Polyandry and sex-specific gene expression

Judith E. Mank¹, Nina Wedell² and David J. Hosken²

¹Department of Genetics, Evolution and Environment, University College London, The Darwin Building, Gower Street, London WC1E 6BT, UK
²Centre for Ecology and Conservation, University of Exeter, Cornwall Campus, Tremough, Penryn TR10 9EZ, UK

Polyandry is widespread in nature, and has important evolutionary consequences for the evolution of sexual dimorphism and sexual conflict. Although many of the phenotypic consequences of polyandry have been elucidated, our understanding of the impacts of polyandry and mating systems on the genome is in its infancy. Polyandry can intensify selection on sexual characters and generate more intense sexual conflict. This has consequences for sequence evolution, but also for sex-biased gene expression, which acts as a link between mating systems, sex-specific selection and the evolution of sexual dimorphism. We discuss this and the remarkable confluence of sexual-conflict theory and patterns of gene expression, while also making predictions about transcription patterns, mating systems and sexual conflict. Gene expression is a key link in the genotype–phenotype chain, and although in its early stages, understanding the sexual selection–transcription relationship will provide significant insights into this critical association.

1. The role of mating and mating system in gene expression

Gene expression represents an important step in the genome–phenotype relationship in both the short- and long-term. Over the short-term, expression of many genes is a dynamic response to stimuli, and this is the way that the static and predefined genome of an individual interacts with changing environments and ecologies over the course of a single lifetime [1,2]. Gene expression also evolves over time [1,3–5], and adaptive changes in gene expression represent an important route to phenotypic evolution [6–8].

Mating system largely defines sex-specific selection, which in turn affects gene expression patterns in males and females in several different ways. Differences in mating system can generate different intensities of sexual selection and sex-specific selection, which in turn can generate sexual conflict when males and females experience different fitness optima for shared traits. It is clear that sexual conflict often plays out at the genetic level through gene expression differences between males and females [9,10], as described in table 1. Conflict can also lead to parent-of-origin genomic imprinting [19,22] when antagonism between the parents over growth and provisioning plays out through the developing offspring. Mating system and sex-specific selection also play a major role in the evolution of sexually dimorphic and sexually selected phenotypes, and many sexually dimorphic traits are encoded by different expression levels of the same genes rather than by different genes in males and females [12,23,24], a phenomenon referred to as sex-biased gene expression. In some extreme cases, expression may be completely absent in one sex leading to sex-limited gene expression; however, this is relatively rare for genes not linked to sex-limited Y or W chromosomes [14]. In addition to expressing different amounts of the same gene, females and males may express different structural arrangements of the same gene, splicing or skipping different exons to create different protein isoforms [16]. Sex-specific alternate splicing is common in many animals, and is key to the sex determination pathway in many insects [15,25,26].
Table 1. Mechanisms of gene expression differences between males and females.

<table>
<thead>
<tr>
<th>type</th>
<th>manifestation</th>
<th>prevalence</th>
<th>mechanism</th>
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<tbody>
<tr>
<td>sex-biased</td>
<td>genes expressed in both males and females, but at</td>
<td>very common, may encompass &gt;50% of genes in the genome [11]</td>
<td>sex-biased genes can be regulated by sex determination pathway [12] or sex-hormone receptors [13]</td>
</tr>
<tr>
<td>gene expression</td>
<td>different levels</td>
<td></td>
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<tr>
<td>sex-limited</td>
<td>genes expressed only in one sex</td>
<td>all Y-linked and W-linked genes are by definition sex-specific; however, sex-limited expression is rare aside from these chromosomes [14]</td>
<td>for loci present in both sexes, regulation by Y or W loci could produce sex-limited expression</td>
</tr>
<tr>
<td>gene expression</td>
<td></td>
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<tr>
<td>alternative splicing</td>
<td>males and females express the same genes, but alter exon order or skip different exons</td>
<td>key element in the sex determination pathway in some insects [15]; also prevalent in mammalian transcriptomes [16]</td>
<td>can occur when splicing is regulated by sex determination pathway or by sex-hormone receptors</td>
</tr>
<tr>
<td>imprinting</td>
<td>either the paternal or maternal allele is expressed in the offspring</td>
<td>involved in sex determination pathway in some insects [17], some medical conditions [18]; may be common in mammals [19,20] but this remains contentious [21]</td>
<td>presumably, methylation patterns in the parental gametes are maintained in the zygote; alternatively, imprinting requires some sort of sensing mechanism to identify parent of origin</td>
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Here, we examine how mating and mating system, with particular emphasis on polyandry, affect gene expression. Our ultimate aim is to understand the relationship between the genome, the transcriptome (the sum output of all expressed genes in an individual) and the phenotype. The complete attainment of this goal is somewhat distant, as the connections linking genome, transcriptome and phenotype remain hazy in many ways; however, initial results have suggested that gene expression studies may be a powerful way to study the effects of sexual conflict and sex-specific selection within the genome. We therefore also extrapolate from phenotypic data in order to predict how polyandry might affect gene expression evolution.

2. Short-term gene expression dynamics in response to courtship and mating

Gene expression is the primary route by which the genome of an individual responds to environmental, ecological and behavioural stimuli, and so it is perhaps not at all surprising that mating behaviours and mating itself have large effects on gene expression. The crucial nature of reproduction, both in terms of physiology and evolutionary fitness, suggests that it should factor heavily in gene expression dynamics. Reproductive maturity is associated with a large number of gene expression changes in both sexes [27–29]. However, sexual maturity is quite a different physiological state than actual reproduction, particularly for females in internally fertilizing species that must assemble either fertilized ova into layable eggs or provision developing embryos. Because courtship and mating always precede reproduction, these can be thought of as cues that initiate the final physiological changes required for successful breeding. From this perspective, courtship- and mating-induced changes in gene expression are the route by which an individual begins to transition physiologically from adult to parent. There are multiple targets and mechanisms by which courtship behaviour and mating induce transcriptomic changes, and although most of the work in this regard involves Drosophila, findings there have been corroborated, albeit with limited evidence from other animals.

There are distinct changes in gene expression that occur in the brain of males and females related to mating, and these can be divided into changes relating to mating behaviours and changes arising owing to mating itself. Visual, auditory and other courtship cues are alone sufficient to cause gene expression changes in the female brain, as observed in both Drosophila melanogaster [30] and songbirds [27,31]. In many ways, this mirrors recently identified changes in cuticular hydrocarbon (CHC) bouquets that occur as a consequence of variation in the social environment and mating status of male D. melanogaster. CHCs are complex sexual signals in Drosophila [32], and these plastic expression responses mean that trait values depend not just on the genotype of focal individuals, but also on the genes being expressed in individuals that make up the social environment [33,34].

Mating itself causes further gene expression changes in both male and female brains [35–37]. In all cases, these transcriptomic changes are subtle, and transcriptomic studies in a range of species suggest sex differences in the brain are relatively modest [13,38–40]. This may be because small changes are sufficient to cause large behavioural differences between the sexes. Alternatively, behaviours are genetically controlled within relatively confined areas of the brain, and because these regions are difficult to isolate, studies that have used whole-brain homogenates almost certainly underestimate local expression differences [4,40].

Beyond the brain, mating elicits a huge response in gene expression in the female reproductive tract [37,41,42]. Part of this is due to the female’s innate transcriptomic preparations for embryo provisioning [43]. In addition to this, the female reproductive system stages an immune response
to the foreign proteins contained in the male’s seminal fluid [43–45]. Finally, part of the changes in the female reproductive tract is in response to manipulative proteins from the male transcriptome in the form of accessory gland proteins (ACPs) [41,46,47], which stimulate female oogenesis and ovulation, as well as impede female remating [47]. ACPs appear to be involved in inter-locus sexual conflict, which involves interactions between antagonistic alleles at two or more loci in males and females, and so they are also discussed in §6.

Most studies of gene expression in relation to mating compare mated and unmated individuals, and so therefore cannot differentiate the effects of monandry from polyandry in the female reproductive tract. Initial evidence suggests that female transcriptomic responses to multiple mating are not cumulative, rather the majority of changes occur with the transition from virgin to non-virgin, and additional matings after the first do little to change the female transcriptome further [45]. However, there may be a temporal aspect to polyandry-induced changes to the female transcriptome. Single-mated females experience a transcriptomic cycle extending for several hours after mating [42], and polyandrous females may experience this cycle multiple times.

3. Long-term gene expression evolution in response to mating system

Specific mating systems are associated with different intensities of sex-specific selection. Selection can alter gene expression or coding sequence to enact phenotypic change [8,48], and if the effect of sex-specific selection in coding sequence is anything to go by [49], then sex-specific selection should also act on gene expression of loci involved in sexual selection and reproduction. Polyandrous mating systems can experience increased levels of male-specific selection, particularly in regard to sperm competition, and this may act on the spermatogenesis transcriptome in several different ways.

Gene expression differences in males under monandrous versus polyandrous mating systems are largely unknown, but we can extrapolate somewhat from the potential for male-specific selection. Given that polyandry is frequently associated with increased sperm competition, we might expect some transcriptomic shift from the production of somatic sexually selected indicators of male quality in monandrous systems [50] to a greater investment in sperm production in order to win fertilizations in polyandrous systems [51,52]. This would manifest as higher expression levels over evolutionary time for genes involved in spermatogenesis, as the sperm production and assembly mechanisms increase their output in polyandrous versus monandrous systems. This is not to say that males in polyandrous species will forgo investment in sexually selected somatic traits that function in mate choice and competition, but they also face post-mating competition that requires transcriptional investment to win. There are two general ways this can be achieved, either by trade-offs between sexual traits, or by trading-off sexual traits against other characters. As males must mate to obtain fitness, we would expect the trade-off to be with non-sexual traits, and hence polyandrous systems might display reductions in gene expression levels that have little obvious connection with sexual fitness. This is consistent with experimental evolution studies in yellow dung flies showing that higher investment in sexual characters such as testis size under polyandry comes at a cost to immune function [53–55].

We might also expect elevated levels of purifying selection on both the gene sequence and gene expression patterns related to sperm production [56,57]. In mating systems with no or very little sperm competition, males need only produce a few viable sperm for each fertilization event. When the sperm competition that results from polyandry conforms to a lottery, where sperm are tickets and fertilizations the prize, males will not only be selected to produce more sperm, but they could also be under selection to produce more high quality sperm to ensure they successfully fertilize ovum [58–60]. This is clear from experimental evolution studies in mice that show an increase in sperm quality under polyandrous conditions compared with monogamy [61]. This increase in sperm quality may manifest in gene expression as lower transcriptional variance for spermatogenesis genes, as variance can be taken as a measure of the strength of purifying selection on gene expression [4].

The haploid nature of sperm offers the possibility that at least some genes associated with sperm function are subject to haploid selection [62]. This would make the effects of purifying selection associated with increased sperm competition even more powerful, especially for recessive or partially recessive variation in expression level. However, in metazoans, the scope for haploid selection appears to be relatively limited [63–65]. This is because DNA compaction during spermatogenesis means very few genes are expressed solely by the haploid sperm cell. Instead, expression more often seems to originate from the testis during sperm formation; therefore each sperm cell, although carrying a haploid genome, is composed of proteins from the diploid genotype, making the sperm population a mix of gene products from both alleles present in the testes.

The effects of female-specific selection in gene expression in polyandrous systems are much more difficult to predict. Polyandry may result in both post-copulatory and pre-copulatory mechanisms of female choice in some systems [59], adding transcriptomic mechanisms of female choice to the reproductive tract. In support of this, a recent study found significant differences in expression levels of ovary and brain genes in female D. melanogaster mated to either preferred or unpreferred males, indicating the involvement of both pre- and post-copulatory female choice mechanisms [66]. Beyond that, female-specific selection to reduce polyspermy may drive the evolution of gamete-recognition proteins on the ovum [67–69].

As with males, polyandry could cause shifts in the focus of female choice to the post-copulatory arena at a cost to pre-copulatory mechanisms of choice, but selection could also favour reductions in investment elsewhere while retaining pre- and expanding post-copulatory choice mechanisms. This may also depend on how easy it is for females to avoid multiple mating when it is not in females’ interests. Again, experimental evolution has shown that females’ ability to circumvent male interests evolves under polyandry and that this comes at a cost to non-sexual immunological fitness [52,70]. Further support for the suggestion that females may divert their investment towards post-copulatory mechanisms comes from evidence that sperm storage can represent a cost to females, again in terms of compromised immune response. In Atta colombiana ants, for example, sperm storage is traded off...
against the ability to mount an immune response, and this effect is exacerbated when the ejaculates from different males are stored, possibly indicating an increased cost of storing and processing genetically different ejaculates [71]. In Drosophila pseudoobscura, a species with polymorphic sperm, non-fertile sperm function to protect the fertile sperm against female-mediated sperm death [72]. Female spermicide in turn may have evolved as a mechanism to reduce the cost of storing sperm from several males.

4. Polyandry and the effects of increased sexual conflict

Although polyandry can potentially reduce the variance in male reproductive success, and thereby reduce the intensity of sexual selection [73–75], in many cases the evolution of polyandry also brings with it greater potential for inter-locus and intra-locus sexual conflict between females and males compared with monogamy. This sexual conflict can play out between the parents directly as males and females seek to manipulate each other’s reproduction. Inter-locus conflict, which involves antagonistic alleles at multiple loci, includes maternal–foetal conflict over resource provisioning in species with internal gestation. Intra-locus conflict, where alleles at a single locus influence male and female fitness in opposite directions can affect a large proportion of the genome [76], and influences gene expression in several ways, ranging from transcriptional differences between the sexes [9,77–79] to parent-of-origin imprinting in the developing offspring [80,81]. At this point, there are few direct connections between gene expression and sexual conflict, although it is becoming increasingly possible to test the existing predictions [77,81,82] with transcriptome data [19,78,79]. What is lacking most is the direct connection between transcriptome data and sex-specific phenotypes, but that will no doubt emerge in time.

5. Mechanisms of differential gene expression

Phenotypes are ultimately composed of protein, and many phenotypic differences are attributable to differences in protein levels [8,48]. The same is true of sex-specific phenotypes, many of which are the result of differential gene expression between males and females, and ultimately many of these must result from conflicting selection between the sexes. Transcription of RNA is a direct predictor of protein translation levels, and because protein chemistry is much more difficult to work with than RNA chemistry, it is often easier to use RNA levels as a proxy for protein levels.

Although the same nuclear genome is present in every somatic cell of an organism, the transcriptome is far more variable, and can vary throughout the body and over a life cycle. This is particularly true for sex-specific transcriptomes, which differ during development [29,83] and among different tissues in the body [84–86]. Transcriptome studies must therefore target the correct part of the body and the correct developmental stage.

Developmental stage is particularly important in this context, because although sexual dimorphisms and sexually selected phenotypes are most evident in adults, a significant proportion of these traits are the product of developmental processes that amplify into adult phenotypes. For example, the horn in adult male horned beetles is the product of larval differences between the sexes that increase over development [23,87,88]. Similarly, the transcriptomic basis of the sword in male swordtails is largely complete by the time the sword manifests fully in the adult phenotype [24]. In cases such as these, it is crucial to sample the individual at the stage at which sexual dimorphisms are being programmed in the phenotype, rather than the adult stage when the dimorphism is simply most evident.

There are several routes by which males and females can express the same gene in different ways and therefore achieve sex-specific phenotypes (table 1). Sex-biased expression is the most commonly assayed form of gene expression differences, and it is clear that many, if not most genes, are expressed at different levels between the sexes at some point in development or in some part of the body [89]. Aside from genes on sex-limited Y or W chromosomes, addressed later, relatively few of these differences are due to sex-limitation, where a gene is completely shut off in one sex or the other. Rather, most sex-biased gene expression is the product of different transcription rates of genes expressed to some degree in both males and females. The rarity of sex-limited expression may be due to the fact that selection to shut off expression progressively weakens as gene expression decreases and therefore produces little phenotypic effect. Theoretically, the difference in transcription between females and males is due to sexual conflict over optimal protein levels [9], and this conflict can be resolved when the correlation in expression between the sexes is broken down.

It is, however, not always possible to resolve sexual conflict, especially when there are ontogenetic [90] or pleiotropic [91] constraints acting on a sexually antagonistic locus. It may be that in these cases, recent gene duplicates take on sex-specific expression patterns [92,93]. Gene duplication may therefore represent a route to the resolution of conflict over genes that perform multiple functions [82,92], but this may also require modifications to genetic architecture [94], in addition to dominance, recombination, sex-linkage and population size [82].

Although there are reasons to think that this might not be an easy or common solution to sexual conflict [95], gene expression studies, particularly of the sex chromosomes, have demonstrated that gene duplication, and duplication-mediated relocation, can provide a route for sexually antagonistic loci to transfer to regions of the genome with beneficial sex-specific selection regimes. The best illustrations come from the sex-limited W and Y chromosomes, which, due to their sex-limited nature, experience only female-specific (in the case of the W) or male-specific (in the case of the Y) selection. Female-benefit genes that cause harm to males relocating to the W chromosome, or present on newly emergent W chromosomes, may experience the immediate resolution of this conflict as these genes are no longer expressed in males, and the same is true for male-benefit genes on Y chromosomes [96]. However, relocation to the Y or W chromosome, associated with loss of the original autosomal, X- or Z-linked locus, will resolve conflict only for some types of genes, particularly those with narrow sex-specific functions, and will not resolve conflicts over optimal expression for genes that function in both sexes. For the latter class of genes, resolution can occur via duplication to the Y and W chromosomes followed by sex-specific
specialization of the W- or Y-linked daughter locus, in concert with retention of the parental copy (figure 1). This route may be more common than relocation for genes with many network connections or multiple functions. Selection for sexual conflict resolution may drive the evolution and turnover of sex chromosome systems [97, 98], as well as gene gain by existing sex chromosomes, as evidenced by the fact that the rate of gene acquisition on the *Drosophila* Y chromosome is nearly ten times greater than the rate of gene loss [99]. We discuss the more complex pattern expected and observed for Z and X chromosomes in §7.

It is not clear at this point how common sexual conflict is at the level of the exon versus the entire transcribed gene; however, there is growing evidence that males and females do not just express genes at different levels, but they also splice many genes differently, combining exons in different order or combinations. Sex-specific alternate splicing is common for many genes [16], and although alternate splicing is key to the sex determination pathway in many insects [15, 25, 26], it is not yet clear how alternative splicing contributes to somatic phenotypic sexual dimorphisms or sexual conflict in a broader sense.

Genomic imprinting occurs when maternally or paternally inherited copies of a gene are expressed differently in the offspring, presumably due to methylation of genes or other regulatory control. Imprinting of single loci is important in the sex determination pathways of some haplodiploid insects [17, 100], and recent assessments have suggested that imprinting affects a large proportion of mammalian genes [19, 20], although the veracity of these results is currently under intense debate [21]. If this mechanism is common, it may represent a means of resolving sexual conflict that plays out in the developing offspring [81].

6. Inter-locus sexual conflict

Inter-locus sexual conflict can generate an arms race of adaptation and counter-adaptation in males and females that can result in severe physiological costs [101], as well as spurring phenotypic evolution [102]. Inter-locus sexual conflict is somewhat difficult to study from a gene expression perspective without some prior knowledge of the loci involved, although work on ACPs and imprinted loci provide some ideas about how the transcriptome may be affected. It may be that increasing levels of sexual conflict under polyandry mean that a greater proportion of loci become entangled in inter-locus arms races between males and females compared with monogamy. However, it may be some time yet before a comprehensive catalogue of the genomic distribution of loci involved in this type of conflict is available. In the meantime, there are several illustrative examples.

Given their role in sexual conflict, the genes encoding ACPs represent potential loci for male benefits in the sexually antagonistic coevolution that can be associated with polyandry. When present in an ejaculate, ACPs act to manipulate female reproduction in the male’s favour and impede female remating [47]. They are therefore subject to particularly strong male-specific selection, which may explain why ACPs are among the most rapidly evolving proteins in the genome [103, 104]. Females show a profound transcriptomic response to ACPs [41, 46, 47], and female-specific selection could act to make females resistant to male manipulation that is harmful to female fitness. Because of this, we might predict that ACPs would evolve more rapidly under polyandry than both monandry and monogamy. Although polyandry is associated with increased rates of evolution of seminal fluid proteins in primates [105], this pattern is not
universal, and polyandry does not explain rates of sequence evolution for ACPs in Heliconius butterflies [106,107]. It may be that polyandry does not affect sequence evolution as much as expression level of ACPs because increasing female resistance may select for increased ACP dosage. Alternatively, inter-locus sexual conflict may result in high turnover of ACPs. This would occur if males do not refine their existing toolkit with which they try to manipulate females, but instead exploit entirely new mechanisms that result in male fitness benefit and female cost. This may explain the vast number of ACPs in D. melanogaster (more than 100). Finally, it may be that other aspects of the ejaculate are responsible for suppressing female receptivity. In butterflies, for example, non-fertile sperm inhibit female remating by filling the female’s sperm-storage organ [108], and male harming of females during copulation is in theory another way to suppress remating [109]. Finally, ACPs may also be beneficial to females when acting as nutrient provisioning. This can still generate sexual conflict over female receptivity, and there is evidence in butterflies that there is extensive genetic variation and genetic correlations between the sexes for ability to suppress female receptivity in males and to withstand manipulation in females [110], although whether this is due to differences in expression level or gene sequences remains to be examined.

The increased level of inter-locus sexual conflict associated with polyandry may in some cases target alleles based on their parent of origin. There is a large body of evolutionary theory that predicts that sexual conflict will foster genomic imprinting [80,81,111–113], where either the maternal or paternal allele at a given locus is expressed in the offspring rather than normal bi-allelic expression. Imprinting of this form allows conflict between the parents to play out in the offspring, and has been shown to be particularly important in foetal growth [18] and murine mating behaviour [114], and underlie sex determination in scale insects [115].

Given the increased potential for inter-locus sexual conflict under polyandry, we might expect to observe a greater proportion of imprinted loci within the genome in polyandrous lineages compared with monogamous species. Until recently, however, surveying whole genomes for imprinted loci was difficult, and although the recent development of methods to scan for allelic imbalance in transcriptomic data may facilitate genome-wide surveys [19,20], this method is not without serious concern [21]. Additionally, there are methods that search RNA data for imprinting by looking for any statistical deviation from bi-allelic expression no matter how slight, and it is unclear how much of this effect is due to variation in cis-regulatory regions that affect expression versus conflict-driven selection for parent-of-origin imprinting. Most importantly, it is not clear how biologically relevant slight deviations from bi-allelic expression are, or how they might manifest phenotypically.


Intra-locus sexual conflict occurs when an allele at a single locus moves one sex away from its fitness optima but the other sex towards its sex-specific fitness peak [116], and has been documented in many traits and species [117]. However, while inter-locus sexual conflict can produce unresolvable arms races, the potential for resolution may be greater for intra-locus sexual conflict. Resolution of intra-locus sexual conflict was thought to occur when the genetic correlation between males and females was broken down, allowing for the evolution of phenotypic sexual dimorphism [77,118]. However, it has become apparent that things are not as straightforward as this [119], primarily because genes do not exist in isolation of other genes. The network and interactive structure of genetic pathways means that selection on a sex-limited gene can still ripple through the genome, and mutations will still accumulate in the sex lacking expression because they are neutral, and these could keep the expressing sex from reaching a fitness optimum [81,94]. For example, recent artificial selection work on the broad-horned flour beetle has shown how selection on a male-limited trait alters female fitness, because the male sex-limited trait is genetically correlated to other traits, and these in turn are genetically correlated between the sexes [90]. As a result, selecting on the male-limit trait, the mandibles, reverberates throughout the genetic network, ultimately affecting female fitness, and although females never develop mandibles, it is this sex-limited trait that lies at the heart of the sexual conflict. Intra-locus sexual conflict over gene expression may therefore not be resolved simply by the evolution of sex-biased or sex-limited gene expression. Furthermore, while it is now possible to measure transcriptomic differences between the sexes to identify a measure of intra-locus sexual conflict within the genome [78], it is not clear whether these differences completely or only partially resolve conflicts.

Despite these concerns, sex-biased gene expression represents possibly the most direct connection linking sexual conflict, mating system and the evolution of sexual dimorphism with the genome, and although work on this front has been possible for little more than a decade, it is surprising how well sex-biased gene expression patterns conform to the predictions from intra-locus sexual conflict theory. For example, the greatest degree of sex-bias is observed in gonad transcriptomes [85,86,89], as is expected given the profound degree of conflict between optimal male and female gamete production and provisioning strategies. Male and female gonad formation and gametogenesis involve many of the same genes used in different ways, and this makes the testis and the ovary the site of maximal sexual conflict within the body. Sex-biased genes, as the encoding loci underlying sex-specific phenotypes, experience the accelerated rates of evolution predicted by sexual selection [11,120,121].

Furthermore, gene expression patterns on the X and Z sex chromosomes show evidence of sex-specific selection [10,79,122], as predicted by their uneven inheritance between males and females [77,123]. Although the pattern is complicated by the effects of sex chromosome regulation related to meiotic sex chromosome inactivation and dosage compensation [122], a general pattern emerges. X chromosomes are more often present in females than males, and therefore more often selected for female-specific effects. This has been tested with gene expression data with the assumption that female-biased genes benefit females and male-biased genes benefit males. Under this framework, the finding that the X chromosomes in both the mouse and Drosophila harbour a combination of excessive female-biased genes and fewer than expected male-biased genes [124] fits with sexual conflict predictions. These observations are supported by a recent population genetic model, showing that female-biased gene duplications preferentially accumulate on the X chromosome [77].
Several objections to the approach of employing sex-biased gene expression to study sexual conflict have been voiced [94,125], including potential limitations of sex-specific regulatory mechanisms and differential male and female allometry. The first concern arises because very few genes lie directly in the sex determination pathway or are proximately located to sex-hormone receptors. This might suggest that there is very little scope for sex-specific gene regulation. However, there is clear evidence that sexual dimorphism of somatic traits can result when gene expression of the trait is ultimately regulated by the sex determination pathway [12,126,127]. Additionally, there are several thousand oestrogen and testosterone receptors in the average metazoan genome [128,129], and the interactive nature of the genetic network implies that even if only a few hundred genes are under direct control of sex hormones, they can transmit sex-biased expression through to thousands of other genes under their regulatory control. This suggests that the potential for sex-specific gene regulation is extensive, if not limitless. It also suggests that the complete resolution of sexual conflict may be difficult because of these extensive gene networks.

Additionally, initial studies of sex-biased gene expression were performed on whole animals [120,121], leading to concerns that sex-biased expression was a result of different sex-specific allometry of constituent body parts. However, the opposite has been observed, as whole-body and aggregate transcriptome studies actually underestimate the degree of sex-bias within constituent parts [40,85,86]. This is particularly problematic for studies of brain transcription: although fine scale analysis of gene expression in the brain has revealed profound sex-bias in limited areas that control sex-specific behaviours [130], whole-brain or partial-brain homogenates reveal very little overall sex-bias [39,40] because sex-specific expression of different genes in different regions is averaged, and localized differences are therefore diluted. This suggests that the initial estimates of whole-body sex-bias in roughly half of all genes are in fact underestimates, rather than overestimates, and that finer-scale dissections will reveal more pervasive patterns of local sex-biased gene expression.

With these concerns allayed, we can now move on to discussing how polyandry influences sex-biased gene expression in light of intra-locus sexual conflict. The short answer is that we have no idea, and this is because no one has yet directly compared monogamous, monandrous and polyandrous species in a phylogenetically controlled study. At this point, we know that sex-biased expression varies among species [131], but this has not yet been put into the context of mating system. Simply doing so, ideally comparing related species with different mating systems, would be a big step. In the absence of any solid data, we can only speculate wildly.

Sex-specific selection and sexual conflict may be stronger in polyandrous than monogamous systems. This is because even under polyandry, a few males more or less monopolize paternity. We therefore expect that phenotypic sexual dimorphism will either be greater in magnitude, or possibly just evolve more quickly, under polyandry compared with monogamy. If protein abundance, and therefore transcription level, holds the key to sexually dimorphic phenotypes, then we might expect either sex-biased gene expression to be prevalent in a larger proportion of genes under polyandry, or alternatively, a limited number of genes will show greater magnitudes of expression difference. Whether greater levels of sexual dimorphism are due to more sex-biased genes or a few genes with greater magnitude of sex-bias depends on how much of the genome is subject to sexual conflict, and to a lesser extent, how much conflict can be resolved via differential expression levels. Similar predictions might be expected to hold for sex-specific alternate splicing, as this is really an exon-specific form of sex-biased gene expression.

If the elevated rates of sequence evolution observed for sex-biased genes [89,120,121] really are the product of sex-specific selection, then we might expect the relative rates of evolution for sex-biased genes to be higher in polyandrous than monogamous systems. Relative rates of evolution for sex-biased genes under different mating systems may need to be determined in adults and embryos separately, as evidence from birds suggests that male-specific selection is strongest on adult-expressed genes while female-specific selection acts maximally on late-embryonic-expressed genes [29]. This is also consistent with studies at the phenotypic level in Drosophila where there is evidence of ontogenetic conflict between males and females [132].

Additionally, if sex chromosomes really are a hotspot of sexually antagonistic selection [10,77,123], then the increased conflict that results from those forms of polyandry might be expected to feminize X chromosomes and masculinize Z chromosomes to a greater degree than in monogamous or monandrous species. If this is true, it would potentially be demonstrated via greater levels of female-biased expression on X chromosomes and male-biased expression on Z chromosomes, and faster rates of gene exodus for male-biased genes from the X and female-biased genes from the Z chromosomes. At this point, these predictions are simple hand-waving based on vague assumptions, but hopefully they will soon be tested critically by studies that span clades with varying mating systems.

A phylogenetic approach will also help determine the time-scale of sex-specific transcriptome evolution. Short-term sex-specific experimental evolution in Drosophila produced little change in sex-biased expression [78]; however, sex-bias varies in both domestic animal breeds that have been subject to sex-specific selection regimes for more than 100 generations [14], and among closely related species [131]. This suggests that transcriptional evolution can occur for many genes over medium- and long-term evolutionary distances, and a phylogenetic study of closely related species with different mating systems will help quantify the rate of sex-specific transcriptomic evolution in response to defined sex-specific selection. This will help answer questions regarding the response mechanisms as well. For example, does the elevated level of sexual conflict in polyandrous systems mean that sex-biased expression evolves more rapidly than in monogamous species? Does polygamy produce more sex-biased genes, or does it produce a greater magnitude of sex-bias in a relatively small number of genes?

8. Conclusions

The recent exponential advances in sequencing capability and reduced cost make the genome and the transcriptome approachable scientific frontiers for the study of model and non-model organisms alike. At the moment, there is a
great deal of effort going into describing the natural history of the genome and transcriptome, and this is required before we can understand how these things interact to form the phenotype. Hopefully soon, this will progress to a more integrated grasp of the ways in which selection, acting on the phenotype, shapes the underlying genes and gene expression patterns.

The comparison of gene expression patterns associated with different mating systems provides a unique opportunity to begin to understand the evolution of sexual dimorphism, quantify the degree and loci of sexual conflict, and unravel the mechanisms by which this conflict can, or cannot be resolved. As such, gene expression differences between the sexes represent possibly the most direct connection linking sexual conflict, mating system and the evolution of sexual dimorphism with the genome.

The rapid expansion of the research frontier in sexual selection and sexual conflict is exciting, and it creates opportunities for both theoretical frameworks for gene expression evolution, as well as empirical tests in a range of organisms with interesting mating systems. Stay tuned for forthcoming developments.

Work in J.E.M.’s laboratory is supported by the BBSRC and the ERC (grant agreement no. 260253); N.W. by a Royal Society Wolfson Merit Award and NERC, and D.J.H. was supported by NERC. We thank the Editors for the invitation to participate in this special issue and the Royal Society for supporting the process. We also thank two anonymous referees for comments, which helped improve the manuscript.

References

32. Sharma MD, Hunt J, Hosken DJ. 2012 Antagonistic responses to natural and sexual selection and the sex-specific evolution of cuticular hydrocarbons in


