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The roles of sexual selection and sexual conflict in shaping patterns of genome and transcriptome variation

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Sexual dimorphism is one of the most prevalent, and often the most extreme, examples of phenotypic variation within species, and arises primarily from genomic variation that is shared between females and males. Many sexual dimorphisms arise through sex differences in gene expression, and sex-biased expression is one way that a single, shared genome can generate multiple, distinct phenotypes. Although many sexual dimorphisms are expected to result from sexual selection, and many studies have invoked the possible role of sexual selection to explain sex-specific traits, the role of sexual selection in the evolution of sexually dimorphic gene expression remains difficult to differentiate from other forms of sex-specific selection. In this Review, we propose a holistic framework for the study of sex-specific selection and transcriptome evolution. We advocate for a comparative approach, across tissues, developmental stages and species, which incorporates an understanding of the molecular mechanisms, including genomic variation and structure, governing gene expression. Such an approach is expected to yield substantial insights into the evolution of genetic variation and have important applications in a variety of fields, including ecology, evolution and behaviour.

Differences in traits between males and females (sexual dimorphism; key terms are defined in Box 1) are widespread in the animal kingdom, and it was the prevalence of such variation within species that motivated Darwin to develop his theory of sexual selection as a force distinct from natural selection[1]. The most conspicuous sexually dimorphic traits are secondary sexual traits, which, broadly speaking, are those traits not directly involved in reproduction[2,3], such as extreme tail lengths in male widowbirds[4], the creation of decorative structures in bowerbirds[5] and pronounced size dimorphism in the hammer-headed fruit bat[6]. These secondary sexual traits are costly to produce and maintain, and can make organisms more obvious to potential predators, decreasing survivorship[7–9], even as they increase reproductive fitness.

Sexual dimorphism frequently emerges due to sex-specific selection, as a way to resolve sexual conflict. Sexual conflict was originally defined by Parker[10] as "a conflict between the evolutionary interests of individuals of the two sexes", in which selection has the possibility to act in opposing directions between males and females. Many sources of sexual conflict exist, including conflict over ecological competition[11], mating rates[12–14] and differential reproductive investment[15–17] (Box 2). Sexual selection, in which some individuals are better able to obtain matings and/or fertilizations than others, is one of the most common forms of sex-specific selection, and a prevalent source of conflict. Sexual selection is often divided into two phases: precopulatory and postcopulatory, which are often modelled separately and might have

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BOX 1

Glossary

Balancing selection. The maintenance of two or more alleles in a population by means of natural selection at frequencies greater than what is expected from genetic drift.

Epistatic interactions/epistasis. Alleles at two or more loci interact in a non-additive manner to produce a phenotype.

Fisherian runaway selection. A mechanism of sexual selection wherein an arbitrary preference for a trait in one sex leads to the rapid evolution of exaggerated secondary sexual traits in the other sex[18,133,134]. This is often considered to be a 'null model' of sexual selection[135], and can result in a runaway positive feedback loop, whereby a preferred trait becomes increasingly exaggerated as indirect selection on the preference increases its frequency.

Good genes model. The traits that one sex prefers when selecting a mate are an honest indicator of that individual's quality and ability to produce offspring with greater survival and reproductive success[136,137].

Interlocus conflict. This phenomenon occurs when there are interactions between sexually antagonistic alleles at different loci in the two sexes, resulting in the displacement of one or both sexes from its phenotypic optimum.

Intralocus conflict. This phenomenon occurs when there are interactions between sexually antagonistic alleles within a single locus.

Pleiotropic functions/pleiotropy. The phenomenon of a single gene influencing one or more phenotypic traits of a living organism.

Purifying selection. The removal of deleterious alleles through natural selection.

Secondary sexual traits. Physical characteristics that emerge in an animal at the onset of sexual maturity or during seasonal breeding cycles.

Sensory bias. A model of sexual selection that posits that female preference for male traits arise due to their sensory system being preadapted to similar stimuli that existed prior to the evolution of the preferred traits[138,139].

Sex-biased gene. A gene with quantitatively different expression in males versus females. In some cases, these genes are expressed exclusively in one sex[53].

Sex-specific selection. A form of selection that occurs when a trait is under selection in only one sex, when the magnitude of selection differs between the sexes, or when selection acts in opposite directions in the two sexes[140].

Sexual conflict. A conflict between the evolutionary interests of individuals of the two sexes, in which selection has the possibility to act in opposing directions between males and females[10].

Sexual dimorphism. The condition in which sexes of the same species display systematic phenotypic differences in morphology, physiology and/or behaviour.

Sexual selection. A form of natural selection in which characteristics of one sex are displayed to attract mates of the other sex (intersexual selection), or are used to compete with members of the same sex for access to mates (intrasexual selection).

Sexually antagonistic selection. Arises when the direction and strength of selection differs between female and male traits, resulting in intralocus conflict[27].

Sexually concordant selection. Arises when the direction of selection is the same for both sexes.

different evolutionary outcomes (for example, postcopulatory sexual selection can result in 'red queen' arms races between the sexes). Sexual selection in either form is expected to be a major agent of evolution[18], with the ability to act alongside local adaptation to drive population divergence and speciation[18–21], although other selective pressures can be required for sexual selection to drive speciation[22]. We might therefore expect that species or populations with higher levels of sexual selection would also experience higher levels of sexual conflict.

A continuum of sexual dimorphism exists, from traits expressed only in one sex at one extreme to monomorphic traits at the other (Fig. 1). This continuum is related to the divergence between male and female trait optima[23,24] and the strength of the intersexual genetic correlation[23,25,26]. If trait optima are not too distant, dimorphism can evolve with minimal conflict—although constraints from shared genetic architecture between males and females may impose limits on the extent to which dimorphism can evolve. However, highly divergent trait optima, and/or highly constrained genetic architecture, are expected to create substantial conflict[27]. Selection acting antagonistically at a single locus, favouring one allele or expression pattern in one sex to the detriment of the other sex, is referred to as intralocus conflict[28,29]. For example, in *Drosophila melanogaster*, an allele conferring pesticide resistance also reduces male reproductive investment but boosts female fecundity, enabling the maintenance of both resistance and susceptibility alleles through intralocus sexual conflict[30]. When antagonistic selection acts on different genes or genetic loci, interlocus conflict emerges instead. An example can again be found in *D. melanogaster*, in which males directly harm females during courtship and mating, and gain fitness benefits by doing so. These direct costs to female fitness outweigh any indirect benefits gained through re-mating in females[31], and thus interlocus conflict arises over female resistance to males (detrimental to the males) and male remating attempts (detrimental to females). In the case of interlocus conflict, factors such as linkage disequilibrium and epistatic interactions become critical in understanding the genetic response to conflict[32–34].

Theory predicts that intralocus conflict can be rapidly resolved through the evolution of sexual dimorphism via mechanisms such as the evolution of sex-specific genetic architecture[35–37] and the evolution of sex-biased gene regulation[38]. However, the resolution of conflict is complicated by genetic constraints, such as genetic correlations (that is, high r_{fm}), pleiotropic functions and epistatic interactions[23,25,26,39,40]. The degree of opposing sex-specific selection, and the degree to which the regulatory expression and the genetic architecture of traits can be decoupled, will determine where along the sexual dimorphism continuum a population will sit. As such, these mechanisms will impact

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BOX 2

Sources of sexual conflict

Evolutionary conflicts of interest arise whenever genetically different individuals interact and the routes they take to maximize fitness differ[74]. For sexual conflict to arise, the optimal outcomes for each sex cannot be achieved simultaneously. Sexual conflict is a fundamental component of sex-specific adaptations and can arise from a variety of sources. In this Box, we briefly describe alternative sources of sexual conflict.

Sexual selection. Sexual selection may cause either intralocus or interlocus conflict, and responses to sexual conflict can in turn generate sexual selection[123]. Multiple models of sexual selection have been proposed (see ref. [74] for a full review). The common element that runs through each model is that of mate choice. Sexual conflict persists when choosiness prevents courters from attaining all possible matings. Traits that increase attractiveness—whether those traits are arbitrary (runaway selection[18,133,134]), exploit a pre-existing preference for aspects of traits (sensory bias[138,139]) or because they provide information on the fitness of an individual ('good genes'[136,137])—are associated with a mate choice preference in the other sex. Sexual conflict arises from these mate choice preferences, as the benefits of choice conferred to the choosy sex consequently reduce the fitness in the competitive sex on average[12].

Mating rates and reproductive investment. Conflict over rates of mating and reproductive investment relates to asymmetries that exist between the sexes as a result of the costs or benefits of mating. Conflicts fit into two broad categories: (1) precopulatory or (2) postcopulatory. Many species are in an intersexual arms race in which males are selected for traits that increase their competitive abilities, which may induce harm in females, and in response, females evolve adaptations that offset the fitness costs of male harm. Thus, a co-evolutionary interlocus arms race results, thereby increasing the potential for reproductive success in one sex while decreasing that potential in the other sex (for example, seminal fluid toxins that reduce female receptivity to enhance oviposition, or plugs that prevent females from remating[141]). Conflict over mating may also result in the joint evolution of costly female mate choice and exaggerated male traits under a wide range of circumstances[12].

Ecological factors and environmental conditions. Environmental conditions and local ecological factors can directly or indirectly shape optimal trait values for the sexes differently and therefore are predicted to modify both the magnitude and direction of both inter- and intralocus sexual conflict[142–148]. For example, small increases in temperature result in decreased female fitness in *D. melanogaster*, exacerbated by social context, as a result of temperature-dependent re-mating rates or other mating costs[149]. Furthermore, consistent fluctuations in environmental conditions such as temperature or precipitation can reduce the strength of sexually antagonistic selection, probably by shifting the proximity of populations to their sex-specific optima[150]. Similarly, populations at the edges of their geographical range are less locally adapted, and therefore more often displaced from their sex-specific trait optima, and therefore experience concordant selection[142,150,151]. However more work is needed to fully connect ecological, environmental and population dynamics with sexual conflict.

the distance from the sex-specific optima to male and female traits, and to the corresponding optimal expression levels (Fig. 1). Additionally, sex-specific expression patterns can arise due to sexually concordant selection as well as sexually antagonistic selection, and through indirect selection[41], further impacting patterns of sex-biased expression.

Evidence suggests a relationship exists between sex-specific expression and sexual conflict, although the nature of that relationship is not well understood. The goal of this Review is to explore the existing knowledge surrounding genetic and transcriptional responses to sex-specific selection on traits to identify gaps in our understanding of sex-specific selection and sexually dimorphic transcriptomes. We start with a focus on sequence and regulatory evolution of sex-biased genes, and the impact of pressures such as balancing selection, to highlight the diversity of ways in which sex-specific expression has evolved. We also describe the various, and sometimes contradictory, results emerging from comparative transcriptomics and population genetics studies. Then, we briefly synthesize the current state of knowledge to propose a holistic approach that considers when genes are able to respond to selection, the different forms of selection acting on various tissues and developmental stages, and whether proxies of sex-specific selection can reflect true strengths of sexual selection.

Sex-specific selection and transcriptome evolution

Although some types of dimorphisms are encoded by genes on the sex chromosomes, the vast majority of sex differences emerge from sex-biased gene expression of shared loci[42,43] (Box 3). Similar to morphological phenotypes, gene expression can be viewed in the same phenotype framework, where opposing selection acting on male and female gene regulation creates sexual conflict and distinct sex-specific fitness optima (Fig. 1). Like sexually dimorphic traits, sex-biased expression can range from quantitative differences in expression (analogous to traits such as body size in Box 4) to completely sex-specific expression (analogous to traits such as the male-specific ornament in Box 4). This conflict is then resolved, at least partially, as regulation between the sexes is decoupled, resulting in sexually dimorphic, or sex-biased, gene expression[44]. Just like phenotypic dimorphism, a logical expectation would be that sexual selection should have a major role in the evolution of sex-biased gene expression[40]. Indeed, many studies have investigated the role of sexual selection and sexual conflict in shaping various molecular aspects of sex-biased genes, which we discuss below.

Importantly for gene expression data, it is difficult to know whether sex bias represents partially or fully resolved sexual conflict, as optima will differ for each locus. Selection on loci tends to increase with expression level[45–48], and therefore sex-specific selection on highly expressed genes will act most strongly on sequence variants expressed in the sex experiencing sex-specific selection. Furthermore, the variation in expression level can differ between males and females, and how selection shapes this expression variance remains an unresolved question. A gap in knowledge is how variation in expression level among individuals of each sex is expected to evolve. If the genetic architecture for a trait experiencing sex-specific selection is shared between the sexes, we generally expect correlated expression patterns between males and females, even if expression is biased towards one sex (Fig. 1b–d). One hypothesis is that selection on these genes will act to remove variance in gene expression in both males and females, regardless of which sex is experiencing stronger selection (Fig. 1b), in an analogous way to how selection removes genetic variants. Alternatively, if sex-specific

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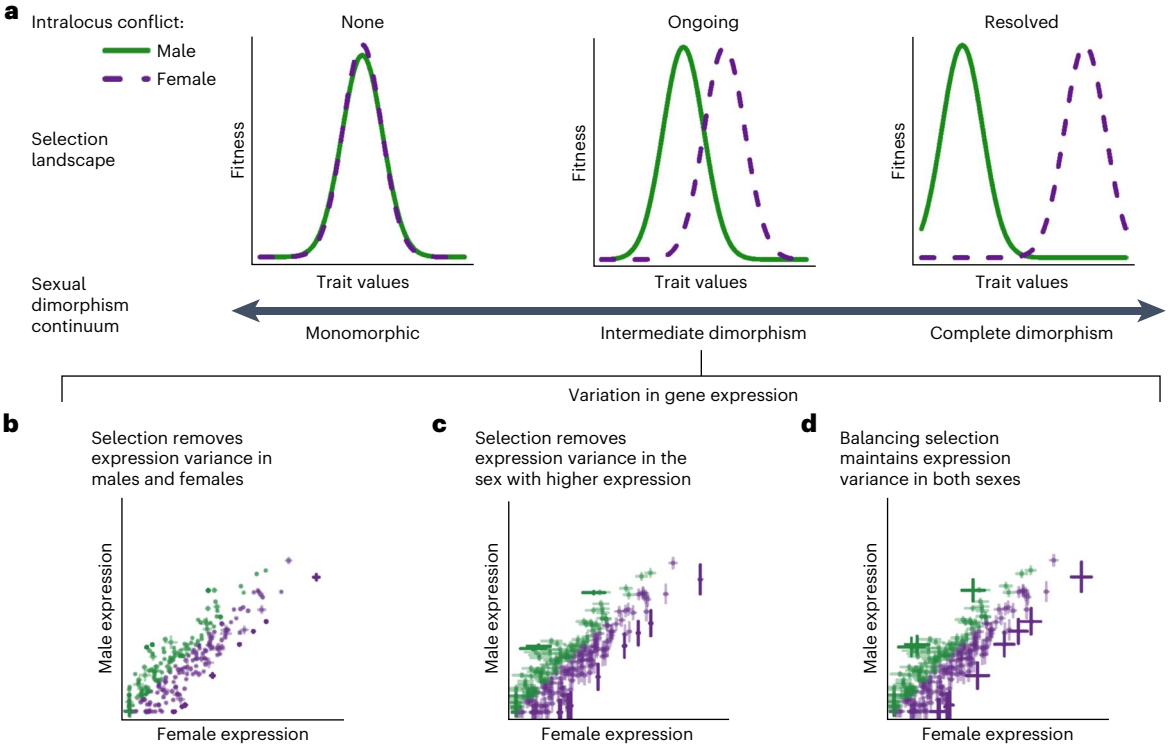

Fig. 1 | The relationship between intralocus sexual conflict, sex-specific selection landscapes, sexual dimorphism and some predictions for how variation in male–female gene expression might be impacted by ongoing intralocus conflict. a, Top left, monomorphic traits do not experience sexual conflict and the traits do not differ between males and females (for example, the spleen in Box 4). Middle, assuming the traits in males and females share a genetic basis, conflict arises once males and females experience diverging selection landscapes. When sex-biased expression only partially resolves conflict, the population displays intermediate sexual dimorphism (for example, the skin in Box 4). Top right, conflict is resolved when males and females reach separate, non-overlapping trait optima. In all cases, some genes will be able to evolve sex-specific expression, but most will be expressed in both sexes, and their expression is likely to be correlated. **b**–**d**, When sexual conflict is ongoing, we can devise three potential hypotheses for how sex-specific selection will shape variance in gene expression (bars represent standard errors around mean expression for each sex). **b**, We might expect selection to reduce variation in expression in both sexes, equivalent to how selection on a sex-specific allele will reduce genetic variation in both sexes in many cases. **c**, Sexual antagonism at loci expressed in both sexes could result in reduced variance in expression in the sex with higher expression, owing to selection being stronger on that sex, but high between-individual variation in the opposite sex because that sex experiences weaker selection and fewer constraints on those genes. **d**, Balancing selection could maintain variation in gene expression in both sexes with ongoing conflict.

selection acts on a male trait with a shared genetic architecture among the sexes, genes that have evolved male bias to partially resolve intralocus conflict should experience stronger selection than unbiased or female-biased genes[49], and therefore selection would be expected to remove variation among male individuals, but not necessarily in females (Fig. 1c). This process would require variation in expression to exist at the outset, which we discuss in more detail below ('Towards a synthesis' section and Fig. 2). If balancing selection acts on expression level, a third possibility includes the maintenance of intra-individual variance in expression in both sexes (Fig. 1d). High levels of variance in expression level could result in heightened interlocus conflict, if the traits are sexually antagonistic, but predictions for the relationship between interlocus conflict and transcriptional variation is currently a gap in the literature. The vast majority of studies of gene expression focus on average expression values in males and females, as described in the remainder of this Review. Although our focus here is primarily on regulatory evolution, the transcriptome does not exist in isolation from the genome, so we first review how genome evolution has impacted sex-specific gene expression patterns.

Sequence evolution of sex-biased genes

Initial studies of sex-biased genes found that genes expressed more highly in males, which were assumed to be important in determining male traits, showed elevated rates of coding sequence evolution[50–52] (see ref. [53] for a full review). These high rates of sequence evolution were initially interpreted as signatures of sexual selection driving rapid evolution in sex-biased genes[53], and sexually selected traits subjected to experimental evolution resulted in nucleotide changes in primarily coding regions that probably removed deleterious variants (potentially also exacerbating sexual antagonism)[54]. However, comparative studies from humans, birds and *Drosophila* suggest that the sequence changes in sex-biased genes are more probably attributed, at least in part, to relaxed constraints and genetic drift[44,55,56]. Theoretical work supports this interpretation of relaxed constraints, as genes with sex-specific expression patterns but not experiencing sexual selection had at least twofold higher rates of sequence evolution compared with constitutively expressed genes in a population genetics model[57,58].

Two key characteristics of sex-biased genes alter the mutation–selection equilibrium expectation compared with genes expressed equally in both sexes. First, since sex-specific genes are, by definition, expressed only in one sex, selection acting on such genes is largely limited to that sex[57,59]. Sex-biased genes might similarly be expected to experience lower selection coefficients—as expression is substantially greater in one sex than the other—and exhibit higher levels of polymorphism, as beneficial alleles are slower to become fixed, compared with other types of genes[57]. Second, sex-biased genes are usually expressed in fewer tissues than unbiased genes[37,60,61], as genes with fewer functional constraints (that is, those genes that are less pleiotropic) are

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BOX 3

Metrics

Sex-biased gene expression. Genes are frequently classified as differentially expressed between the sexes if the fold change is significantly higher than a designated threshold[55,61,87,97,152], usually using a false discovery rate to correct for thousands of multiple tests[153]. In addition, high fold-change thresholds can reduce false-positive rates that result from sex differences in cell type abundance within a tissue[64].

Tissue-specific expression. The tissue specificity index (τ) can help to identify genes experiencing pleiotropic constraints[61]. The value of τ ranges from 0 to 1, with higher values representing a greater level of tissue specificity[61]. This statistic can be calculated for a given gene using expression levels standardized to transcripts per million (TPM) for each tissue, i, as follows:

$$\tau = \frac{\sum_i^n \left[1 - \ln\left(\mathrm{TPM}_i\right) / \ln\left(\mathrm{TPM}_{\max}\right)\right]}{n-1}.$$

Here, n is the number of tissues and $\mathrm{TPM}_{\max}$ is the highest expression level for a given gene across all tissues analysed. An alternative approach to investigating pleiotropy is to identify genes with fold change between different tissues above a threshold[97]. These indices can identify genes with functions across multiple tissues but cannot highlight genes with multiple functions within a single tissue, which could also constrain adaptation and evolution.

Nucleotide diversity. Tajima's D can be used to estimate the proportion of polymorphic nucleotides within a given sequence in a population, which is a signature of balancing selection[75,87,89,97,154,155]. Negative Tajima's D values often indicate purifying selection[75,156], while positive values indicate balancing selection due to elevated polymorphism[75]. Comparisons across multiple species necessitate calculating relative measures of Tajima's D to avoid biases arising from variables like demographic changes in population size[97], for example, by calculating Tajima's D relative to the neutral estimate for each species[97]. Other analyses can be used to infer sex-specific selection on gene sequences, especially when multiple species or lineages are considered, for example, comparisons of non-synonymous to synonymous substitution rates between lineages[55,63] and analysis of variance (ANOVA)-based methods for variance partitioning within versus among lineages[63,157].

Male–female (or intersexual) fixation index. Following Mendelian inheritance, allele frequencies are expected to be equal between the sexes at conception, with genetic divergence between the sexes increasing within a generation due to forces such as sex-specific viability selection, but returning to equal at the start of the next generation. To track these within-generation selective events, differences in allele frequencies between adult breeding males and females can be calculated using fixation indices, for example, F_{ST}. Intersexual F_{ST} is calculated from nucleotide data, ideally only from sites found in more than 50% of individuals in each sex to ensure that sex-limited genes are excluded[87,97]. Several estimators can be used to calculate F_{ST}, some of which are less sensitive to small sample sizes[87,97]. When combined with Tajima's D, intersexual F_{ST} can determine whether within-generation conflict is originating from differences in reproductive fitness versus mortality[75,87,88].Factors including the high degree of statistical noise relative to biological signal could result in many false positives in empirical studies using F_{ST} as sex-specific selection estimator[92,93]. However, reanalyses of genome-wide intersexual F_{ST} estimates from some studies[89,90] probably capture signals of sex-specific selection[76]. Implementing controls to reduce impacts of sex-linked regions, recent duplications onto sex chromosomes[94], and sex-specific population structure[157]—alongside using high-quality reference genomes to avoid mismapping between autosomal and sex chromosomes[76,92]—can increase the accuracy of F_{ST} estimates, but caution is advised when interpreting intersexual F_{ST}.

more likely to respond to divergent selective pressures between the sexes[44]. The reduced functional constraint that permits sex-biased expression might also produce higher rates of neutral evolution than genes with broader expression profiles, but reduced pleiotropy might also allow genes to evolve under positive selection (that is, accumulate beneficial mutations)[62]. This selection on tissue- and/or sex-biased genes might also result in reduced expression variability (Fig. 1), although in *D. melanogaster* brains, tissue-specific genes had more variable expression than broadly expressed genes[63]. A further complication is the fact that cell types can differ in abundance between the sexes within a tissue type, potentially obfuscating true signatures of selection on sex-specific expression patterns[64]. Additional work—both theoretical and empirical—is required to resolve whether sex-biased genes generally evolve due to neutral or selective processes.

Regulatory evolution of sex-biased genes

Instead of acting on coding sequences, several studies have suggested that sex-specific selection acts primarily on gene regulation[44,55,65,66]. In birds, sexual selection acts more on gene regulation than coding sequence[55], a finding consistent with studies in *Drosophila* showing that sexual conflict can be at least partially resolved through regulatory modifications[30]. In jewel wasps, the most highly expressed gene in both sexes—but with 800-fold higher expression in males—is a key enzyme involved in sex pheromone production, indicative of sexual selection acting on expression levels of the gene and genetic constraints preventing the loss of expression in females entirely[67]. Experimental manipulations of sex ratio, a key driver of sexual conflict and sexual selection, have also produced changes in sex-biased gene expression, although the direction varies across *Drosophila* species and with different experimental designs that alter the softness of selection experienced by females[14,65,66]. These results highlight how gene expression can respond rapidly to different forms of sex-specific selection, and indeed sex-biased expression patterns are highly dynamic with differences across tissues and life history stages (see 'Towards a synthesis' for more detail).

Another form of regulatory evolution that could resolve conflict is alternative splicing[68], which can occur through a variety of mechanisms[69]. Alternative splicing can correspond to different levels of expression of the same gene between males and females[70], resulting in patterns of sex-biased expression of the gene. Another scenario exists where genes are able to maintain an overall constant level of expression, but produce different transcripts and proteins, ultimately resulting in phenotypic dimorphism[71]. In birds, alternative splicing can allow genes to respond to differential selection in males and females at the level of the exon, with different isoforms of the same gene being expressed in each sex[49].

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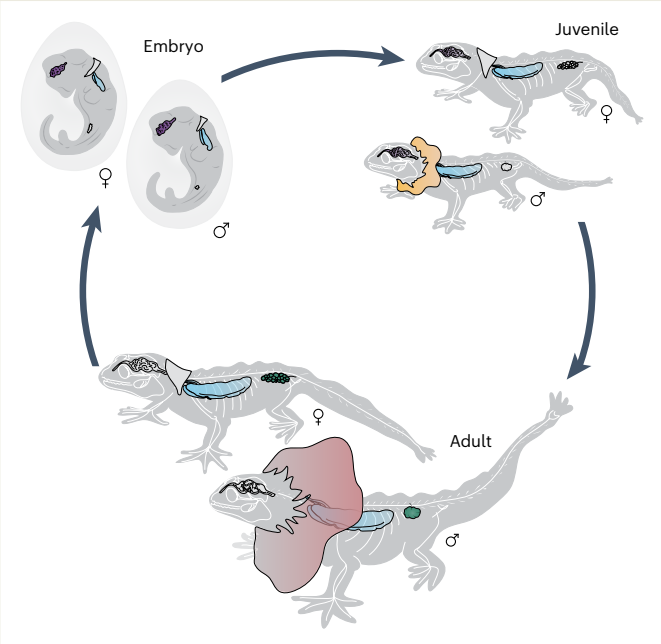
BOX 4

Hypothetical creature

In the figure, we present a hypothetical animal to visually demonstrate a simple case for how selection pressures might differ across tissues, stages of development and sexes. Different selection pressures are denoted by the different colours. This hypothetical animal is a sexually dimorphic reptile, with a large male ornamental neck frill used in intra- and intersexual competition. Across all life stages, the lungs (blue) experience concordant selection, wherein there is little to no variation in selection between the sexes. This or similar tissues can act as a metric for measuring baseline genetic variation observed between males and females. At the embryonic stage, the brain (purple) will predominantly experience sex-specific natural selection as it develops into the more mature brain found in later stages. Here, we would expect some amount of sex-biased gene expression as sex determination occurs in the embryo.



As the hypothetical creature moves into its juvenile state, the most prominent form of selection transitions to viability selection acting on the male neck frill ornament (yellow). The bright colours and exaggerated size of the ornament make males more obvious to potential predators, and thus males with smaller and less obvious ornaments are favoured by selection. The unornamented females would not experience the same selection. Upon sexual maturity, the male ornament is exposed to precopulatory sexual selection (pink). Here, because females are able to perceive the sexual ornament, the more extravagant skin flaps correspond to a higher rate of mating success for males. Further, at this stage the creature is too large to experience the same predation as seen in the juvenile state, and thus viability selection would no longer act on mature males. Following successful mating, additional sexual selection pressures can be observed in the form of postcopulatory sexual selection (for example, via sperm competition and cryptic female choice). This form of sexual selection would primarily act on mature gonadal tissues (green).

Sex-specific selection only acts on genes expressed in the tissues and at the developmental stages during which selection occurs. In our hypothetical example, the clearest case of sex-specific selection acts on the creature's ornament. Consider precopulatory sexual selection on a locus impacting the colour of the creature's ornament. Imagine that allele A1 gives our creature a colour preferred by females but disfavoured by males (that is, females prefer males with that colour but males dislike females of that colour). This creates conflict at the gene, with A1 benefitting males and harming females but A2 benefitting females and harming males. If sex-biased expression evolves to resolve this conflict, those expression patterns will only be necessary in the skin where the colour pattern is expressed, and not in any other tissues. Therefore, if researchers investigated sex-biased expression using adult gonadal tissues, they would miss this conflict resolution. This hypothetical example serves to demonstrate the importance of considering the life history, natural history and context of sex-specific selection and sex-biased expression. Integrating an appreciation for these features of the natural history of a species with the complexities of the molecular mechanisms impacting the resolution of sexual conflict (Fig. 2) will be a powerful approach to understanding the sources of noise in many transcriptomic datasets.

These examples demonstrate that the evolution of sex-biased gene expression can be an important and dynamic mechanism in resolving conflict, but gene expression is probably not sufficient to explain sexual dimorphism on its own. For instance, there are multiple examples whereby knocking out a single gene in mice (and therefore standardizing expression at that locus to be zero) has resulted in sexually dimorphic mutant phenotypes[24]. Additionally, if expression is primarily sex-limited, selection on the regulatory elements could be impeded in the same way it is on coding sequences by acting on only one sex[57,59]. Furthermore, in both *D. melanogaster* and *D. simulans*, the targets of sexually antagonistic selection are generally not enriched for highly sex-biased genes, suggesting that regulatory evolution can resolve conflict at many loci, but that constraints exist at some loci that prevent complete resolution of sexual conflict[72].

Genetic diversity and sex-biased genes

In many instances when sexual conflict arises over alleles at the same locus (intralocus conflict), the allele with the greater net fitness will go to fixation, even if this results in a negative impact on population fitness due to deleterious effects in one sex[38,73,74]. However, when the absolute value of the effects in each sex are relatively similar, balancing selection may occur under certain circumstances, favouring different alleles in males and females, and resulting in the maintenance of higher genetic diversity than loci not under balancing selection[75–77]. Balancing selection is least likely to occur when both sexes have been displaced from their respective fitness optima, but it still has the potential to shape genetic variation as populations adapt[77].

Sex-specific dominance reversal can broaden the parameter space in which balancing selection can maintain sexually antagonistic alleles[78]. In these cases, if A_1 is a male-beneficial allele and A_2 is a female-beneficial allele, A_1A_2 males experience fitness outcomes most similar to A_1A_1 males while A_1A_2 females resemble A_2A_2 females in their fitness[35]. While only a few examples exist for sex-specific dominance reversals in empirical systems (salmon[79], trout[80], seed beetles[81] and *Drosophila*[82]), they can also be difficult to detect in wild populations. As such, dominance reversals could be a more prevalent process that might account for observed levels of sexually antagonistic variation observed. Partial sex-specific dominance reversals can even persist

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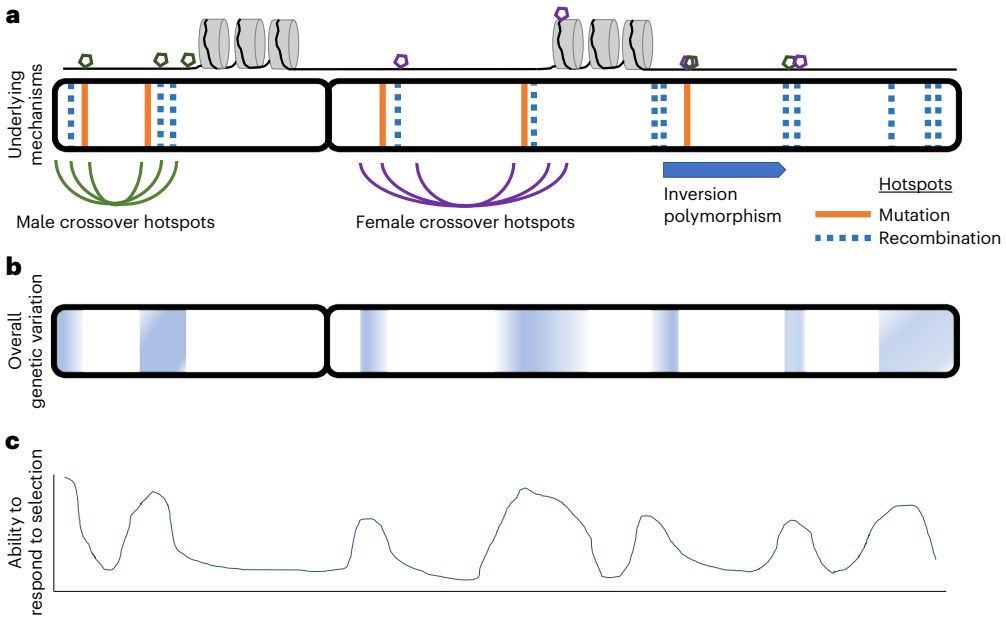

Fig. 2 | A schematic of mechanisms that facilitate genetic variation, transcriptional variation and the ability to respond to selection. a, Variation in gene expression can arise due to mutation and recombination hotspots, inversions, differential crossovers between males and females, and DNA modifications such as methylation (coloured pentagons; purple, female; green, male) and histones (grey cylinders), depending on the organism. **b**, Overall genetic variation, with darker blue corresponding to hotspots of variation and white corresponding to background rates of variation. **c**, The general ability of loci to respond to selection (that is, sufficient variation at the protein level as a substrate for selection) varies in response to **a** and **b**.

if the sexually antagonistic selection is historical and the population currently experiences sexually concordant selection[82]. In general, sex-specific dominance parameters can partially resolve conflict[83,84], but will require the other mechanisms such as gene duplication and sex-specific expression[36] to fully resolve sexual conflict.

In the cases when balancing selection does maintain sexually antagonistic variation, this intralocus sexual conflict results in polymorphisms in coding regions that can persist across evolutionary timescales. Consistent with this, balancing selection has been observed in genes underlying traits subject to opposing selection in the sexes[30,85,86], and some coding sequences have experienced persistent patterns of balancing selection across populations and species of *Drosophila*[72]. Integrating population genetic signatures beyond balancing selection can help differentiate the sources of sexual conflict[87]. If the intralocus conflict occurs over reproductive fitness—such as conflict arising from sexual selection[42]—then major allele frequency differences between the sexes are unlikely[75,87], and we would only expect excess polymorphism. By contrast, if intralocus conflict emerges over viability or survival, with a given allele influencing male and female mortality in opposite directions, observable differences in allele frequencies between the sexes can accumulate within a single generation[75,76,88–91], although selection is required to be strong[92,93] and spurious signals can emerge during analysis—especially if signals emerge at autosomal genes that have been recently duplicated to sex chromosomes[94] (Box 3). Therefore, we would expect a signature at these loci of both excess polymorphism due to balancing selection, and allelic differences between males and females (see Box 3 for estimation methods). Indeed, an excess of polymorphism has been observed at certain loci in a range of species, such as Soay sheep[86], *Drosophila* spp.[30,95] and bank voles[96].

When genome- or transcriptome-wide patterns of balancing selection and male–female allele frequencies have been investigated, and related back to sex-biased expression, several patterns have emerged. Various authors have reported that highly sex-biased genes can have large genetic differentiation between adult males and females across a range of taxa[72,87–89,97], although the power of the tests was often low[97]. This consistency could imply that conflict over survival is more closely related to sex-biased expression than conflict over reproduction, even if the majority of genes are experiencing differential fitness effects related to reproduction (that is, have high balancing selection and low male–female differentiation), as seen in guppies[87] and collared flycatchers[89]. However, arriving at this conclusion may be premature as few studies have robustly compared these two sources of conflict using comparable fitness estimates.

Towards a synthesis

Putting all of this evidence together, can we make any general conclusions about the impacts of sex-specific selection on genome sequences and gene expression? First, in some cases, coding sequence evolution might be a by-product of a relaxation from constraints rather than a response to sex-specific selection pressures. Second, sex-biased regulation, through expression, splicing, or both, often evolves and resolves conflict, and may interact with sex-specific genetic architecture to shape phenotypic dimorphism. Finally, loci experiencing intralocus sexual conflict can retain genetic variation through balancing selection, and small shifts in allele frequencies within a generation are sometimes detected if this conflict arises over viability, but statistically this effect is often difficult to identify.

Several questions remain unresolved, despite the progress towards understanding the impacts of sex-specific selection on the transcriptome. For instance, how much of sex-biased expression can be attributed to the resolution of conflict over sexual selection? To what extent do the mechanisms driving sexual selection, such as Fisherian runaway selection, sensory drive and good genes models (Box 2), influence the transcriptomic and genomic patterns observed? Similarly, how often does sexual selection result in persistent sexual conflict, with signatures of ongoing conflict, as opposed to the resolution of conflict through the evolution of phenotypic dimorphism that allows males and females to reach their respective fitness optima?

To address these questions, we suggest taking a holistic and integrative approach, which incorporates knowledge of the study species

(for example, from behavioural or ecological studies) with developmental and genome biology. Specifically, to differentiate between conflict over sexual selection versus other sources (including different types of sexual selection), we highlight the need to consider when genes are able to respond to sex-specific selection pressures, which tissues are likely to experience different forms of sex-specific selection, when during the life cycle different forms of sex-specific selection are likely to create conflict and whether proxies of sex-specific selection reflect true strengths of sexual selection. To investigate the persistence of conflict, we highlight the need for a comparative approach (across tissues, developmental stages and species) if we want to fully decipher the relationship between sex-specific selection and sexually dimorphic transcriptomes.

The chromosomal view of selection

Just as pleiotropic expression patterns can constrain genes from responding to sex-specific selection[26,60,61], so too can the physical location of a gene constrain or facilitate the response to sex-specific selection. Recombination and mutation rates are not uniformly distributed across chromosomes, with some regions acting as hotspots of recombination[98], mutation[99–101] or both[99]. Structural variants such as chromosomal inversions can create further heterogeneity in recombination rates across chromosomes—impacting the amount of genetic variation available to selection[98]—and in some cases creating 'supergenes' encoding sex- and morph-specific phenotypes[102–104]. Recombination rates can also differ between the sexes, resulting in potential sexual conflict over recombination rate and hotspot location[105]. These factors interact with genetic drift and selection—including sexually antagonistic selection[106]—to create linkage disequilibrium[107], which will impact the ability of the genes within an affected region to respond rapidly to sex-specific selection, potentially resulting in conflict hotspots and coldspots based on chromosome architecture (Fig. 2).

Furthermore, methylation, histone modifications and other components of the packaging of DNA molecules can impact the expression of genes, and methylation often differs between the sexes[108]. The contribution of methylation to sexual dimorphism appears to vary across species, with some species having methylation patterns associated with sex-specific expression[67] and others showing no clear relationship between methylation and sex-biased genetic regulation[108,109]. In *D. melanogaster*, regulatory polymorphisms on the Y chromosome can alter the chromatin components that impact the expression of genes on other chromosomes, even in tissues where Y-linked genes are not expressed[110]. Furthermore, these regulatory patterns might be a mechanism through which sexual conflict acts, as genes with altered expression patterns are those associated with traits experiencing ongoing conflict[110]. As such, not only will methylation and other modifications impact observed patterns of gene expression—and potentially contribute to conflict resolution—but might also have a direct role in the perpetuation of sexual conflict as well.

Appreciating these molecular mechanisms (among others, including cell type abundances[64]) will be critical in understanding the impact of sex-specific selection on sex differences in gene expression (Fig. 2). Interrogating transcriptome datasets for patterns of linkage disequilibrium, or identifying chromosomal regions with elevated sex-biased expression across multiple linked genes, will bring us closer to answering unresolved questions about the mechanisms enabling conflict to be resolved or that allow sexually discordant variation to be maintained in a population.

Tissue specificity and interactions with selection

The pattern and extent of sex-biased expression differs across tissue types[111], and we can leverage these tissue-level patterns to gain further insight into how sex-specific selection drives genomic and transcriptomic evolution. Comparing patterns of sex-biased expression and signals of balancing selection across tissues makes it possible to identify signals of resolved conflict associated with specific types of sex-specific selection. For example, comparing gonadal tissue to skin tissue in our hypothetical organism (Box 4) would allow us to differentiate between postcopulatory sexual selection (in the gonads) and precopulatory selection (in the skin). Furthermore, by also investigating population genetic patterns (Box 3) in a tissue expected to experience concordant selection between the sexes, background levels of genetic variation can also be investigated to provide a baseline that reflects demography and evolutionary history.

Development and timing of selection

Likewise, genes show vastly different patterns of expression across developmental stages. All studies explicitly investigating the molecular mechanisms underlying sexual selection and/or sexual conflict have studied adults. This focus is understandable as strong sexual selection and sex-biased genes occur predominantly after sexual development[24]. Yet, we know that mechanisms such as sex-biased gene expression, have been shown to be dynamic throughout development[112–121]. Some transcriptional differences between males and females appear to be conserved throughout all life stages[122], however many more exhibit stage-specific expression with genes either transiently sex-biased throughout development[117,118], or switch from being biased in one sex to biased in the other at different stages[120,121]. Identifying sex bias across development provides insights into sex differentiation and the different routes in which conflict resolution could be constrained or facilitated (for example, through sex-limited trait development, gene duplication, sex-specific dominance and sex-specific gene regulation[123]). Additionally, by examining gene expression at stages prior to sex determination, we can determine whether there are inherent differences between the transcriptome of males and females not necessarily associated with sexual development[113].

The dynamic nature of sex-biased expression may also indicate fluctuations in sex-specific selection throughout development (Box 4). As it is generally observed that sexual dimorphism increases throughout development, we may expect a corresponding increase in sexual conflict as the evolutionary interests of the two sexes diverge. Sexually antagonistic selection is likely to be strongest in developmental stages when sex-specific traits are being produced, such as during sex determination early in ontogeny[120] or at the onset of sexual maturity[117,118]. There is, however, a paucity of studies examining the progression of sexual conflict, sexually antagonistic selection and sexual dimorphism across developmental stages. The few studies that have examined sex-specific selection through development to some degree highlight the importance of understanding the natural and life history of study species, as patterns that emerge can greatly differ across organisms[27,124–128]. By focusing on components of selection at a single developmental stage, we risk overlooking important variation and signals of resolved conflict related to specific types of sex-specific selection. While sampling every tissue at every developmental stage is not feasible for the majority of organisms, choosing appropriate tissues and life history stages is a critical component of experimental design when investigating patterns of gene expression.

Estimating selection strengths across species for a comparative approach

Alongside a holistic approach with regard to selection, tissues and developmental stages, comparative approaches are particularly powerful tools to study patterns of selection on traits alongside transcriptome data[129]. Few studies directly tie estimates of fitness or selection to expression patterns[30], and often proxies for sexual selection are used[55,97], potentially introducing additional uncertainty into already noisy datasets. Quantitative estimates of strengths of selection at various episodes in a population's life cycle[130,131], identification of the traits involved in sexual selection and assessments of whether pre- or postcopulatory selection is stronger for a given species can help to guide

transcriptome studies aimed at understanding sexual conflict and sexual selection. Additionally, pairing analyses of sex-biased expression with an assessment of tissue specificity (to estimate pleiotropy) and genetic variation (that is, whether balancing selection is acting) will enhance interpretations of whether genes are likely to be responding to sex-specific selection pressures. Furthermore, if a comparative approach is taken, species can be sorted by quantitative estimates of sexual selection strengths, and rates of molecular evolution at genes responding to sex-specific selection pressures could be assessed. Combining data and inference from multiple levels (genomic variation, transcriptomic expression and elements of natural history such as the form that sexual selection takes) is required for answering major outstanding questions.

However, it is not always feasible to directly estimate strength of selection. Instead of focusing on univariate traits as a proxy for selection, we suggest leveraging existing data on behaviours, multivariate traits and life histories to create a more holistic picture of the relative strengths of sex-specific selection, for example, by estimating the strength of mate preferences from wild populations[132]. The comparative approach is particularly useful when combined with phylogenetic relationships so that evolutionary histories can be included in the analysis.

Conclusions and outlook

Recent studies examining the effects of sex-specific selection and sexual conflict on genome and transcriptome variation have revealed that sex-biased gene regulation and sex-specific genetic architecture frequently interact to resolve sexual conflict, with balancing selection retaining genetic polymorphisms in only some cases. Despite this progress, some outstanding questions remain: (1) are some forms of conflict more likely to be resolved through sex-biased expression than others, and how important is conflict resolution? (2) Can we differentiate between signatures of sexual selection and other sources of conflict using genomic and transcriptomic datasets? (3) How consistent and persistent are the mechanisms resolving sexual conflict across populations and closely related species?

In this Review, we provide an integrative framework for answering these questions that brings together the molecular mechanisms governing sex-biased expression, the known transience of expression patterns through development and across tissues, and leveraging natural variation in traits and natural histories across closely related species. By using this holistic and comparative approach, we believe that answering these outstanding questions is within our grasp.

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Author contributions

S.P.F. conceived this work and all concepts were shaped by discussions with N.M.T., E.R.B., B.B.M.W. and J.E.M.; S.P.F., N.M.T. and E.R.B. wrote the first draft of the manuscript, and N.M.T., E.R.B., B.B.M.W., J.E.M. and S.P.F. edited subsequent drafts.

Competing interests

The authors declare no competing interests.

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