama. *F*, Red jungle fowl. *G*, Common pheasant. *H*, Wild turkey. *I*, Indian peafowl. *J*, Guinea fowl. *K*, Swan goose. *L*, Mallard duck. Genes are binned into increments of 0.1 $\log_2$ m:f, and mean values per bin are plotted. Quadratic regressions, shown in black, are weighted by number of genes in each bin. $P > .05$ for the linear components of the quadratic fit in all populations and species. The intercept differs from 0 in all cases ($P < .0001$). Robust regressions for male-biased genes ($\log_2$ m:f > 0) are shown in blue and female-biased genes ($\log_2$ m:f < 0) in red. Three asterisks, $P < .001$.

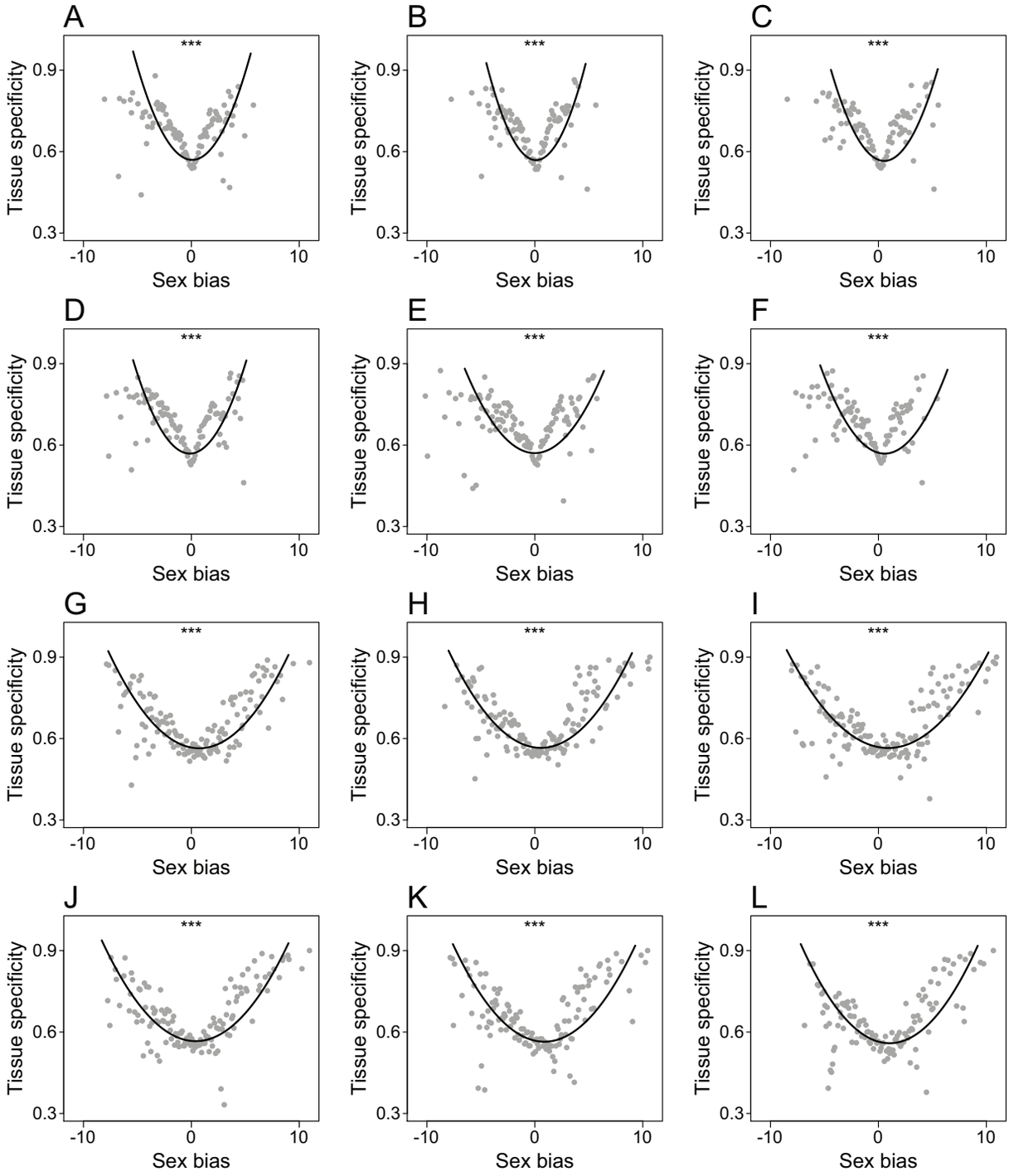

Figure 2: Tissue specificity (τ) and sex bias ($\log_2$ male:female [m:f] expression ratio) across populations (*A–F*) and species (*G–L*). *A*, Black Minorcan. *B*, White Leghorn. *C*, Rhode Island red. *D*, Oxford Old English. *E*, Yokahama. *F*, Red jungle fowl. *G*, Common pheasant. *H*, Wild turkey. *I*, Indian peafowl. *J*, Guinea fowl. *K*, Swan goose. *L*, Mallard duck. Genes are binned into increments of 0.1 $\log_2$ m:f, and mean values per bin are plotted. Quadratic regressions, shown by the black line, are weighted by number of genes in each bin. Three asterisks, $P < .001$.

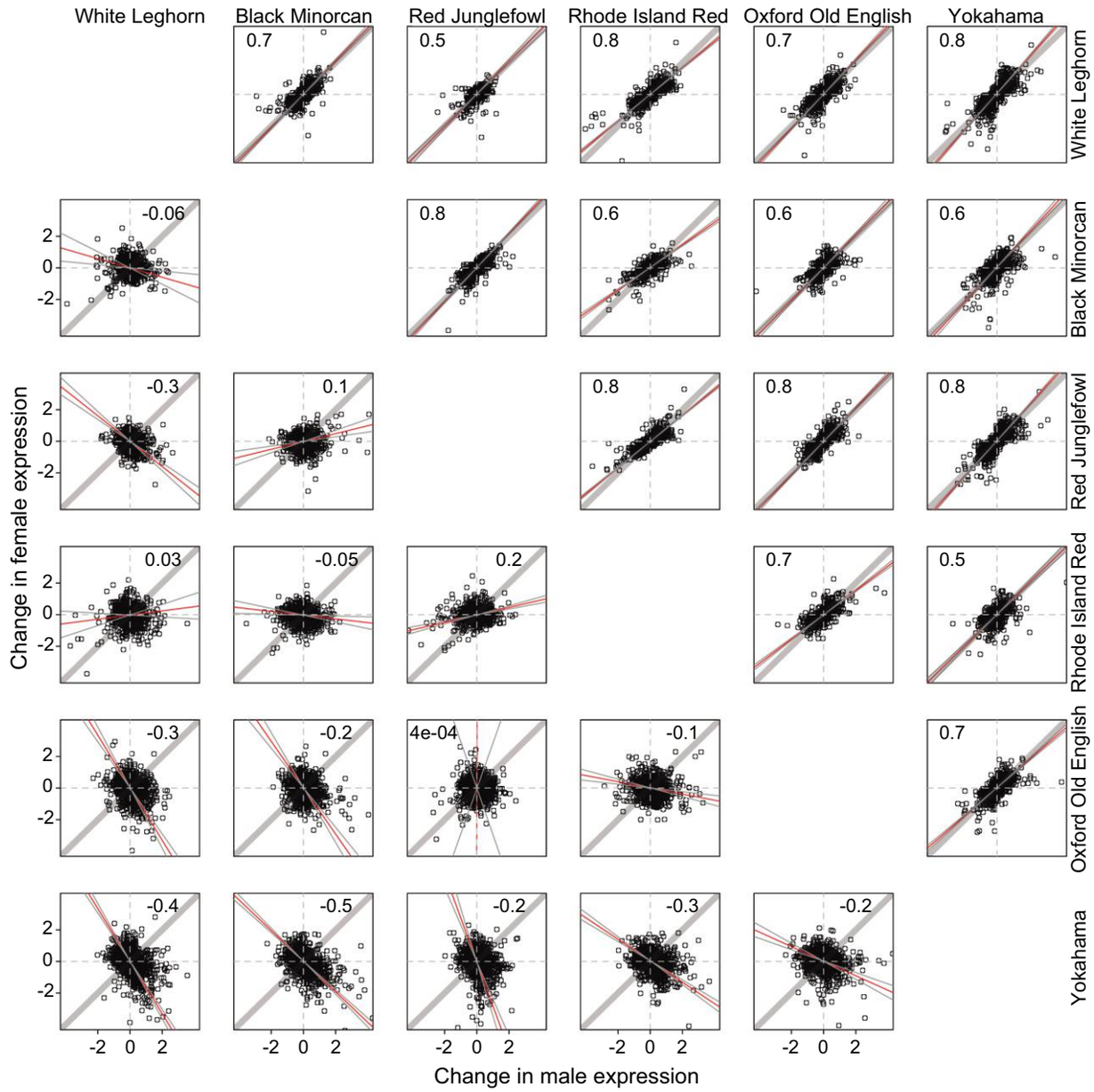

Figure 3: Change in $\log_2$ gene expression in the gonad between males and females for each pairwise comparison between populations. Genes with a high C_{mf} (within the top quartile) are shown in the plots above the diagonal, and genes with a low C_{mf} (within the bottom quartile) are shown in the plots below the diagonal. The red line is the major axis regression, with the 95% confidence interval in gray. The thick gray line has a gradient of 1. The correlation coefficient (r) for the major axis regression is presented in each graph.

Intersexual Genetic Correlation

We find that C_{mf} declines as the magnitude of sex bias increases. Such a pattern could be interpreted as consistent with intersexual genetic correlations constraining the evolution of sexual dimorphism (Poissant et al. 2010; Griffin et al. 2013); however, although there is a statistical association, not all genes strictly conform to this relationship, with some genes having high C_{mf} yet pronounced sex bias (fig. 1). Moreover, in general, there is greater variance in C_{mf} as sex bias increases. This suggests that sex bias in many cases can evolve without a substantial reduction in C_{mf} and that low

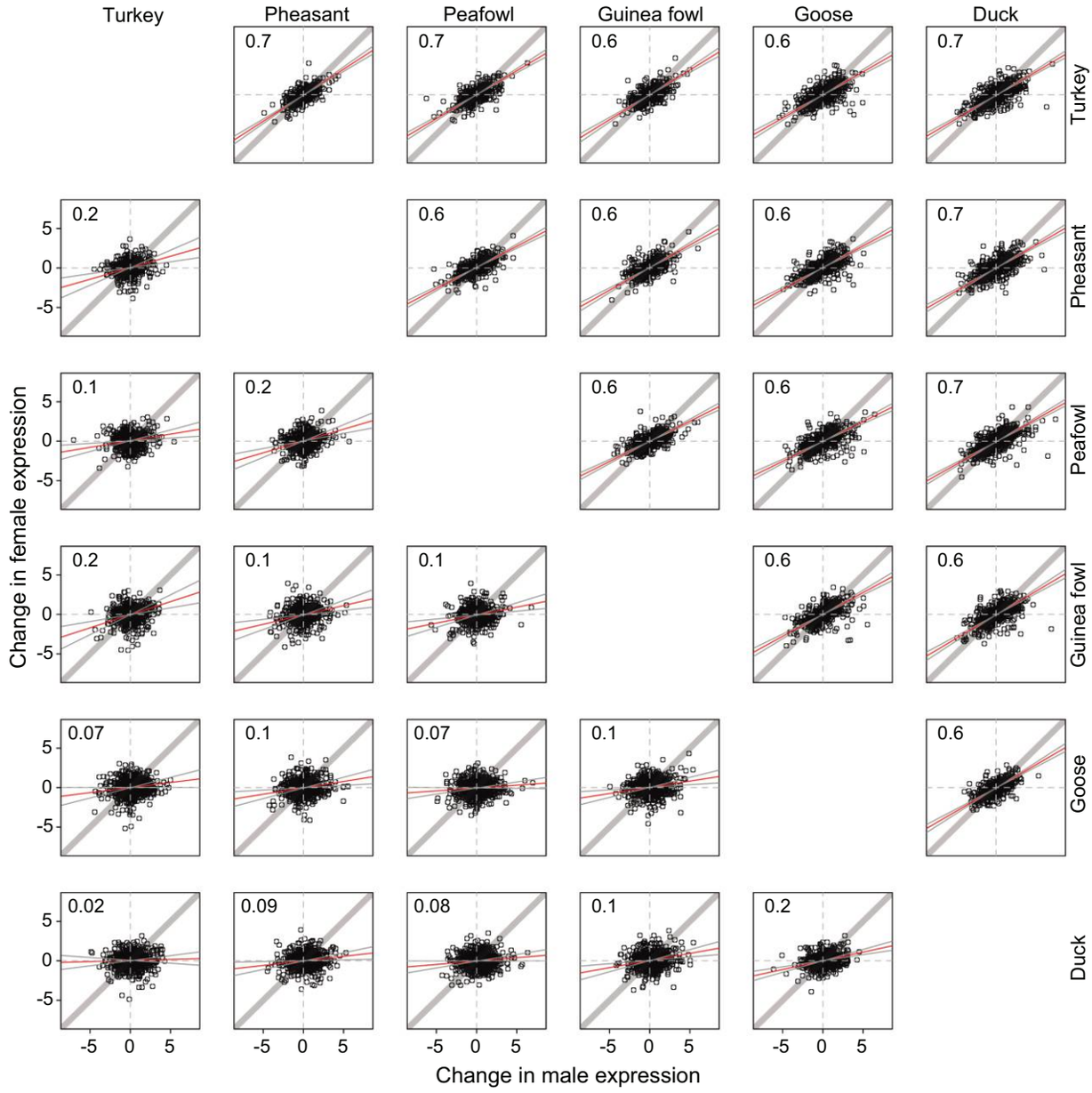

Figure 4: Change in $\log_2$ gene expression in the gonad between males and females for each pairwise comparison between species. Genes with a high C_{mf} (within the top quartile) are shown in the plots above the diagonal, and genes with a low C_{mf} (within the bottom quartile) are shown in the plots below the diagonal. The red line is the major axis regression, with the 95% confidence interval in gray. The thick gray line has a gradient of 1. The correlation coefficient (r) for the major axis regression is presented in each graph.

C_{mf} is not always the result of past selection for sex-biased gene expression.

It is perhaps perplexing as to how sex bias can evolve for loci with high intersexual correlations. However, in the species data set, we found that loci with high C_{mf} show correlations significantly <1 between male and female expression (fig. 4). This is consistent with selection driving changes in male gene expression with a reduced response in female expression (Harrison et al. 2015). More importantly, this suggests that even for genes with high intersexual transcriptional correlation, a substantial amount of regulatory variation is sex specific in its effects. Our results therefore show that for genes with high C_{mf}, expression change in one sex need not produce the same magnitude of change in the

other sex, providing a route to sex-biased expression while maintaining intersexual genetic correlation. However, it is worth noting that ancestral sex bias could influence C_{mf} for genes that have recently evolved similar transcription in males and females.

Another important consideration is that phenotypic expression relies on protein levels, and the genome-wide correlations between mRNA and protein abundance can vary. Although some report low estimates of the correlation in protein and mRNA abundance—for example, 0.4 by Schwanhausser et al. (2011)—other estimates are substantially higher and can range from 0.60 to 0.70 (Futcher et al. 1999; Greenbaum et al. 2003; Lundberg et al. 2010). More importantly, overall patterns of sex bias appear to be broadly conserved between mRNA and protein (Uebbing et al. 2015). Estimates for RNA abundance are generally more accurate, and our data had high pairwise correlations among within-sex replicates (Moghadam et al. 2012; Harrison et al. 2015). However, there was substantial variation in C_{mf} among genes within each expression class, and this could influence our estimates for extreme sex bias, given the restricted number of loci in these categories. The influence of this variance is reduced by our analysis of expression (bins) and by the large number of expressed loci overall (9,698 for the population-level analysis and 2,729 for the species-level analysis).

Additionally, intersexual genetic correlations may not be a rigid genetic architecture (Delph et al. 2011), and experimental evolution to reduce the genetic correlation in plants found that it could be rapidly broken down, albeit using a selection pressure that may be unlikely to occur often in natural populations (Delph et al. 2011). In this study, we found that sex-biased genes at the population level showed a similar—if not more pronounced—reduction in C_{mf} than sex-biased genes within the species data set, suggesting that selection can change C_{mf} even across short evolutionary timescales.

This study was conducted only on autosomal genes because there are few female-biased genes on the avian Z chromosome as a result of the incomplete dosage compensation in birds (Mank and Ellegren 2009). However, sex chromosomes could play an important role in sex-specific transregulation of autosomal genes (Stocks et al. 2015) because they have high rates of expression evolution (Meisel et al. 2012; Dean et al. 2015) and are thought to be important for sexual antagonism (Rice 1984; Gibson et al. 2002; Dean et al. 2012), although whether this special role of the sex chromosomes extends to sexually dimorphic phenotypes is still questionable (Dean and Mank 2014). Despite hosting an enrichment of sex-biased genes (Parisi et al. 2003; Mank 2009; Dean and Mank 2014), sex chromosomes tend to show only a small reduction (Griffin et al. 2013) or no significant reduction (Dean et al. 2015) in the intersexual genetic correlation, further suggesting that there are alternative routes to the evolution of sexual dimorphism that do not require the breakdown of the intersexual genetic correlation.

Tissue Specificity

Tissue specificity is expected to affect evolvability because genes expressed in many tissues are subject to greater overall regulatory constraint through greater pleiotropy. We explored how tissue specificity (τ), a proxy for pleiotropy, affects the breakdown of intersexual genetic correlations. As previously observed (Mank et al. 2008; Meisel 2011), we found that sex-biased genes have greater tissue specificity, and this reduced pleiotropic constraint may mean that intersexual genetic correlations can be more rapidly broken down for tissue-specific genes than those with broader expression patterns. In contrast, unbiased genes have lower tissue specificity, and pleiotropy may constrain the breakdown of C_{mf} in genes that are expressed in a number of different tissues. Genes with narrow expression may be more able to respond to sex-specific selection than genes with broad expression. However, it is important to note (1) that our measures of tissue specificity were derived from chicken and may have changed across species and (2) that even after controlling for tissue specificity, a negative relationship between sex bias and intersexual genetic correlation is still observed (Griffin et al. 2013).

Concluding Remarks

Although the problem of encoding two sexes from a shared genome theoretically poses potential conflict between males and females over trait expression (Pennell and Morrow 2013), a single genome manages to encode multiple different tissues with little apparent problem (Khaitovich et al. 2006). This suggests that tissue-specific regulatory variation can overcome genetic correlations among tissues. Our data suggest that sex-specific regulatory variation can act in a similar fashion. Sex-specific or tissue-specific regulatory variation is clearly key in the evolution of regulatory diversity.

In conclusion, we find a negative relationship between the magnitude of sexual dimorphism in gene expression and the genetic correlation in transcription between males and females, similar to the relationship reported in previous studies (Reeve and Fairbairn 2001; Bonduriansky and Rowe 2005; Poissant et al. 2010; Griffin et al. 2013). However, we find substantial variation, and not all genes conform to this relationship. Expression breadth and pleiotropy are also likely to be important factors affecting the constraint on gene expression evolution. Our results suggest that tissue-specific expression and sex-specific regulatory variation may permit the evolution of sexual dimorphism and provide resolution to the paradox of how sex differences can evolve

from a genome that is largely shared between males and females.

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Extreme sexual dimorphism, as exhibited in the peafowl, is able to evolve from a genome that is largely shared between males and females. Sex-specific gene expression regulation and tissue-specific gene expression may be key in the evolution of regulatory diversity. Photo credit: Yvonne and Greg Dean.