

DR. ALISON WRIGHT (Orcid ID : 0000-0003-2479-5250)

Article type : Original Article

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

Alison E. Wright^{1*}, Thea F. Rogers¹, Matteo Fumagalli², Christopher R. Cooney¹ & Judith E.
Mank^{3,4,5}

1 Department of Animal and Plant Sciences, University of Sheffield, United Kingdom

2 Department of Life Sciences, Silwood Park Campus, Imperial College London, United Kingdom

3 Department of Genetics, Evolution and Environment, University College London, United Kingdom

4 Department of Organismal Biology, Uppsala University, Sweden

5 Department of Zoology, University of British Columbia, Canada

* Corresponding author: a.e.wright@sheffield.ac.uk

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

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mec.15115

This article is protected by copyright. All rights reserved.

Accepted Article

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

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

Furthermore, a major challenge in evolutionary biology is to explain the maintenance and variation in genetic diversity across many species. The existence of elevated genetic diversity relative to neutral expectations across species is puzzling, as directional selection and drift are both expected to erode variation. However, there is increasing evidence that intra-locus sexual conflict, through balancing selection, can significantly increase genome-wide patterns of variability (Chippindale, et al. 2001; Foerster, et al. 2007; Delcourt, et al. 2009; Mokkonen, et al. 2011; Hawkes, et al. 2016; Lonn, et al. 2017). Therefore, variation in sexual conflict across lineages, likely mediated by mating systems, could drive variation in genetic diversity across species and resolve this apparent paradox. However, the exact nature of the relationship between sexual conflict, mating system and genetic 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 platyrhynchos*), wild turkey (*Meleagris gallopavo*), common pheasant (*Phasianus colchicus*), helmeted guinea fowl (*Numida meleagris*), Indian peafowl (*Pavo cristatus*) and swan goose (*Anser cygnoides*) (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 < 2 FPKM 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 1×10^{-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 1×10^{-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 GO terms 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.

Accepted Article

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 ($TD \sim \text{Tissue bias} + \log(tW) + \log(\text{Gene length}) + \log(\text{GC}) + \log(\text{Gene expression level})$). 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.

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

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

We contrasted intersexual F_{ST} for gonad and spleen-biased genes using three approaches. First, we found no significant difference in median F_{ST} for unbiased genes expressed primarily in the gonad relative to those expressed broadly across both the gonad and spleen (Table S4). We observed the same pattern in the spleen, with the exception of the goose and turkey where F_{ST} was elevated marginally. Second, there was no significant difference in the number of unbiased genes with elevated intersexual F_{ST} that were expressed primarily in the gonad compared to those with non-tissue-specific expression patterns (Table 1). We observe the same result in the spleen, with the exception of the turkey. However, all of these differences become non-significant when we analyse tissue-biased genes identified from female expression data (Tables S5 & S6). Lastly, we found no significant effect of tissue bias on F_{ST} after accounting for gene length, average expression level, GC content and

Watterson's theta in a multiple regression ($TD \sim \text{Tissue bias} + \log(tW) + \log(\text{Gene length}) + \log(\text{GC}) + \log(\text{Gene expression level})$) (Table S11).

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

Accepted Article

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

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

Negative Tajima's D can be interpreted in the context of positive selection, where selective sweeps can result in lower estimates. A greater frequency of selective sweeps in sex-biased genes could therefore explain our finding that Tajima's D is lower in the gonad than the spleen. Furthermore, the positive correlation between Tajima's D and sexual dimorphism we observe in the gonad could also be due to more intense positive selection in species with less sexual dimorphism. However, elevated positive selection is unlikely to explain our results, as previous research on the same dataset found no significant evidence for positive selection acting on sex-biased genes in the gonad, or any evidence for variation in the magnitude of positive selection across species based on mating system (Harrison, et al. 2015). Therefore, we conclude that lower Tajima's D is indicative of lower levels of balancing selection and resolved intra-locus conflict, likely mediated by the evolution of sex-biased gene expression.

Population genomic measures of intersexual F_{ST} and Tajima's D can be influenced by a number of demographic events, not just sexual conflict, including sex-biased migration, sex-biased predation and changes in population size (Hartl and Clark 2006). By conducting comparisons of population genomic statistics within each species, instead of directly comparing across species, we controlled for the effect of population contractions or expansions, and our use of captive populations further minimizes the effects of sex-biased migration or predation. Furthermore, samples were taken from all individuals during their

Accepted Article

first breeding season, effectively controlling for age differences that can confound measures of intersexual F_{ST} or lead to high levels of regulatory variation. However, we note that due to statistical noise, likely due to low sample sizes, we could not reliably identify specific loci subject to sexual conflict, and instead compare large groups of genes to determine broad trends across tissues and species. Our analyses of intersexual F_{ST} are particularly limited by sample size and therefore we urge caution when interpreting these in the light of sexual conflict. However, while we do find loci with elevated intersexual F_{ST} , which has previously been interpreted as evidence for ongoing sexual conflict (Cheng and Kirkpatrick 2016; Lucotte, et al. 2016; Dutoit, et al. 2018), the number of loci with elevated F_{ST} do not appear to differ between the gonad and spleen, despite the obvious differences in function and role in survival between the two tissues.

Interestingly, our failure to detect differences in conflict over viability between the tissues is consistent with recent theoretical work (Kasimatis, et al. 2017) suggesting 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.

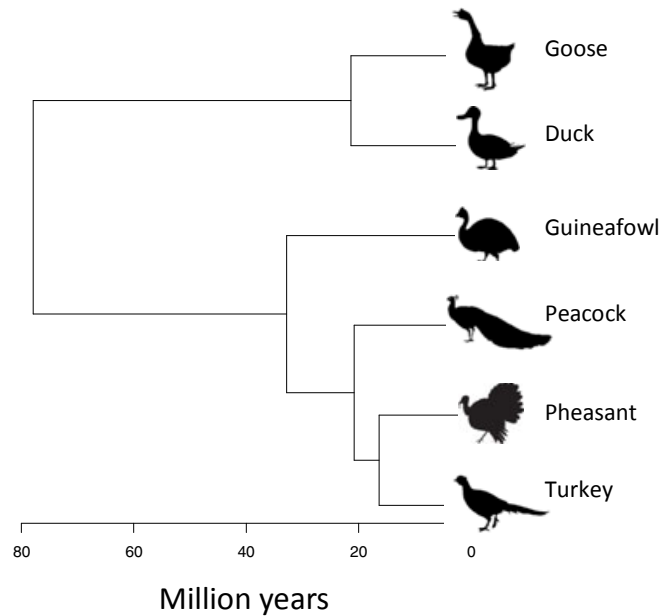


Figure 1. Phylogenetic relationships across the six avian species in this study.

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.

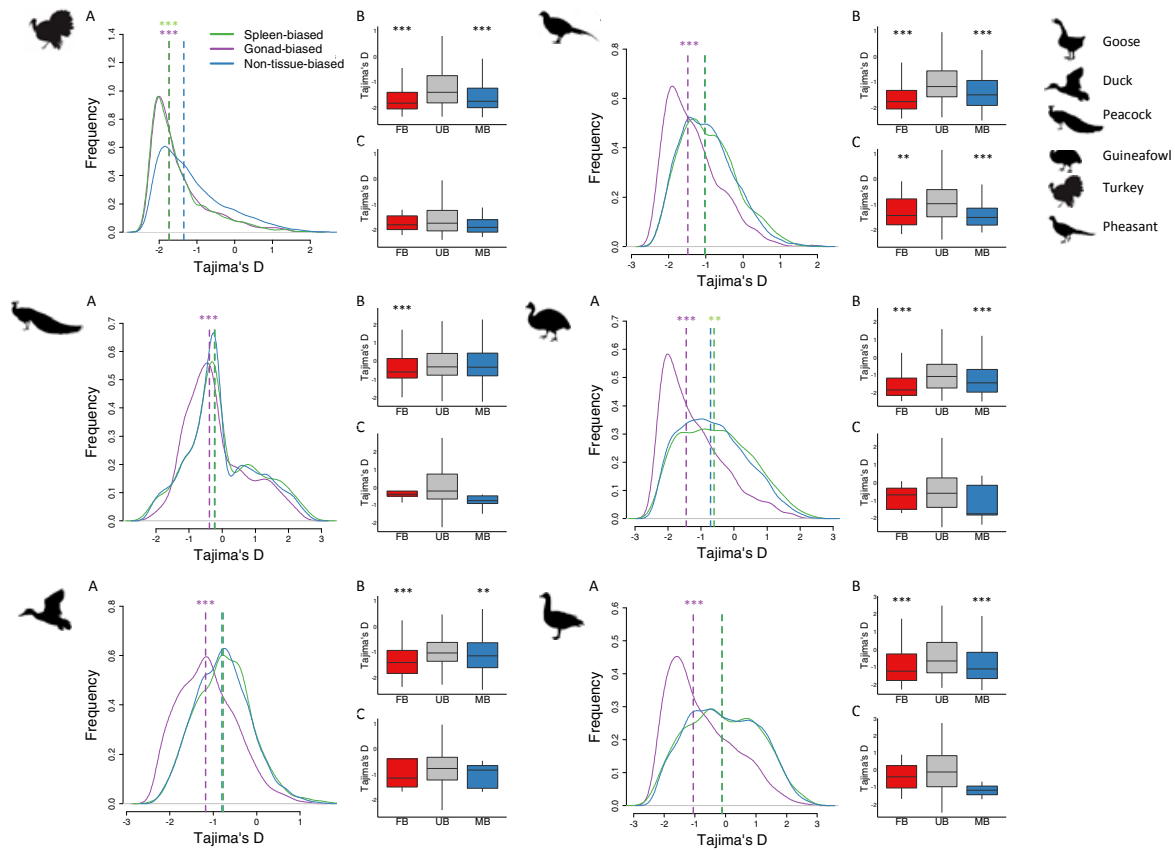


Figure 2. Patterns of Tajima's D for tissue-biased and sex-biased genes across species.

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.

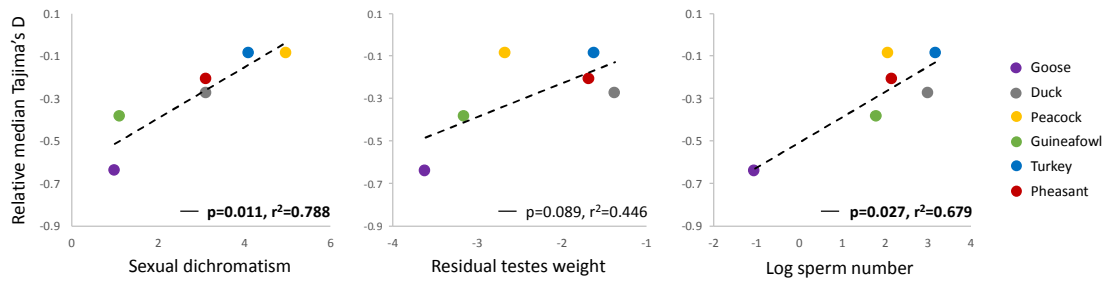


Figure 3. Phylogenetically controlled regression between proxies of sperm competition and Tajima's D in the gonad. Relative D is shown for autosomal genes with unbiased expression between males and females in the gonad. Relative D is calculated as the difference between median D for tissue-biased genes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

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.

REFERENCES

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403-410.
- Andersson M. 1994. *Sexual selection*: Princeton University Press.
- Bhatia G, Patterson N, Sankararaman S, Price AL. 2013. Estimating and interpreting FST: The impact of rare variants. *Genome Research* 23:1514-1521.
- Birkhead T, Moller AP. 1998. *Sperm competition and sexual selection*: Academic Press Inc.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114-2120.
- Candolin U, Heuschele J. 2008. Is sexual selection beneficial during adaptation to environmental change? *Trends in Ecology & Evolution* 23:446-452.
- Charlesworth D. 2006. Balancing selection and its effects on sequences in nearby genome regions. *PLOS Genetics* 2:e64.
- Cheng C, Kirkpatrick M. 2016. Sex-specific selection and sex-biased gene expression in humans and flies. *PLOS Genetics* 12:e1006170.
- Chenoweth Stephen F, Appleton Nicholas C, Allen Scott L, Rundle Howard D. 2015. Genomic evidence that sexual selection impedes adaptation to a novel environment. *Current Biology* 25:1860-1866.
- Chippindale AK, Gibson JR, Rice WR. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proceedings of the National Academy of Sciences* 98:1671-1675.
- Connallon T, Knowles LL. 2005. Intergenomic conflict revealed by patterns of sex-biased gene expression. *Trends in Genetics* 21:495-499.
- Czorlich Y, Aykanat T, Erkinaro J, Orell P, Primmer CR. 2018. Rapid sex-specific evolution of age at maturity is shaped by genetic architecture in Atlantic salmon. *Nature Ecology & Evolution* 2:1800-1807.
- Dapper AL, Wade MJ. 2016. The evolution of sperm competition genes: the effect of mating system on levels of genetic variation within and between species. *Evolution* 70:502-511.
- Delcourt M, Blows MW, Rundle HD. 2009. Sexually antagonistic genetic variance for fitness in an ancestral and a novel environment. *Proceedings of the Royal Society B: Biological Sciences* 276:2009-2014.
- Dutoit L, Mugal CF, Bolívar P, Wang M, Nadachowska-Brzyska K, Smeds L, Papoli H, Gustafsson L, Ellegren H. 2018. Sex-biased gene expression, sexual antagonism and levels of genetic diversity in the collared flycatcher (*Ficedula albicollis*) genome. *Molecular Ecology* 0.
- Ellegren H, Parsch J. 2007. The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics* 8:689.
- Foerster K, Coulson T, Sheldon BC, Pemberton JM, Clutton-Brock TH, Kruuk LEB. 2007. Sexually antagonistic genetic variation for fitness in red deer. *Nature* 447:1107-1110.
- Fumagalli M, Vieira FG, Linderoth T, Nielsen R. 2014. ngsTools: methods for population genetics analyses from next-generation sequencing data. *Bioinformatics* 30:1486-1487.
- Gershoni M, Pietrokovski S. 2014. Reduced selection and accumulation of deleterious mutations in genes exclusively expressed in men. *Nature Communications* 5:4438.
- Ghosh R, Andersen EC, Shapiro JA, Gerke JP, Kruglyak L. 2012. Natural variation in a chloride channel subunit confers avermectin resistance in *C. elegans*. *Science* 335:574-578.
- Gilks WP, Abbott JK, Morrow EH. 2014. Sex differences in disease genetics: evidence, evolution, and detection. *Trends in Genetics* 30:453-463.

- Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 29:644.
- Harrison PW, Wright AE, Zimmer F, Dean R, Montgomery SH, Pointer MA, Mank JE. 2015. Sexual selection drives evolution and rapid turnover of male gene expression. *Proceedings of the National Academy of Sciences* 112:4393-4398.
- Hartl DL, Clark AG. 2006. *Principles of population genetics*: Sinauer Associates, Inc.
- Hawkes MF, Gamble CE, Turner ECR, Carey MR, Wedell N, Hosken DJ. 2016. Intralocus sexual conflict and insecticide resistance. *Proceedings of the Royal Society B: Biological Sciences* 283.
- Hedrick PW. 2011. Population genetics of malaria resistance in humans. *Heredity* 107:283-304.
- Ingleby FC, Flis I, Morrow EH. 2015. Sex-biased gene expression and sexual conflict throughout development. *Cold Spring Harbor Perspectives in Biology* 7:a017632.
- Innocenti P, Morrow EH. 2010. The sexually antagonistic genes of *Drosophila melanogaster*. *PLoS Biology* 8.
- Itoh Y, Melamed E, Yang X, Kampf K, Wang S, Yehya N, Van Nas A, Replogle K, Band MR, Clayton DF, et al. 2007. Dosage compensation is less effective in birds than in mammals. *Journal of Biology* 6:2-2.
- Jacomb F, Marsh J, Holman L. 2016. Sexual selection expedites the evolution of pesticide resistance. *Evolution* 70:2746-2751.
- Karp NA, Mason J, Beaudet AL, Benjamini Y, Bower L, Braun RE, Brown SDM, Chesler EJ, Dickinson ME, Flenniken AM, et al. 2017. Prevalence of sexual dimorphism in mammalian phenotypic traits. *Nature Communications* 8:15475.
- Kasimatis KR, Nelson TC, Phillips PC. 2017. Genomic signatures of sexual conflict. *Journal of Heredity* 108:780-790.
- Kasimatis KR, Ralph PL, Phillips PC. 2019. Limits to genomic divergence under sexually antagonistic selection. *Biorxiv*. <https://doi.org/10.1101/591610>
- Korneliussen TS, Albrechtsen A, Nielsen R. 2014. ANGSD: Analysis of next generation sequencing data. *BMC Bioinformatics* 15.
- Korneliussen TS, Moltke I. 2015. NgsRelate: a software tool for estimating pairwise relatedness from next-generation sequencing data. *Bioinformatics* 31:4009-4011.
- Lewontin RC, Krakauer J. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics* 74:175-195.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078-2079.
- Lonn E, Koskela E, Mappes T, Mokkonen M, Sims AM, Watts PC. 2017. Balancing selection maintains polymorphisms at neurogenetic loci in field experiments. *Proceedings of the National Academy of Sciences* 114:3690-3695.
- Lucotte EA, Laurent R, Heyer E, Ségurel L, Toupance B. 2016. Detection of allelic frequency differences between the sexes in humans: A signature of sexually antagonistic selection. *Genome Biology and Evolution* 8:1489-1500.
- Lumley AJ, Michalczyk Ł, Kitson JJN, Spurgin LG, Morrison CA, Godwin JL, Dickinson ME, Martin OY, Emerson BC, Chapman T, et al. 2015. Sexual selection protects against extinction. *Nature* 522:470-473.

- Mank JE. 2017. Population genetics of sexual conflict in the genomic era. *Nature Reviews Genetics* 18:721-730.
- Mank Judith E. 2009. Sex chromosomes and the evolution of sexual dimorphism: Lessons from the genome. *The American Naturalist* 173:141-150.
- Mank JE, Ellegren H. 2008. All dosage compensation is local: Gene-by-gene regulation of sex-biased expression on the chicken Z chromosome. *Heredity* 102:312–320.
- Meiklejohn CD, Parsch J, Ranz JM, Hartl DL. 2003. Rapid evolution of male-biased gene expression in *Drosophila*. *Proceedings of the National Academy of Sciences* 100:9894-9899.
- Mokkonen M, Kokko H, Koskela E, Lehtonen J, Mappes T, Martiskainen H, Mills SC. 2011. Negative frequency-dependent selection of sexually antagonistic alleles in *Myodes glareolus*. *Science* 334:972-974.
- Moller AP. 1991. Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *The American Naturalist* 137:882-906.
- Mostafavi H, Berisa T, Day FR, Perry JRB, Przeworski M, Pickrell JK. 2017. Identifying genetic variants that affect viability in large cohorts. *PLoS Biology* 15:e2002458.
- Nielsen R, Korneliussen T, Albrechtsen A, Li Y, Wang J. 2012. SNP calling, genotype calling, and sample allele frequency estimation from new-generation sequencing data. *PLOS ONE* 7:e37558.
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2013. caper: comparative analyses of phylogenetics and evolution in R. R package version 0.5.2.
- Parker GA, Partridge L. 1998. Sexual conflict and speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 353:261-274.
- Parsch J, Ellegren H. 2013. The evolutionary causes and consequences of sex-biased gene expression. *Nature Reviews Genetics* 14:83-87.
- Pitcher TE, Dunn PO, Whittingham LA. 2005. Sperm competition and the evolution of testes size in birds. *Journal of Evolutionary Biology* 18:557-567.
- Pröschel M, Zhang Z, Parsch J. 2006. Widespread adaptive evolution of *Drosophila* genes with sex-biased expression. *Genetics* 174:893-900.
- Rice WR. 1984. Sex chromosomes and the evolution of sexual dimorphism. *Evolution* 38:735-742.
- Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:139-140.
- Rowe L, Chenoweth SF, Agrawal AF. 2018. The genomics of sexual conflict. *The American Naturalist* 192:274-286.
- Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J. 1999. Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. *Nature* 400:667-671.
- Stewart AD, Rice WR. 2018. Arrest of sex-specific adaptation during the evolution of sexual dimorphism in *Drosophila*. *Nature Ecology & Evolution* 2:1507-1513.
- Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, Lee T-h, Jin H, Marler B, Guo H, et al. 2012. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Research* 40:e49-e49.
- Waterhouse RM, Seppey M, Simão FA, Manni M, Ioannidis P, Klioutchnikov G, Kriventseva EV, Zdobnov EM. 2018. BUSCO applications from quality assessments to gene prediction and phylogenomics. *Molecular Biology and Evolution* 35:543-548.
- Wright AE, Fumagalli M, Cooney CR, Bloch NI, Vieira FG, Buechel SD, Kolm N, Mank JE. 2018. Male-biased gene expression resolves sexual conflict through the evolution of sex-specific genetic architecture. *Evolution Letters* 2:52-61.

- Accepted Article
- Wright AE, Harrison PW, Zimmer F, Montgomery SH, Pointer MA, Mank JE. 2015. Variation in promiscuity and sexual selection drives avian rate of Faster-Z evolution. *Molecular Ecology* 24:1218-1235.
- Wright AE, Mank JE. 2013. The scope and strength of sex-specific selection in genome evolution. *Journal of Evolutionary Biology* 26:1841-1853.
- Wright AE, Moghadam HK, Mank JE. 2012. Trade-off Between Selection for Dosage Compensation and Masculinization on the Avian Z Chromosome. *Genetics* 192:1433-1445.
- Zerbino DR, Achuthan P, Akanni W, Amode M R, Barrell D, Bhai J, Billis K, Cummins C, Gall A, Girón CG, et al. 2018. Ensembl 2018. *Nucleic Acids Research* 46:D754-D761.
- Zhang Y, Sturgill D, Parisi M, Kumar S, Oliver B. 2007. Constraint and turnover in sex-biased gene expression in the genus *Drosophila*. *Nature* 450:233–237.