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Wright, A.E., Rogers, T.F., Fumagalli, M. et al. (2 more authors) (2019) Phenotypic sexual dimorphism is associated with genomic signatures of resolved sexual conflict. Molecular Ecology. ISSN 0962-1083

https://doi.org/10.1111/mec.15115

PHENOTYPIC SEXUAL DIMORPHISM IS ASSOCIATED WITH GENOMIC SIGNATURES OF RESOLVED SEXUAL CONFLICT

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ABSTRACT

Intra-locus sexual conflict, where an allele benefits one sex at the expense of the other, has an important role in shaping genetic diversity of populations through balancing selection. However, the potential for mating systems to exert balancing selection through sexual conflict on the genome remains unclear. Furthermore, the nature and potential for resolution of sexual conflict across the genome has been hotly debated. To address this, we analysed *de novo* transcriptomes from six avian species, chosen to reflect the full range of sexual dimorphism and mating systems. Our analyses combine expression and population genomic statistics across reproductive and somatic tissue, with measures of sperm competition and promiscuity. Our results reveal that balancing selection is weakest in the gonad, consistent with the resolution of sexual conflict and evolutionary theory that phenotypic sex differences are associated with lower levels of ongoing conflict. We also demonstrate a clear link between variation in sexual conflict and levels of genetic variation across phylogenetic space in a comparative framework. Our observations suggest that this conflict is short-lived, and is resolved via the decoupling of male and female gene expression patterns, with important implications for the role of sexual selection in adaptive potential and role of dimorphism in facilitating sex-specific fitness optima.

INTRODUCTION

Males and females in many species often have divergent evolutionary interests and are subject to conflicting selection pressures (Andersson 1994). However, with the exception of the sex chromosomes, the sexes share an identical genome, and this can give rise to intra-locus sexual conflict, where an allele benefits one sex at the expense of the other (Parker and Partridge 1998). This shared genomic architecture is thought to hamper males and females simultaneously evolving towards their respective fitness peaks, and in turn acts as a constraint in the evolution of sexual dimorphism (Mank 2017; Rowe, et al. 2018; Stewart and Rice 2018).

Recently, studies have used population genomic statistics to detect the signature of sexual conflict across the genome (Cheng and Kirkpatrick 2016; Lucotte, et al. 2016; Mank 2017; Mostafavi, et al. 2017; Dutoit, et al. 2018; Rowe, et al. 2018; Wright, et al. 2018). Ongoing sexual conflict can arise from a number of different factors and these leave distinct population genomic signatures in sequence data (Mank 2017; Wright, et al. 2018). Sexual conflict can result over reproduction, where an allele increases the reproductive fitness of one sex at a cost to the other (Barson, et al. 2015; Lonn, et al. 2017). Alternatively, sexual conflict can result when an allele has differential effects on survival between males and females (Czorlich, et al. 2018). Both of these scenarios are predicted to result in elevated genetic diversity and higher Tajima's D, a population genomic statistic that estimates the proportion of polymorphic nucleotide sites in a given sequence within a population.

To distinguish between sexual conflict arising over reproduction or survival, it is necessary to employ contrasts with intersexual F_{ST} (Lewontin and Krakauer 1973), which measures divergence in allele frequency between males and females within a generation. As allele frequencies are identical between the sexes at conception, different allele frequencies in male and female adults are assumed to be the result of sexual conflict over survival. Elevated F_{ST} can therefore be used to identify alleles that have differential effects on survival parameters, including viability, mortality or predation. By contrasting these two population genomic statistics, it is possible to determine the relative importance of conflict over reproduction, which only leads to

increased Tajima's D, versus conflict over survival, which leads to elevated Tajima's D and intersexual F_{ST} (Mank 2017; Wright, et al. 2018).

Population genomic approaches such as these have made it possible to investigate the manifestation of different types of intra-locus sexual conflict at the genomic level and the mechanisms by which they can be resolved. In theory, sexual conflict should be most prevalent in genes with similar expression patterns in males and females, where mutational inputs will be manifest in both sexes. Ultimately, sexual conflict is thought to be resolved via the evolution of sex-biased gene expression (Connallon and Knowles 2005; Ellegren and Parsch 2007), which, because of primary expression in one sex or the other, in principle allows for the emergence of male- and female-specific fitness optima (Mank 2017). However, the exact nature of the relationship between sex-biased gene expression and resolved sexual conflict has been hotly debated, with some recent studies suggesting that sex-biased genes are subject to ongoing sexual antagonism (Cheng and Kirkpatrick 2016; Dutoit, et al. 2018). If true, this suggests that sexual conflict can persist even after gene expression diverges between males and females, and is potentially an unrelenting constraint on sex-specific optima. It would also suggest that, although expressed primarily in one sex, sex-biased genes function similarly in both males and females, and are therefore not appropriate for studying molecular signatures of sex-specific selection, as is often done (Ellegren and Parsch 2007).

Moreover, the signature of balancing selection for sex-biased genes detected by recent studies is discordant with the rapid molecular evolutionary rates of directional selection (Meiklejohn, et al. 2003; Pröschel, et al. 2006; Zhang, et al. 2007) and relaxed constraint (Gershoni and Pietrokovski 2014; Harrison, et al. 2015; Dapper and Wade 2016) observed in this class of genes across a wide variety of species. At the same time, and consistent with the molecular signatures observed, other work has suggested that sex-biased genes represent resolved conflict, and therefore exhibit lower average levels of balancing selection than unbiased genes (Connallon and Knowles 2005; Mank 2009; Innocenti and Morrow 2010; Wright, et al. 2018). If broadly true, this suggests that conflict is prevalent in genes with similar expression patterns between the sexes, and is primarily resolved through regulatory

121 decoupling of males and females into separate male and female genetic
122 architectures. This conclusion is intuitively concordant with the fact that sex-biased
123 genes are primarily expressed in either males or females, and also suggests that
124 sexual conflict is a short-lived constraint, given the rapid turn-over in sex-biased
125 gene expression across related species (Zhang, et al. 2007; Harrison, et al. 2015).

126 Importantly, recent theoretical work indicates that implausibly large selective
127 pressures and mortality loads are required to generate the patterns of intersexual
128 F_{ST} observed in the literature attributed to ongoing sexual antagonism (Kasimatis, et
129 al. 2017; Kasimatis, et al 2019). This calls into question the application of F_{ST} based
130 approaches for detecting sexual conflict arising from survival differences between
131 the sexes. Consistent with this, a recent study found evidence that elevated
132 intersexual F_{ST} for sex-biased genes is actually the product not of sexual conflict, but
133 of sex-specific genetic architecture (Wright, et al. 2018), where an allele only affects
134 one sex or the other. Sex-specific genetic architecture invokes relatively lower
135 genetic loads, and there is increasing evidence that many loci exhibit profound sex
136 differences in their phenotypic effects (Gilks, et al. 2014; Dapper and Wade 2016;
137 Karp, et al. 2017). Similarly, recent analyses of large genomic datasets indicated only
138 a very small number of loci subject to antagonistic selection on survival (Mostafavi,
139 et al. 2017; Czorlich, et al. 2018).

140 Furthermore, a major challenge in evolutionary biology is to explain the
141 maintenance and variation in genetic diversity across many species. The existence of
142 elevated genetic diversity relative to neutral expectations across species is puzzling,
143 as directional selection and drift are both expected to erode variation. However,
144 there is increasing evidence that intra-locus sexual conflict, through balancing
145 selection, can significantly increase genome-wide patterns of variability
146 (Chippindale, et al. 2001; Foerster, et al. 2007; Delcourt, et al. 2009; Mokkonen, et
147 al. 2011; Hawkes, et al. 2016; Lonn, et al. 2017). Therefore, variation in sexual
148 conflict across lineages, likely mediated by mating systems, could drive variation in
149 genetic diversity across species and resolve this apparent paradox. However, the
150 exact nature of the relationship between sexual conflict, mating system and genetic
151 diversity remains unclear. Sexual conflict also has important implications for sexual

selection, adaptation and evolvability. For instance, on the one hand, balancing selection would be expected to slow rates of sequence evolution arising from directional selection. However, balancing selection can also facilitate rapid adaptation from standing variation by maintaining multiple alleles within the population at high allele frequencies (Charlesworth 2006; Hartl and Clark 2006).

In order to assess the degree to which sex-biased genes exhibit signatures of unresolved conflict and the potential for mating systems to exert balancing selection through sexual conflict on the genome, it is necessary to compare population genomic patterns of species and tissues with different levels of sexual dimorphism. We therefore estimated population genomic statistics for genes expressed in reproductive and somatic tissue across six avian species spanning the full range of mating systems and sexual selection in birds. Reproductive tissue has multiple sex-specific functions and is phenotypically more sexually dimorphic, whereas the function of many somatic tissues is largely similar in males and females. By exploiting natural variation in the magnitude of sexual conflict across the body plan within individuals, as well as across mating systems between species, we were able to study the manifestation and resolution of sexual conflict, and subsequent genomic and phenotypic consequences. Our results reveal that the resolution of genomic sexual conflict is associated with the evolution of phenotypic sex differences. We demonstrate a clear link between variation in sexual conflict over reproduction and levels of genetic variation across phylogenetic space in a comparative framework.

MATERIALS & METHODS

Tissue collection

We previously extracted RNA from the left gonad and spleen of individuals with the RNeasy Kit (Qiagen), following the manufacturer's instructions, from the following captive avian populations; mallard duck (*Anas platyrynchos*), wild turkey (*Meleagris gallopavo*), common pheasant (*Phasianus colchicus*), helmeted guinea fowl (*Numida meleagris*), Indian peafowl (*Pavo* cristatus) and swan goose (*Anser cynoides*) (Harrison, et al. 2015) (Figure 1). These captive populations are not maintained with sterile or biosafety conditions. Samples were collected during the first breeding season from five males and five females of each species, with the exception of the

pheasant, where six male gonad and spleen samples were collected, and turkey where four male and two female spleens were collected.

These six species were deliberately chosen to reflect a full range of sexual dimorphism, ranging from monogamous and sexually monomorphic species such as the swan goose and guineafowl, to polygynous and sexually dimorphic species such as the peafowl and wild turkey. We estimated the intensity of sexual conflict in each species using three proxies of sperm competition and male promiscuity; sexual dichromatism score, sperm number and relative testes size, obtained from Harrison et al. 2015.

Transcriptome assembly

Samples were sequenced on an Illumina HiSeq 2000 with 100 bp paired-end reads and are available in the NCBI SRA (BioProject ID PRJNA271731). We assembled and filtered transcriptomes for each species using previously implemented approaches (Harrison, et al. 2015). Briefly, we quality filtered RNA data using Trimmomatic v0.36 (Bolger, et al. 2014) to filter reads containing adaptor sequences and trim reads if the sliding window average Phred score over four bases was <15 or if the leading/trailing bases had a Phred score <3. Reads were removed post filtering if either read pair was <36 bases in length. We assembled a *de novo* transcriptome for each species using Trinity v2.4.0 (Grabherr, et al. 2011) with default parameters. We then filtered each transcriptome to remove spurious and low confidence genes. First, we selected the 'best isoform' per gene to avoid redundancy. We used the Trinity script align_and_estimate_abundance.pl to map RNA-seq reads to transcriptomes using bowtie2 and to quantify expression for each sample using RSEM. We suppressed unpaired and discordant alignments for paired reads. We then picked the most highly expressed isoform per gene to obtain a set of 'best isoforms' for each species. RNA-seq reads were remapped to the set of 'best isoforms' in each species using the same approach as above to ensure consistency between expression and sequence data. Second, we filtered the transcriptome to remove lowly expressed genes. Specifically, we removed genes with expression < 2FPKM in half or more of the individuals in either tissue. We assessed the

completeness of our transcriptome assembly using eukaryota_odb9 BUSCO v3.0.2 (Waterhouse, et al. 2018) (Table S1).

Identification of orthologs

We used BLAST (Altschul, et al. 1990) to identify orthologous genes across the six species. First, we identified pairwise reciprocal orthologs between the chicken reference genome (Gallus_gallus-5.0) and the wild turkey, common pheasant, helmeted guinea fowl, and Indian peafowl, and between the duck reference genome (BGI_duck_1.0) and mallard duck, and swan goose (Zerbino, et al. 2018). We downloaded cDNA sequences from Ensembl (Zerbino, et al. 2018) and selected the longest transcript per gene. We ran reciprocal BLASTn with an e-value cut-off of 1 x 10^{-10} and selected the best hit reciprocal ortholog using a minimum percentage identity of 30% and the highest bitscore following previous approaches (Harrison, et al. 2015; Wright, et al. 2018). If two hits shared the same highest bitscore, then the hit with the highest percentage identity was chosen. If both hits had the same highest bitscore and percentage identity, the gene was discarded.

For the wild turkey, common pheasant, helmeted guinea fowl, and Indian peafowl, we assigned chromosomal location and gene position from the pairwise reciprocal ortholog in the chicken reference genome. Chromosomal positional information is not available in the duck reference genome and so we used a synteny based approach to obtain chromosomal location using MScanX (Wang, et al. 2012). Briefly, we downloaded chicken and duck protein sequences from Ensembl, selected the longest protein per gene in each species, and then conducted a reciprocal BLASTp with an e-value cut-off of 1 x 10^{-10}. We restricted the number of BLASTp hits for each gene to the top five, generated gff files, and concatenated the duck and chicken results as recommended by MScanX. We then identified syntenic regions between the duck and chicken reference genome using MScanX run with default parameters. For the mallard duck and swan goose, we assigned chromosomal location and gene position from the syntenic information available for the pairwise reciprocal ortholog in the duck reference genome. For all species, we split genes into autosomal or Z-linked based on location in the chicken reference genome (Table S1) as evolutionary

forces including sexual conflict act differently across these genomic regions (Rice 1984; Wright and Mank 2013).

Second, we identified reciprocal orthologs using the same approach across all species using the chicken and duck reference genomes to assign chromosomal location. This resulted in 1,457 autosomal reciprocal orthologs, which we used to contrast population genetic statistics across species. Finally, potential immune loci were identified from GOterms in Biomart in the chicken and duck reference genomes (Zerbino, et al. 2018). Specifically, we removed all loci with the terms 'immune' or 'MHC' in their Gene Ontology annotations from subsequent analyses. This was to reduce any potential confounding effects as heterozygote advantage in immunity can produce patterns of balancing selection independent of sexual conflict (Stahl, et al. 1999; Hedrick 2011; Ghosh, et al. 2012).

Gene expression analyses

Read counts for autosomal and Z-linked genes were extracted for all gonad and spleen samples and normalized using TMM in EdgeR (Robinson, et al. 2010). We identified gonad-biased, spleen-biased, and non-tissue-biased genes using a standard $\log_2$ fold change value of 2 (Wright, et al. 2018) in each species (Tables S2 & S3). The gonad is transcriptionally more sexually dimorphic than the spleen and so we identified tissue-biased genes in each sex separately instead of combining all samples to avoid biasing our analyses against highly sex-biased or sex-limited genes. We report results from tissue-biased genes identified in males in the main text but results based on tissue-biased genes identified from female expression data are fully detailed in SI. The results are qualitatively identical unless otherwise indicated. Sex-biased genes were identified in each set of tissue-biased genes separately using a $\log_2$ fold change value of 1. We identified tissue-biased genes on the Z chromosome separately due to the unique expression profile of the avian Z chromosome arising from incomplete dosage compensation (Itoh, et al. 2007; Mank and Ellegren 2008; Wright, et al. 2012).

Filtering data for population genomic analyses

Population genomic analyses were conducted on BAM files generated by mapping RNA-seq data to the set of 'best isoforms' in each species with RSEM. For each

individual, we merged the spleen and gonad BAM files using SAMtools (Li, et al. 2009). The exception was the turkey, where the spleen and gonad were not sequenced for all individuals so we used only gonad data for subsequent analyses.

We used ANGSD (Korneliussen, et al. 2014) to estimate population genetic summary statistics, following our previous approach (Wright, et al. 2018) as ANGSD implements methods to account for sequencing uncertainty and is appropriate for uneven sequencing depth associated with transcriptome data. We filtered BAM files to discard reads if they did not uniquely map, had a flag >=256, had a mate that was not mapped or had a mapping quality below 20. Bases were filtered if base quality fell below 13 or there was data in less than half the individuals. Mapping quality scores were adjusted for excessive mismatches and quality scores were adjusted around indels to rule out false SNPs.

We identified and removed related individuals (four peacock, two wild turkey and two swan goose individuals) from our analyses using ngsRelate (Korneliussen and Moltke 2015) to avoid violating Hardy Weinberg assumptions, and calculated inbreeding coefficients using an EM algorithm with the ngsF package in ngsTools (Fumagalli, et al. 2014) (full details in SI Methods). For all species, inbreeding coefficients were <0.03 with the exception of the peacock where we identified two inbred individuals. We incorporated inbreeding coefficients for the peacock in subsequent analyses.

Calculating Tajima's D

ANGSD was used for each species to calculate sample allele frequency likelihoods at each site from genotype likelihoods calculated with the SAMtools model. We calculated allele frequency likelihoods separately for the Z chromosome and the autosomes as they are subject to different evolutionary pressures and differ in ploidy. The Z chromosome is diploid in males yet haploid in females, therefore, we used only male samples to estimate allele frequency to avoid violating Hardy Weinberg assumptions. Next, we estimated the overall unfolded site frequency spectrum (SFS) for each species (Nielsen, et al. 2012) (Figure S1). Specifically, at each site we randomly sampled an allele frequency according to its likelihood, as calculated by ANSGD. Finally, we computed genetic diversity indices, including allele

frequency posterior probability and Tajima's D using the site frequency spectrum as prior information with ANGSD thetaStat (Korneliussen, et al. 2014).

For each species, we calculated a relative measure of Tajima's D for spleen-biased and gonad-biased genes. Specifically, we quantified median D relative to non-tissue-biased genes, our neutral estimate of D for each species. Calculating a relative measure of Tajima's D makes it possible to circumvent problems arising from demographic changes in population size that would otherwise bias comparative analyses of population genetic statistics across species.

Calculating intersexual F_{ST}

Intersexual F_{ST} was calculated using the same procedure and filtering criteria as Tajima's D, except that RNA-seq data were instead filtered to remove bases where we had data in less than half the individuals in males and females separately. This ensures we do not exclude sex-limited genes from the analysis. Hudson's F_{ST}, which is less sensitive to small sample sizes (Bhatia, et al. 2013), was estimated as implemented in ANGSD (Korneliussen, et al. 2014). Estimates across loci were obtained using weighted averages (see Fumagalli et al 2013, equations 4 and 12), where per-gene F_{ST} is the ratio between the sum of the between-populations variance across loci and the sum of the total variance across loci. Given the Z chromosome is haploid in females, we do not have the power to analyze patterns of F_{ST} across the Z chromosome in this study.

RESULTS

Lower levels of ongoing sexual conflict in reproductive versus somatic tissue

Reproductive tissue, such as the gonad, has many sex-specific functions whereas the function of somatic tissue, such as the spleen, is more aligned between male and female fitness. In order to test whether phenotypic sexual dimorphism is associated with resolved sexual conflict at the genomic level, we contrasted population genomic statistics between genes expressed in the gonad versus the spleen.

As heterozygote advantage in immunity can produce patterns of balancing selection independent of sexual conflict (Stahl, et al. 1999; Hedrick 2011; Ghosh, et al. 2012),

we removed all loci with potential immune function from downstream analyses. We found that median Tajima's D is significantly lower for gonad-biased genes relative to genes expressed in both tissues in all species across the autosomes (Figures 2 & S2, panels A). This result is consistent with lower levels of ongoing sexual antagonism in the gonad. In contrast, we found no significant difference in Tajima's D between spleen-biased genes and loci expressed in both tissues in the majority of species. We observe consistent patterns on the Z chromosome (Figure S5), however, our power to detect statistically significant differences is reduced due to limited numbers of tissue-biased Z-linked genes (Table S1).

The proportion of sex-biased genes varies across the spleen and gonad (Harrison, et al. 2015) and sex-biased genes are subject to different selective pressures (Ellegren and Parsch 2007; Harrison, et al. 2015) as well as distinct patterns of balancing selection relative to unbiased genes (Cheng and Kirkpatrick 2016; Dutoit, et al. 2018; Wright, et al. 2018). In order to ensure that differences in the number of sex-biased genes between the two tissues are not responsible for the lower Tajima's D we observe in gonad-biased genes, we repeated the analyses using Tajima's D calculated only from unbiased genes in each tissue. We find a consistent pattern across the majority of species, where Tajima's D is significantly lower in gonad-biased but not spleen-biased genes relative to loci expressed similarly in both tissues (Figure S3). However, these species differ in mating system, which could explain the variation in the strength of balancing selection we observe across species, addressed in more detail below.

It is important to note that multiple factors can influence population genetic statistics for any particular locus. Therefore, we tested whether our results could also be attributed to the effect of covariates that might vary across tissue-biased genes. We incorporated measures of gene length, average expression level, GC content and Watterson's theta into a multiple regression (TD ~ Tissue bias + log(tW) + log(Gene length) + log(GC) + log(Gene expression level)). Tissue-bias remains a significant factor in explaining variation in Tajima's D once accounting for these covariates (Table S11). However, the effect size in some species is relatively small, indicating that the pattern we detect is subtle and influenced by multiple factors.

366 ***Limited power of intersexual F_{ST} to detect sexual conflict arising over survival***

367 We tested the power of intersexual F_{ST} to detect sexual conflict arising over survival
368 through contrasts between the spleen and gonad. Given its role in the lymphatic
369 system and in filtering blood components, we might expect the spleen to be subject
370 to viability selection more so than the gonad, whose role is primarily reproductive.
371 We removed sex-biased genes from this analysis to avoid biasing the results, as the
372 abundance of sex-biased expression differs between reproductive and somatic tissue
373 and previously we have shown that intersexual F_{ST} is often elevated for sex-biased
374 genes (Cheng and Kirkpatrick 2016; Dutoit, et al. 2018; Wright, et al. 2018).

375 We contrasted intersexual F_{ST} for gonad and spleen-biased genes using three
376 approaches. First, we found no significant difference in median F_{ST} for unbiased
377 genes expressed primarily in the gonad relative to those expressed broadly across
378 both the gonad and spleen (Table S4). We observed the same pattern in the spleen,
379 with the exception of the goose and turkey where F_{ST} was elevated marginally.
380 Second, there was no significant difference in the number of unbiased genes with
381 elevated intersexual F_{ST} that were expressed primarily in the gonad compared to
382 those with non-tissue-specific expression patterns (Table 1). We observe the same
383 result in the spleen, with the exception of the turkey. However, all of these
384 differences become non-significant when we analyse tissue-biased genes identified
385 from female expression data (Tables S5 & S6). Lastly, we found no significant effect
386 of tissue bias on F_{ST} after accounting for gene length, average expression level, GC
387 content and Watterson's theta in a multiple regression (TD ~ Tissue bias + log(tW) +
388 log(Gene length) + log(GC) + log (Gene expression level)) (Table S11).

389 Intriguingly, despite the limited potential role of the gonad in survival, elevated
390 intersexual F_{ST} has been previously detected in gonad expressed genes in flycatchers
391 (Dutoit, et al. 2018). Consistent with this, we find a weak relationship between
392 intersexual F_{ST} and sex-biased gene expression in the gonad, where F_{ST} is significantly
393 elevated in sex-biased genes in some species (Figures S7, Table S12). However, it is
394 important to note that our power to quantify intersexual F_{ST} is limited by our sample
395 size. Whilst our results are consistent with flycatchers, the associated effect sizes are
396 weak (sex-bias and F_{ST} for gonad-biased genes r^2 =0.000-0.042, spleen-biased genes

r^2 =0.000-0.008). Most importantly, our results are consistent with theoretical work suggesting that intersexual divergence in allele frequency may not always be a reliable indicator of ongoing sexual conflict over viability (Kasimatis, et al. 2017; Kasimatis, et al 2019), particularly in studies with low numbers of samples.

Regulatory evolution is associated with resolved conflict over long evolutionary timeframes.

We contrasted population genomic statistics across sex-biased and unbiased genes to test the role of regulatory variation in sexual conflict resolution. We found that autosomal sex-biased genes expressed in the gonad have significantly lower Tajima's D than unbiased genes across all six species, consistent with largely resolved sexual conflict (Figures 2 & S2). However, male and female-biased genes also have significantly elevated intersexual F_{ST} in many species (Figures S7), even after accounting for potential covariates (Table S12). These results are consistent with a potential role of regulatory evolution in conflict resolution via the evolution of sex-specific architecture (Wright, et al. 2018). We observed a similar pattern across spleen-biased genes (Figures 2 & S2), however, the differences are non-significant, likely because of reduced power due to limited numbers of sex-biased genes in somatic tissue.

Employing discrete thresholds to identify sex-biased genes has been shown to have a major effect on the number of genes identified (Ingleby, et al. 2015). We therefore next investigated the relationship between Tajima's D and sex-bias using a polynomial approach (Cheng and Kirkpatrick 2016). These results confirmed our finding that sex-biased genes have lower Tajima's D (Tables S7, S8, S9 & S10). It is important to note that the variance in Tajima's D that is accounted for by these associations is extremely low (sex-bias and *D* for gonad-biased genes r^2 = 0.007-0.147, spleen-biased genes r^2 = 0.000-0.018), similar to findings of previous somatic studies in fish (Wright, et al. 2018), likely resulting, at least in part, from the inherent noise in Tajima's D estimates.

In order to quantify the pervasiveness of sexual conflict and extent to which balancing selection shapes patterns of genetic diversity across related species, we identified reciprocal orthologs across the six species, which last shared a common

ancestor 90 million years ago. Across reciprocal orthologs on the autosomes, we identified genes with elevated Tajima's D in all species; specifically, where Tajima's D was in the top 10% quantile in each species separately. The average range of Tajima's D values for this highest 10% class across species was 1.41-3.26. Using ancestral reconstructions of gene expression levels (Harrison, et al. 2015) (SI Methods), we identified gonadal genes that were ancestrally and universally either sex-biased or unbiased across all six species. We found that gonadal genes that were ancestrally sex-biased across the clade were significantly less likely to show elevated Tajima's D across all six species than expected from random permutations (245 genes, χ^2 $p<0.001$, 1000 permutes). In contrast, universally unbiased genes were significantly enriched in genes with elevated Tajima's D across all species (141 genes, χ^2 $p<0.001$, 1000 permutes). Our results are robust across multiple quantile thresholds used to define elevated Tajima's D (SI Results). This indicates that sexual conflict can shape patterns of genetic diversity in certain sets of sex-biased genes across evolutionary time frames.

Conflict over reproductive potential is greatest in sexually dimorphic species.

To investigate the relationship between sexual conflict and levels of genetic diversity across the genome, we conducted a phylogenetically controlled comparative analysis of Tajima's D across species that vary in mating system and sexual dimorphism. Specifically, we used phylogenetic generalized least squares (PGLS) from the R package caper (Orme, et al. 2013) to test the relationship between Tajima's D and measures of sexual dimorphism, while accounting for the observed level of phylogenetic signal in the data. For each species, we quantified median Tajima's D for spleen-biased and gonad-biased genes relative to non-tissue-biased genes. Tajima's D cannot be compared directly across species or populations, as demographic history has a major influence on genetic diversity, and therefore Tajima's D estimation. Calculating a relative measure of Tajima's D makes it possible to circumvent problems arising from demographic changes in population size. There are a number of phenotypic indices of sexual conflict, including degree of sexual dichromatism, sperm number, and residual testes weight, that are widely used indicators of post-copulatory sexual selection and therefore a measure of variance in

male mating success in birds (Moller 1991; Birkhead and Moller 1998; Pitcher, et al. 2005). We recovered a significant and positive relationship between relative Tajima's D in the gonad and sexual dichromatism (r^2=0.890, p=0.003) after correcting for phylogeny, and marginally non-significant positive associations with both sperm number (r^2=0.491, p=0.073) and residual testes weight (r^2=0.298, p=0.152).

The proportion of sex-biased genes varies with mating system across these species (Harrison, et al. 2015), which together with the fact that sex-biased genes have distinct patterns of Tajima's D (Cheng and Kirkpatrick 2016; Dutoit, et al. 2018; Wright, et al. 2018) and are subject to different selective pressures relative to unbiased genes (Ellegren and Parsch 2007; Harrison, et al. 2015), may confound the pattern we observe. We therefore repeated the analyses using relative median Tajima's D calculated using only unbiased genes in each tissue. In doing so, we found that relative Tajima's D in the gonad becomes significantly and positively correlated with sexual dichromatism (r^2=0.788, p=0.011), and sperm number (r^2=0.679, p=0.027) after correcting for phylogenetic relationships (Figure 3), and marginally non-significantly associated with residual testes weight (r^2=0.446, p=0.089). In contrast, there was no significant association with Tajima's D in the spleen and measures of sexual dimorphism (Figure S4).

Interestingly, we found no significant relationship between Tajima's D and phenotypic sexual conflict for Z-linked genes in either tissue (Figure S6). Given there are fewer genes on the Z chromosome relative to the autosomes, this pattern might simply be a consequence of smaller sample sizes and therefore greater uncertainty around the median. In order to assess the role of gene number in our population genetic parameter estimates, we subsampled tissue-biased genes on the autosomes to the equivalent number of the Z-linked genes in each species 1000 times. The Pearson's correlation coefficients for the relationship between Tajima's D and sexual dichromatism, testes weight, and sperm number for gonad-biased Z-linked genes are smaller relative to the subsampled dataset (p=0.027, p=0.048, p=0.168). The slope of the regression is also smaller than the subsampled data (p=0.024, p=0.058, p=0.121). This indicates that our failure to observe a significant relationship between Tajima's

D and sexual conflict on the Z is not a consequence of reduced gene numbers relative to the autosomes.

DISCUSSION

The manifestation, resolution, and consequences of intra-locus sexual conflict have been the subject to considerable recent debate. To address this, we exploited natural variation in the magnitude of sexual conflict across the body plan within individuals, and across mating systems between species, in a clade of birds that diverged 90 million years ago.

The role of regulatory variation between males and females in the resolution of sexual conflict has received substantial attention in recent literature, with population genomic studies suggesting that sex-biased genes are subject to ongoing sexual antagonism (Cheng and Kirkpatrick 2016; Dutoit, et al. 2018) and others indicating that they represent resolved conflict (Innocenti and Morrow 2010; Wright, et al. 2018). Sex-biased genes in the guppy tail, particularly male-biased genes, resolve conflict arising over reproduction through the evolution of separate sex-specific genetic architectures (Wright, et al. 2018). However, as this tissue is heavily implicated in female mate choice and therefore primarily affects male reproductive fitness, it is possible that the relative importance of male versus female expression is unusual in this tissue and that sex-biased genes play equal roles in most species. Contrary to this, Dutoit *et al*. (2018) suggest that ongoing sexual antagonism is more prevalent in male-biased than female-biased genes in the gonad, potentially hinting at an important role for female-biased expression in conflict resolution. However, without a direct comparison between sex-biased and unbiased genes, the relationship remains unclear. Finally, both male- and female-biased genes in humans show elevated F_{ST} measures (Cheng and Kirkpatrick 2016), although it is not clear how much of this signal is due to somatic versus gonadal expression, or whether this was associated with elevated Tajima's D.

Here, we find that balancing selection is weaker in sex-biased genes relative to unbiased genes, consistent with an important role for sex-biased expression in the resolution of sexual conflict. Lower Tajima's D in sex-biased genes is consistent with the rapid rates of evolution in this class of genes observed across many species

(Ellegren and Parsch 2007; Parsch and Ellegren 2013; Mank 2017; Rowe, et al. 2018), either through positive selection (Meiklejohn, et al. 2003; Pröschel, et al. 2006; Zhang, et al. 2007), or relaxed purifying selection (Gershoni and Pietrokovski 2014; Harrison, et al. 2015; Dapper and Wade 2016; Dutoit, et al. 2018). Balancing selection, which slows the fixation of alleles, is inconsistent with accelerated rates of sequence evolution observed for sex-biased genes (Wright and Mank 2013; Harrison, et al. 2015). In contrast, resolved conflict, which results in sex-specific selection and separate male and female genetic architectures suggested by our data, is expected to lead to the higher levels of standing diversity and faster rates of evolution observed across sex-biased genes in a broad array of taxa (Dapper and Wade 2016).

Whereas identifying the mechanisms responsible for the resolution of genomic sexual conflict has received considerable attention, the consequences for phenotypic evolution have been comparatively understudied. This is in part due to the difficulties in identifying specific loci subject to sexual conflict and establishing their phenotypic effects from genome scans alone. Our study adds considerably to this goal by using different levels of dimorphism within the body plan and across related species to determine the relationship between population genetic and phenotypic measures of sexual conflict.

Relative to the spleen, the gonad is more phenotypically sexually dimorphic, has higher levels of sex-biased gene expression, and has evolved many sex-specific functions. If sexual dimorphism represents resolved sexual conflict, we might expect gonad-biased genes to have lower levels of balancing selection than spleen-biased genes and loci expressed similarly in both tissues. Consistent with this prediction, we find reduced balancing selection in the gonad, indicative of lower levels of ongoing sexual conflict. This supports the theory that resolved sexual conflict facilitates the evolution of phenotypic sex differences. It is plausible that the large numbers of sex-biased genes in the gonad relative to somatic tissue act to resolve conflict through regulatory decoupling of male and female expression and the evolution of sex-specific architecture.

While we found that intra-locus sexual conflict is resolved in the gonad, we found a significant and positive correlation between the magnitude of sexual conflict, arising

551 from differences in mating system, and balancing selection in the gonad but not the
552 spleen. Whilst this may appear initially contradictory, this relationship is in fact
553 consistent with an ephemeral nature of sexual antagonism and rapid turnover of
554 sexual conflict loci. This is in line with previous work showing that sex-biased genes
555 exhibit rapid rates of evolution and turnover (Zhang, et al. 2007; Harrison, et al.
556 2015). Our results suggest that unbiased genes are the locus of ongoing sexual
557 conflict due to mating system, and that increasing levels of sexual conflict over
558 reproduction result in elevated levels of genetic diversity across a greater proportion
559 of genes. In contrast, relative Tajima's D in spleen-biased genes is not associated
560 with any phenotypic measure of sexual conflict, suggesting that sexual conflict over
561 reproduction has the greatest potential to contribute significantly to variation in the
562 maintenance of genetic diversity across species. This has important consequences
563 for understanding the relationship between sexual conflict and adaptation, where
564 higher levels of conflict promote genetic diversity and provide genetic fuel for
565 adaptive opportunities (Candolin and Heuschele 2008; Chenoweth, et al. 2015;
566 Lumley, et al. 2015; Jacomb, et al. 2016).

567 In contrast, we observed no significant relationship between mating system and
568 balancing selection on the Z chromosome. Previously, we showed that the adaptive
569 potential of the Z chromosome is compromised by increasing sexual selection, which
570 decreases the relative effective population size of the Z compared to autosomes
571 (Wright, et al. 2015), leading to increased levels of genetic drift. This means that Z-
572 linked genes in sexually dimorphic species are subject to higher levels of genetic drift
573 (Wright and Mank 2013). Our results indicate that the potential for sexual conflict to
574 shape patterns of genetic diversity on the Z chromosome might be counteracted by
575 the depleting forces of genetic drift, and that sexual conflict may not play a
576 disproportionally greater role in Z chromosome evolution compared to the rest of
577 the genome.

578 Negative Tajima's D can be interpreted in the context of positive selection, where
579 selective sweeps can result in lower estimates. A greater frequency of selective
580 sweeps in sex-biased genes could therefore explain our finding that Tajima's D is
581 lower in the gonad than the spleen. Furthermore, the positive correlation between

582 Tajima's D and sexual dimorphism we observe in the gonad could also be due to
583 more intense positive selection in species with less sexual dimorphism. However,
584 elevated positive selection is unlikely to explain our results, as previous research on
585 the same dataset found no significant evidence for positive selection acting on sex-
586 biased genes in the gonad, or any evidence for variation in the magnitude of positive
587 selection across species based on mating system (Harrison, et al. 2015). Therefore,
588 we conclude that lower Tajima's D is indicative of lower levels of balancing selection
589 and resolved intra-locus conflict, likely mediated by the evolution of sex-biased gene
590 expression.

591 Population genomic measures of intersexual F_{ST} and Tajima's D can be influenced by
592 a number of demographic events, not just sexual conflict, including sex-biased
593 migration, sex-biased predation and changes in population size (Hartl and Clark
594 2006). By conducting comparisons of population genomic statistics within each
595 species, instead of directly comparing across species, we controlled for the effect of
596 population contractions or expansions, and our use of captive populations further
597 minimizes the effects of sex-biased migration or predation. Furthermore, samples
598 were taken from all individuals during their first breeding season, effectively
599 controlling for age differences that can confound measures of intersexual F_{ST} or lead
600 to high levels of regulatory variation. However, we note that due to statistical noise,
601 likely due to low sample sizes, we could not reliably identify specific loci subject to
602 sexual conflict, and instead compare large groups of genes to determine broad
603 trends across tissues and species. Our analyses of intersexual F_{ST} are particularly
604 limited by sample size and therefore we urge caution when interpreting these in the
605 light of sexual conflict. However, while we do find loci with elevated intersexual F_{ST},
606 which has previously been interpreted as evidence for ongoing sexual conflict (Cheng
607 and Kirkpatrick 2016; Lucotte, et al. 2016; Dutoit, et al. 2018), the number of loci
608 with elevated F_{ST} do not appear to differ between the gonad and spleen, despite the
609 obvious differences in function and role in survival between the two tissues.

610 Interestingly, our failure to detect differences in conflict over viability between the
611 tissues is consistent with recent theoretical work (Kasimatis, et al. 2017) suggesting
612 that the magnitude of sexual conflict, and associated mortality load, required to

generate patterns of intersexual F_{ST} across large numbers of loci are implausibly high. This suggests that they may be a result of alternative demographic processes or statistical noise arising from low sample sizes, instead of ongoing sexual conflict. Instead, our previous work indicates that divergence in allele frequencies between males and females in somatic tissue could instead be indicative of the evolution of sex-specific architectures, which would invoke weaker genetic loads.

In conclusion, our findings suggest that mating system can significantly increase standing diversity across the genome via sexual conflict. More importantly, our results suggest that sexual conflict is short-lived, and is resolved via the decoupling of male and female gene expression patterns. Our results are consistent both across a gradient of sexual dimorphism within the body plan and across species, and have important implications about the role of sexual selection in adaptive potential (Candolin and Heuschele 2008; Chenoweth, et al. 2015; Lumley, et al. 2015; Jacomb, et al. 2016), the persistence of sexual conflict over evolutionary time-scales, and role of dimorphism in facilitating sex-specific fitness optima.

ACKNOWLEDGEMENTS

This work was supported by a NERC Independent Research Fellowship to A.E.W, and the European Research Council (grant agreements 260233 and 680951) to J.E.M, who also gratefully acknowledges funding from a Canada 150 Research Chair. We thank Thorfinn Korneliussen for help with ANGSD and ngsTools, Ida Koltke with ngsRelate, Jon Slate, Kai Zeng and Toni Gossmann for productive discussions, and four anonymous reviewers for helpful comments. The authors acknowledge the use of the UCL Legion High Performance Computing Facility (Legion@UCL), and associated support services, in the completion of this work.

DATA ACCESSIBILITY

RNA-seq data is publicly available in the NCBI SRA (BioProject ID PRJNA271731). Transcriptome assemblies are available via Dryad. Statistics for autosomal genes in each species are in SI data files.

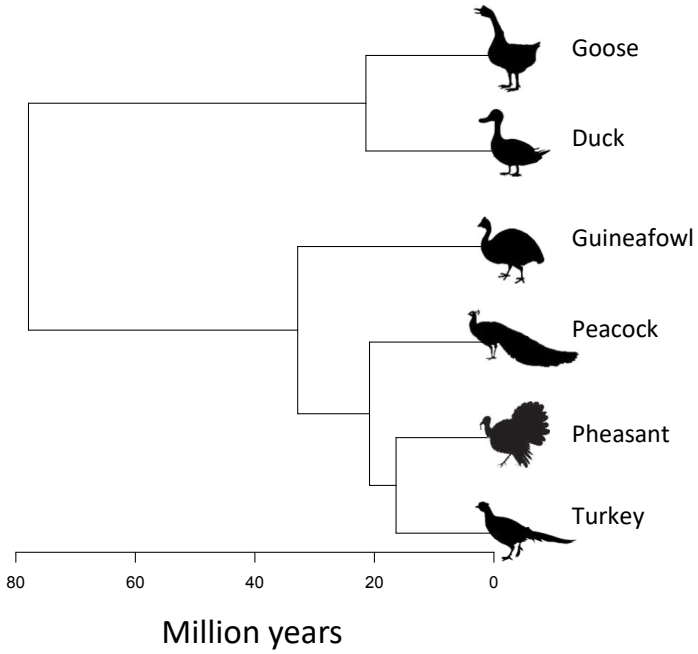


Figure 1. Phylogenetic relationships across the six avian species in this study. These species were chosen to reflect the full range of mating system and sexual dimorphism. The intensity of sexual conflict in each species was estimated using three proxies; sexual dichromatism score, sperm number and relative testes size.

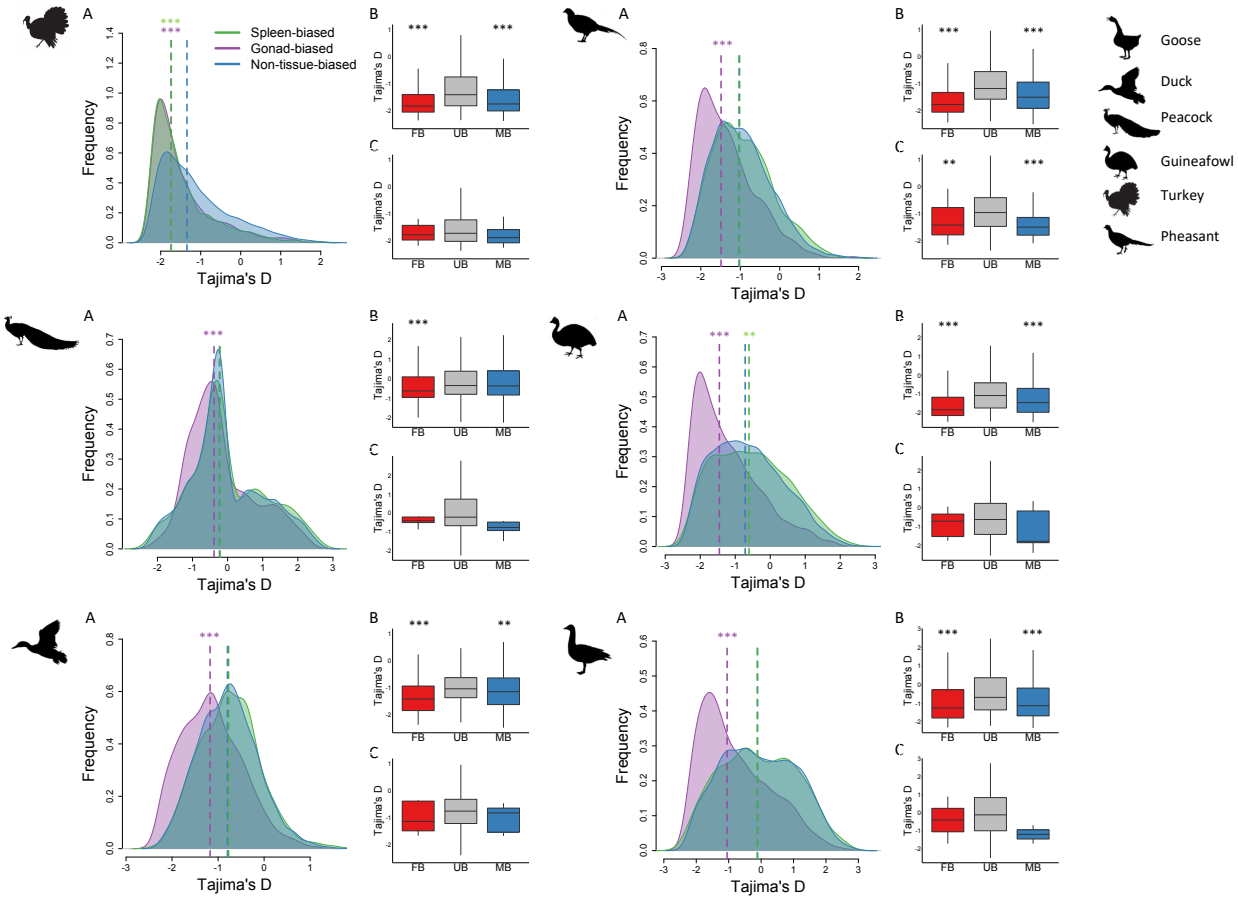
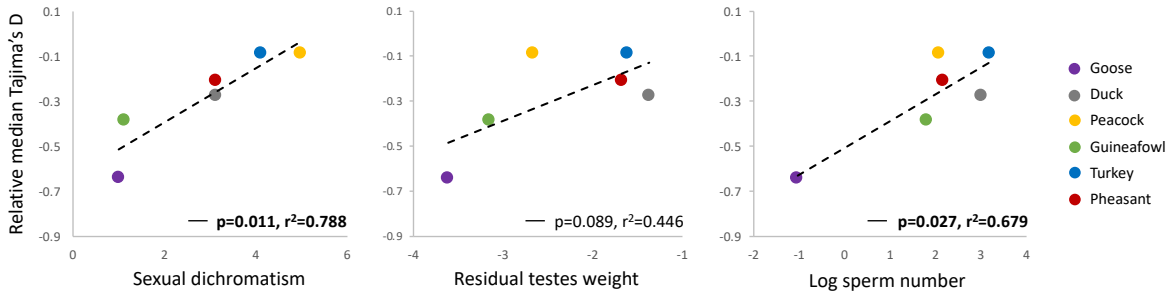


Figure 2. Patterns of Tajima's D for tissue-biased and sex-biased genes across species. Panels A show the distribution of *D* for autosomal genes for spleen-biased, gonad-biased and non-tissue-biased genes. Dotted lines show median *D* for each set of genes and *,**,*** denote a significant difference relative to non-tissue-biased genes (Wilcoxon test, $p < 0.05$, $p < 0.01$, $p < 0.001$). Tissue-biased genes were identified from male expression data. Panels B and C show the relationship between *D* and expression for genes with gonad-biased expression (panel B) or spleen-biased expression (panel C). *,**,*** denote a significant difference relative to unbiased genes (Wilcoxon test, $p < 0.05$, $p < 0.01$, $p < 0.001$). FB, UB, MB refer to female-biased, unbiased and male-biased genes respectively.



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681 **Figure 3. Phylogenetically controlled regression between proxies of sperm**
682 **competition and Tajima's D in the gonad**. Relative *D* is shown for autosomal genes
683 with unbiased expression between males and females in the gonad. Relative *D* is
684 calculated as the difference between median *D* for tissue-biased genes compared to
685 non-tissue-biased genes. Tissue-biased genes were identified from male expression
686 data. We tested the relationship between Tajima's D and measures of sexual
687 dimorphism, while accounting for the observed level of phylogenetic signal in the
688 data.

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Table 1: Observed and expected number of genes with intersexual $F_{ST} > 0$ across tissue-biased genes

Species	Gonad-biased			Spleen-biased		
	E	O	p-value	E	O	p-value
Mallard duck	116	118	0.875	112	111	0.956
Swan goose	56	65	0.248	56	70	0.056
Wild turkey	166	160	0.644	204	236	**0.026**
Common pheasant	165	163	0.520	187	174	0.532
Guineafowl	112	124	0.269	151	142	0.461
Indian peafowl	200	209	0.520	217	208	0.532

Only unbiased genes were used in this analysis. Tissue-biased genes were identified from male expression data. Only autosomal genes are included in the analyses. Expected number of genes with intersexual $F_{ST} > 0$ were calculated from observations of F_{ST} in non-tissue-specific genes. P-values were calculated using chi-squared tests.

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