The evolution of sex-biased genes and sex-biased gene expression

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Abstract | Differences between males and females in the optimal phenotype that is favoured by selection can be resolved by the evolution of differential gene expression in the two sexes. Microarray experiments have shown that such sex-biased gene expression is widespread across organisms and genomes. Sex-biased genes show unusually rapid sequence evolution, are often labile in their pattern of expression, and are non-randomly distributed in the genome. Here we discuss the characteristics and expression of sex-biased genes, and the selective forces that shape this previously unappreciated source of phenotypic diversity. Sex-biased gene expression has implications beyond just evolutionary biology, including for medical genetics.

Sexual dimorphism
Phenotypic differences between males and females of the same species.

Sexual selection
The process of natural selection acting on traits related directly to mating or reproductive success.

Sexual antagonism
Conflict arising from traits that are beneficial to one sex but harmful to the other.

Sex-biased genes
A gene that is expressed predominantly or exclusively in one sex.

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Females and males often differ dramatically in appearance and behaviour. Most of these differences, collectively referred to as sexual dimorphism, are the result of natural and/or sexual selection for traits that influence the fitness of each sex. Sex-specific natural selection favours traits that increase the survival or general reproductive success of individuals of the respective sex, whereas sexual selection favours traits involved specifically in mating (or fertilization) success. This includes traits that are relevant to within-sex competition, such as male–male or sperm competition, as well as those related to mating preference, such as female mate choice.

Despite the extensive phenotypic differences between the sexes, females and males are nearly identical genetically. Indeed, in species without genetic sex determination, they are identical. In most other species, the male and female genomes differ by only a few genes located on sex-specific chromosomes (such as the Y chromosome of mammals). This implies that the vast majority of sexually dimorphic traits result from the differential expression of genes that are present in both sexes[1,2]. It also implies that these genes will be subject to different levels of selection in the two sexes, and might even be subject to conflicting selective pressures in females and males. This latter scenario, known as sexual antagonism[3], describes the situation in which expression of a gene is beneficial to one sex but harmful to the other (FIG. 1). Experimental work in *Drosophila* species has confirmed the frequent genomic occurrence of sexually antagonistic alleles and has demonstrated their response to selection[4–6].

For convenience, genes with sexually dimorphic expression are often referred to as sex-biased genes, although it should be noted that it is not the genes themselves that are biased but, rather, their expression. These genes include those that are expressed exclusively in one sex (sex-specific expression), as well as those that are expressed in both sexes but at a higher level in one sex (sex-enriched expression). The sex-biased genes can be further separated into male-biased and female-biased genes, depending on which sex shows higher expression. Genes with equal expression in the two sexes are referred to as unbiased. Thanks to recent advances in 'omics' fields, it is now possible to identify genes that are differentially expressed between males and females and investigate their evolutionary patterns (BOX 1). Importantly, it has been demonstrated that sex-related differences in gene expression are extensive across a range of taxa, including insects, nematodes, birds and mammals.

The goal of this Review is to bring together these recent findings and highlight the common patterns that are emerging from studies of sex-biased genes. We examine the genetic and genomic differences between sex-biased and unbiased genes, as well as those between male-biased and female-biased genes. First, we compare rates of evolution, and show that male-biased genes consistently show the greatest divergence between species at both the sequence and expression levels. Second, we examine codon bias, which tends to be reduced in male-biased genes. Third, we look at the chromosomal distribution of sex-biased genes and how their density differs between autosomes and sex chromosomes. Finally, we explore the origins of new sex-biased genes, for which there seems to be an overabundance of new and duplicate genes that are expressed in male reproductive tissues.

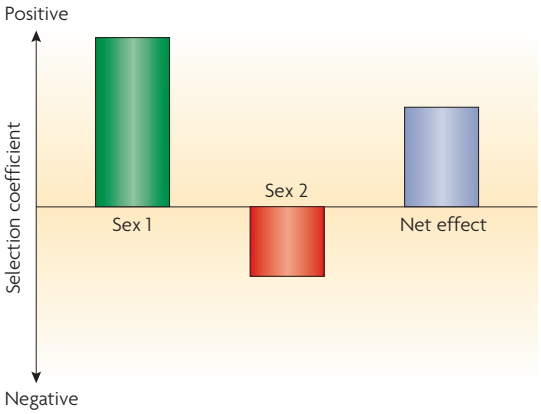


Figure 1 | **Fitness trade-offs for a sexually antagonistic mutation.** When autosomal, a sexually antagonistic mutation can go to fixation if the benefits to one sex exceed the detrimental effects that are incurred to the other sex.

The occurrence of sex-biased gene expression

How many genes differ in expression between females and males? This is a difficult question to answer, because the number of differentially expressed genes that will be detected between two samples depends on several factors, including the species and tissue being investigated, the experimental methodology, the degree of replication and the statistical criteria that are chosen to define differential expression. In any case, the number of sex-biased genes seems to be substantial. For example, when whole adult females and males of *Drosophila melanogaster* are compared, up to 57% of the genes show sex-biased expression[7], with the vast majority of these differences being attributable to genes that are expressed in reproductive tissues[8]. Although comparisons using whole organisms or combined tissue samples provide valuable information about general patterns of sex-biased gene expression, they cannot distinguish between two potential causes for an expression difference between the sexes: a broader expression pattern (that is, expression in more tissues) in one sex relative to the other; or a higher concentration of an mRNA in one sex when the same tissue is compared between the sexes. Recent work indicates that the second factor is a major contributor to intersexual differences in gene expression[9]. As a consequence, the number of genes that will be documented as showing sex-biased expression will increase with the number of tissues being investigated individually. In one of the most extensive studies to be conducted to date, particularly in its statistical power, Yang and colleagues detected more than 10,000 genes with sex-biased expression when screening several somatic tissues in mouse[9]. These findings underscore the importance of controlling for sex in gene expression studies, as the extensive between-sex differences in gene expression can obscure expression differences caused by other genetic, environmental or experimental factors that are the subject of investigation.

Less is known about how the global patterns of sex-biased gene expression change throughout life and between different developmental stages[10], and further research in this area will be valuable. It seems reasonable to assume, although it has not been shown, that sex-biased gene expression becomes most pronounced after sexual differentiation. Generally, the design of expression microarray experiments aims at reducing the influence of factors other than the parameter of interest; for instance, by rearing and/or treating individuals as homogeneously as possible. However, if the purpose of the study is to characterize sex-biased gene expression in natural conditions, it is more important to expose individuals to the sex-specific life history and behaviours that are normal in the wild.

Finally, it should be noted that little is known about how well sex-biased gene expression corresponds to sex-biased protein synthesis. Although the default prediction would be that sex-specific mRNA and protein levels are highly correlated, the possibility of sex-specific regulation occurring at the translational level cannot be excluded.

Coding-sequence evolution of sex-biased genes

Because the vast majority of expression studies have focused exclusively on protein-coding genes, comparison of coding sequences between species has been used as a standard way to detect differences in evolutionary rate between genes with different expression patterns. In addition, statistical analysis of changes that occur within coding sequences can reveal the type and strength of selection acting on genes. Below we discuss the key findings that have come from coding sequence analyses of sex-biased genes.

Rapid evolution of sex-biased genes. A common pattern that has emerged from large-scale expression and comparative genomic studies is that sex-biased genes, especially those with male-biased expression, tend to evolve rapidly in protein sequence[11]. For example, *Drosophila melanogaster* genes with male-biased expression are more functionally divergent (measured as the ratio of the non-synonymous substitution rate to the synonymous substitution rate, d_N/d_S) between species than those with female-biased, or unbiased expression (FIG. 2a). Male-biased genes also have the lowest fraction of identifiable orthologues between *D. melanogaster* and *Drosophila pseudoobscura*, which diverged 25–50 million years ago (FIG. 2b). Furthermore, whole-genome comparison of these two species, in combination with EST sequencing data, revealed that genes that are expressed exclusively in males showed the greatest amino-acid divergence of all the functional classes studied[12]. Thus, the evidence for rapid evolution of male-biased genes in *Drosophila* is convincing. However, it should be noted that the above studies used expression data obtained from only *D. melanogaster* to define sex-biased and sex-specific genes. A recent SAGE (serial analysis of gene expression) analysis of *D. pseudoobscura* gave slightly different results: genes with male-biased expression in both species or only in *D. melanogaster* showed high levels of

Codon bias
The non-random use of synonymous codons to encode a protein.

d_N/d_S
The ratio of the non-synonymous (amino-acid altering) and synonymous substitution rates, used as a standard measure of the rate of evolution of a protein-encoding sequence.

EST sequencing
Large-scale sequencing of clones from a cDNA library obtained from mRNA.

Box 1 | **How are sex-biased genes identified?**

In principle, detecting sex-biased gene expression does not differ from quantification of mRNA levels in general. For instance, real-time PCR amplification of cDNA prepared from male and female samples is a useful approach for the study of relative levels of expression of individual genes in the two sexes. However, the realization that sex-biased gene expression occurs widely on a genomic scale was not made until the introduction of microarrays for transcriptome profiling. Most of the literature that is pertinent to global patterns of sex-biased gene expression derives from the use of microarrays, including the various types of arrays that are used in many other applications (for example, cDNA or oligonucleotide arrays, competitive two-colour or direct one-colour hybridization).

An obvious limitation with the microarray approach is the availability of spotted arrays for hybridization experiments. Although these are commercially available for several model organisms, studies of less well-characterized genomes are hampered by the lack of genomic sequence data or of cDNA clones that are necessary for array construction. This should be less of a problem in the future, as genomic sequence data are accumulating at an unprecedented speed. Moreover, to some extent, the lack of genomic resources for a non-model organism can be overcome by the use of cross-species hybridization to heterologous arrays that are available from a related organism[73]. Such experiments come with reduced hybridization efficiency owing to coding-sequence divergence between the focal species (on the array) and the species of interest (the hybridizing sample); in many cases this might be considered acceptable. However, a potential ascertainment bias that could arise under such circumstances is that male-biased and female-biased genes evolve at different rates (see the main text), meaning that one might underestimate the occurrence of genes from one or the other of these categories.

An alternative approach for studying sex-biased gene expression is to estimate transcript abundance from EST sequencing of cDNA libraries made specifically from males or females[50,67,68]. As this should be done using non-normalized libraries to provide an unbiased estimate of the relative levels of transcripts in males and females, the approach requires extensive effort and is costly (abundant transcripts will be sequenced over and over again). The amount of sequencing can be reduced by concatenating 10–20 bp 'tags' from the 3′ end of transcripts in an approach known as SAGE (serial analysis of gene expression)[74]. However, this approach is useful only when the genome sequence is available and the tags can be traced back to their corresponding genes. New technology based on massive parallel sequencing of solid-phase or emulsion-generated amplicons is likely to offer more effective and accurate detection of sex-biased gene expression. Concepts such as the 454 technology[75] and Solexa's[76] sequencing-by-synthesis can already generate extremely deep coverage and, in theory, a means for unbiased expression profiling and mRNA quantification. This has the benefit of allowing the analysis of all the expressed sequences in a certain tissue, not only those that are targeted on an expression array. Large-scale sequencing also has the advantage of being able to distinguish among alternative transcripts of a gene, which can differ between the sexes. This is not possible in most microarray or SAGE experiments, although microarray platforms have been designed to detect the abundance of alternative transcripts of genes[77,78].

divergence between the two species, whereas those with male-biased expression in *D. pseudoobscura* alone were about half as divergent — a similar level to female-biased and unbiased genes[13]. Thus, patterns of sex-biased gene evolution seem to have changed since the split of the *D. melanogaster* and *D. pseudoobscura* lineages. However, it is also possible that the above result is an artefact of low SAGE sequencing depth. This uncertainty should be resolved in the near future, when expression studies using species-specific microarrays are combined with whole-genome comparisons of multiple species from across the *Drosophila* genus.

The rapid evolution of sex-biased genes has also been reported for soil nematodes of the genus *Caenorhabditis*. These worms provide an interesting system for the study of sex-biased genes because they are androdioecious. Detailed expression data from *Caenorhabditis elegans*[14] and comparative genomic data from *C. elegans* and *Caenorhabditis briggsae* have been used to investigate the relationship between expression pattern and rate of molecular evolution[15]. Overall, genes expressed during spermatogenesis showed the fastest rate of evolution, with the genes expressed exclusively in male spermatogenesis evolving faster than those expressed in hermaphrodites, or those that are shared between males and hermaphrodites. Genes expressed in oocytes evolved slower than those expressed in sperm, but still at above-average rates. In the soma, genes expressed exclusively in males evolved faster than those expressed in hermaphrodites. Furthermore, genes expressed in sperm or the male germ line showed a significant excess of 'orphans' — that is, genes that did not give a significant BLAST[16] match between the two species. Collectively, these results uphold the pattern of rapid evolution of male-biased genes, even in species in which the two sexes can coexist in a single individual.

Comparative genomic studies of mammals have provided similar results to those seen for flies and worms. For example, a comparison of human and mouse orthologous genes found that genes expressed specifically in spermatozoa had non-synonymous substitution rates of more than twice that of genes expressed in other tissues[17]. A subsequent study using microarray data, which quantified gene expression levels across nine stages of mouse spermatogenesis[18], and comparative genomic data from mouse and rat explored this pattern on a finer scale[19]. Here it was found that genes expressed early in spermatogenesis had an average d_N/d_S that was similar to (but slightly higher than) the genome average. By contrast, genes expressed late in spermatogenesis, especially those genes that are specific to the later stages, had a much higher d_N/d_S, averaging about twice the genome average. Comparison of the human and chimpanzee genomes has shown that the rapid evolution of male-biased genes also extends to primate lineages: genes expressed in testis, especially those that are specific to testis, are the fastest evolving of all genes for which tissue-based expression profile has been investigated so far[20].

The role of positive selection. The consistently fast rate of evolution of male-biased genes could have two causes. One possibility is that these genes are under less selective constraint, and therefore accumulate many amino-acid replacements that have no effect on fitness. Alternatively, male-biased genes might experience more positive selection, driving the rapid replacement of amino acids. Two recent observations in *Drosophila* support the second explanation. First, there is a positive correlation between d_N/d_S and local recombination rate for male-biased genes[21]. This is expected in cases in which frequent adaptive evolution occurs, because recombination reduces interference among positively selected mutations at different sites within a gene, thereby increasing the rate of substitution. Second, male-biased genes show an excess of non-synonymous differences between species relative to non-synonymous polymorphisms within species,

Transcriptome profiling
A characterization of the mRNA molecules that are expressed in a certain tissue.

Androdioecious
A population consisting of hermaphrodites, which contain both male and female reproductive tissues, and males, which contain only male reproductive tissues.

which is a hallmark of positive selection[22,23] (FIG. 3). This is because positively selected mutations are expected to go to fixation rapidly within a species, and therefore contribute to interspecific divergence more than to intraspecific polymorphism. There is also evidence for the action of positive selection on at least some male-biased genes after the split of the human and chimpanzee lineages, as genes expressed in testis or with known functions in spermatogenesis are overrepresented among the class of genes that show evidence for adaptive evolution between these two species[24].

Although male-biased genes collectively show rapid rates of coding-sequence evolution, this property is not universal. For instance, a recent study of the *D. melanogaster* sperm proteome revealed that the structural and developmental proteins that are expressed at high levels in sperm did not show high divergence between species[25]. However, despite this low divergence, population genetic analyses revealed evidence for adaptive evolution of at least 3 out of the 11 sperm proteome genes that were investigated. Similarly, although female-biased genes tend to evolve more slowly than male-biased genes (FIG. 2), they also show evidence for increased adaptive evolution relative to unbiased genes (FIG. 3). In general, female-biased genes have not received the attention that has been given to male-biased genes, but there is growing evidence that they might also be frequent targets of positive selection[26,27].

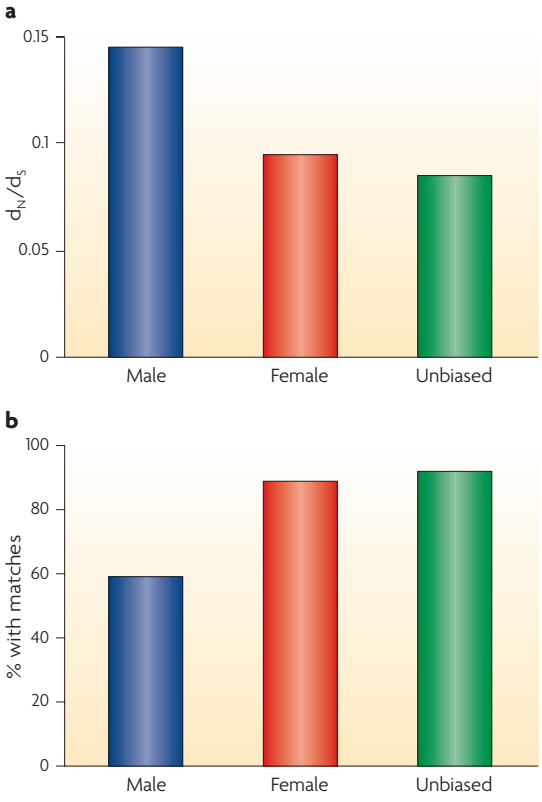


Figure 2 | **Divergence of sex-biased genes between *Drosophila* species. a** | For closely related species, for which almost all genes can be aligned with their orthologues, male-biased genes show the greatest divergence between species. Shown is the ratio of the non-synonymous and synonymous substitution rates (d_N/d_S) for a whole-genome comparison between *Drosophila melanogaster* and *Drosophila simulans*. **b** | For more distantly related species, it is often difficult to identify orthologous genes. Shown is the percentage of significant BLAST[16] matches ($E < 10^{-9}$) when all *D. melanogaster* genes are aligned against the *Drosophila pseudoobscura* genome. Male-biased genes show the fewest matches, indicating that they are the least conserved. Genes were assigned to sex-bias categories using microarray data and a twofold expression cutoff[69].

Although the molecular evolutionary analyses described above provide evidence for the action of positive selection on sex-biased genes, they do not reveal its underlying cause. That is, the molecular genetic data cannot distinguish between the possibilities of natural selection, sexual selection or sexual antagonism. A full understanding of the relative contributions of these forces will require gene-specific and species-specific studies of molecular function, physiology, behaviour and ecology. This is a great challenge that will require much work over the coming years. However, some of the functional and experimental data that are already available hint at the importance of sexual selection and sexual antagonism. For example, the strongest signal of positive selection is typically seen for genes that are expressed specifically in male reproductive tissues, such as testes and accessory glands, which immediately suggests a role for sexual selection and/or sexual antagonism. The fact that the signal of adaptive evolution is stronger in male-biased than female-biased genes suggests that sexual selection arising from male–male competition is the more important force. This would be expected in polygamous species like *Drosophila* and many mammals, for which there is strong mating and sperm competition among males. This strong selection pressure to maximize paternity could lead to the fixation of alleles that are harmful to females, which in turn will lead to selection in females for alleles that can counteract their effect. Such sexually antagonistic co-evolution has been demonstrated in laboratory populations of *Drosophila*, in which sexually selected males have been shown to reduce the lifespan of their mates[4], possibly explaining why female genes show a greater signal of adaptive evolution than unbiased genes.

The influence of reproductive biology. Sexual selection and sexual antagonism with respect to gamete recognition (the interaction of surface proteins on sperm and egg cells) are likely to be common and important driving forces behind the rapid evolution of many of the sex-biased genes that are involved in reproduction. However, the impact of these forces might vary with the biology of the reproductive system in different organisms. A classical example of sexual conflict is that between sperm competition among males for rapid rates of fertilization and females' interest in retaining a moderate rate of fertilization to prevent the detrimental effects of polyspermic fertilization[28]. This is expected to introduce a co-evolutionary arms race, increasing the rate of evolution of sperm and egg surface proteins involved with gamete

Positive selection
Positive selection is key to adaptive evolution and implies that an advantageous allele gives its carrier a higher fitness and is therefore favoured by natural selection.

Fixation
When a new mutation is eventually spread to all individuals in the population.

Accessory glands
In insects, such as *Drosophila*, these are male reproductive tissues that produce and secrete seminal fluid proteins that are transferred to the female during copulation.

recognition[29,30]. Consistent with this prediction, both male and female gamete-recognition proteins of several organisms have been shown to evolve rapidly under positive selection[28]. However, in birds, normal fertilization involves physiological polyspermy with multiple sperm penetrating the egg at the inner perivitelline layer[31]. There is also relatively limited species specificity between avian sperm and eggs[32]. This probably explains the observation that the female zona pellucida gamete-recognition protein, which was shown to evolve rapidly in other organisms, does not display a signature of positive selection in birds[33].

Gene expression evolution of sex-biased genes

The evolution of expression of sex-biased genes has been given less attention than the coding-sequence evolution of such genes. An important insight is derived from studies of adult gene expression profiles in *D. melanogaster* and *D. simulans*[7]. Here it was found that sex-biased genes are significantly overrepresented among genes that are differentially expressed between the two species. Notably, the pattern of sex-specific expression seems labile. Among 2,283 genes with an interspecific difference in expression, 1,903 were sex-biased. Of these, 952 showed a quantitative change in the level of sex bias, 931 gained or lost sex-biased expression, and 20 displayed a reversal in sex bias. Furthermore, of the genes that maintained the same sex bias between species, those with male-biased expression showed the greatest interspecific expression difference. Similar patterns have also been reported in mammals: genes expressed in testis show high expression divergence between humans and chimpanzees, as well as among mouse species, when compared to genes expressed in tissues such as the heart, liver, kidney or brain[20,34].

What evolutionary forces drive this rapid divergence of genes expressed predominantly in males and male-specific tissues? As in the case of coding sequences, a comparison of within-species polymorphism to between-species divergence can help to distinguish between the alternatives of positive selection and relaxed selective constraint: positive selection is expected to disproportionately increase interspecific divergence relative to intraspecific polymorphism, as described above. In *Drosophila*, although male-biased genes show relatively high levels of expression polymorphism within species, their ratio of expression divergence to expression polymorphism is significantly greater than that of female-biased or unbiased genes[35]. Similarly, human and chimpanzee testis-expressed genes show low intraspecific expression polymorphism, but high interspecific expression divergence[20]. Thus, there is evidence for adaptive evolution of male-biased genes not only at the level of coding sequence, but also at the level of expression. Changes in sex-biased gene expression are therefore likely to be a major contributor to adaptive phenotypic divergence between species. One interesting possibility is that a decoupling of male and female gene expression circumvents constraints that would otherwise be associated with changes in gene expression.

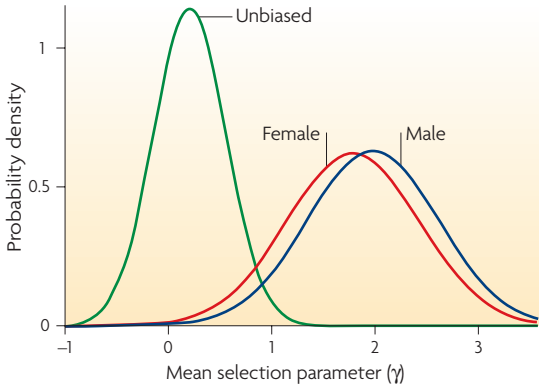


Figure 3 | **Adaptive evolution of sex-biased genes.** Distribution of the mean selection parameter, γ, for 33 male-biased, 28 female-biased and 30 unbiased genes based on polymorphism within *Drosophila melanogaster* and divergence from *Drosophila simulans*. A γ value of zero corresponds to neutral evolution, whereas positive values indicate positive selection[89]. Data reproduced from REF. 22.

Codon bias of sex-biased genes

Although there are often several codons that encode the same amino acid, in most genomes analysed to date, certain codons are used more frequently than would be expected to occur by chance, whereas others are used less frequently. This phenomenon is known as codon bias and, in many species, it is thought to result from natural selection for codons that are translated more efficiently and accurately. This is supported by the observations that codon bias is strongest in highly expressed genes[36–39], and that the most frequently used codons tend to correspond to the most abundant tRNAs for a given amino acid[40–43]. As more data become available, it is increasingly clear that codon bias also differs between the genes that are expressed in the different sexes. Here it should be noted that there is a distinction between sex-biased expression and overall expression level. The former is determined by the abundance of a gene's mRNA in one sex relative to the other, whereas the latter is determined by the abundance of a gene's mRNA (usually averaged over both sexes) relative to that of other genes in the genome. As such, it is possible for a gene to show highly sex-biased expression, but have a low overall expression level (or the opposite can also be true).

In *Drosophila*, male-biased genes have significantly less codon bias than either female-biased or unbiased genes, whereas the last two groups have nearly equal codon bias[44]. So, the difference between the sex-biased genes seems to be caused by a reduction in codon bias of the male-biased genes. This difference cannot be explained by the general molecular genetic factors that are known to influence codon bias, such as expression level, protein length, chromosomal location, mutational bias or local recombination rate. Furthermore, although little is known about tRNA abundance in various *Drosophila* tissues, the difference in codon bias between male-biased and female-biased genes does not seem to be caused by differences in the translational environment of the cells in which

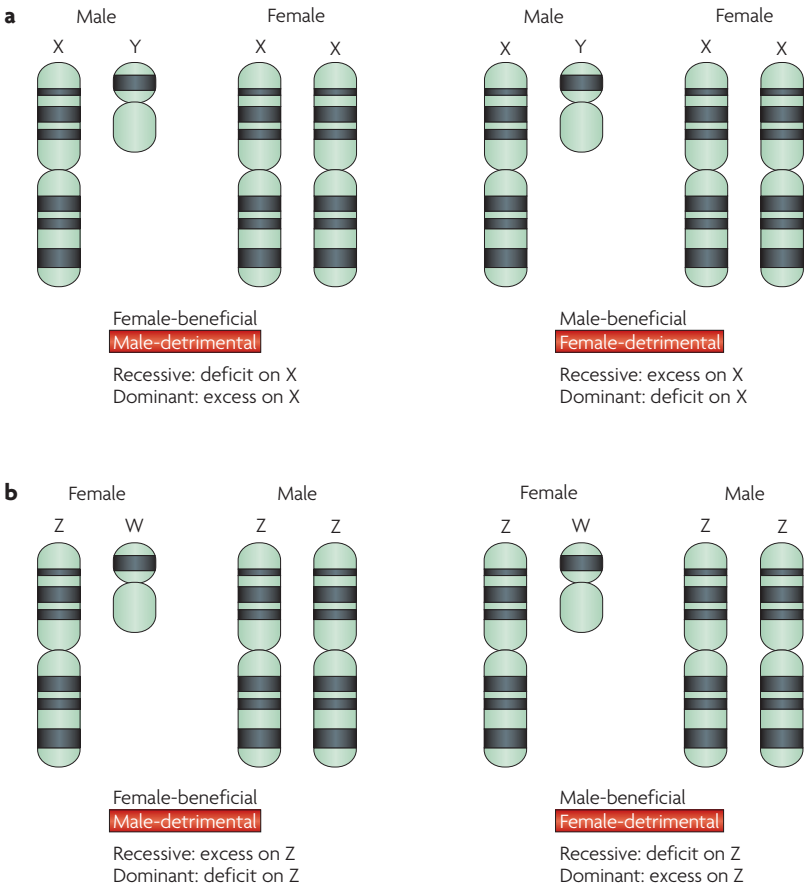


Figure 4 | **Scenarios for the accumulation and underrepresentation of sexually antagonistic mutations on sex chromosomes. a** | In a male heterogametic system, an X-linked female-beneficial but male-detrimental recessive mutation will not be positively selected in females until it has become common enough that is starts to appear in homozygous form. However, it will be directly exposed to negative selection in males, reducing the likelihood of its spread in the population. An X-linked female-beneficial but male-detrimental dominant mutation will occur in females two-thirds of the time, and thus be positively selected more often than it is negatively selected. Ultimately, this leads to the prediction of an excess of female-beneficial genes on X if mutations are generally dominant, and a deficit if mutations are recessive. Correspondingly, for an X-linked male-beneficial but female-detrimental mutation, positive selection will occur directly in males if the mutation is recessive. If dominant, negative selection in females will occur more often than positive selection in males. **b** | The same principles are valid for female heterogametic systems, although the sex roles are reversed.

Purifying selection
Negative selection against a deleterious or slightly deleterious mutation.

Heterogametic sex
The sex that produces two different types of gametes with respect to sex chromosome content (for example, XY).

Homogametic sex
The sex that produces one type of gamete with respect to sex chromosome content (for example, XX).

the genes are expressed (for example, ovaries versus testes), because the same pattern is seen for genes that are expressed in somatic cells. This suggests that there are general selective differences between female-biased and male-biased genes, with less-effective selection for synonymous codon usage in male-biased genes. This would be expected if male-biased genes were under less constraint for efficient translation. Such a relaxation of purifying selection would allow more synonymous mutations to non-optimal codons to become fixed in the species, and thereby reduce codon bias. Alternatively, if positive selection acts frequently on non-synonymous mutations in male-biased genes, interference between linked mutations would cause selection to be less efficient in removing slightly deleterious synonymous mutations, and would lead to a reduction in codon bias. The observation of frequent adaptive evolution at non-synonymous sites in male-biased genes supports this explanation[22]. However, these two scenarios are not mutually exclusive, and it is likely that both contribute to the differences in codon bias between male-biased and female-biased genes.

A recent study of codon bias in sex-biased genes of corn and wheat has extended our knowledge to the plant kingdom[45]. Here it was found that genes expressed in male reproductive cells (sperm and anther) showed significantly less codon bias than those expressed in female reproductive cells (egg and ovary). Thus, plants exhibit the same pattern as that seen in *Drosophila*. Furthermore, as with *Drosophila*, the codon bias differences between male-expressed and female-expressed genes in plants could not be explained by other factors known to correlate with codon bias, such as expression level or protein length, suggesting that selective differences between the two classes of genes are responsible.

The genomic distribution of sex-biased genes

Sex differences in the optimal phenotypic value for many traits imply that some genes, or mutations, can favour one sex at the expense of the other. When autosomal, a sexually antagonistic mutation is expected to go to fixation only if the benefits to one sex exceed the costs incurred to the other (FIG. 1). However, when mutations are sex-linked, several cost–benefit scenarios are possible[46,47]. Briefly, as sex-linked recessive mutations are always exposed to selection in the heterogametic sex, their spread in the population will be facilitated if they are beneficial, and hampered if they are detrimental, to the heterogametic sex (FIG. 4). For instance, in the case of a male-beneficial, female-detrimental X-linked mutation in an XY system, the new allele will have a head start because it will be directly selected for as soon as it is present in a male. By contrast, purifying selection will not occur until the mutation is present homozygously in females, the likelihood of which is relatively low until the allele has reached an appreciable frequency. Everything else being equal, the fixation probability of such a mutation is higher when X-linked than when autosomal, and there will be a fair chance of the allele going to fixation even if the absolute value of the selection coefficient is higher in females than in males.

For fully or partly dominant sex-linked mutations, the homogametic sex has the biggest influence on the fate of a new sexually antagonistic allele. For example, consider the case of a female-beneficial, male-detrimental X-linked mutation. Given that a particular X chromosome is (about) twice as likely to be found in a female than in a male, the new mutation will be selected for roughly two-thirds of the time and selected against about one-third of the time. It would therefore be possible for such a mutation to go to fixation even when fitness is significantly diminished in males. In the end, the fixation probability of sexually antagonistic mutations will depend on their dominance coefficient. For sex-linked loci, this probability will be the same as that for autosomal genes at a

specific degree of dominance, the precise magnitude of which depends on the relative cost and benefit to males and females, respectively.

It follows that we would expect to see an enrichment of different mutations on sex chromosomes and autosomes. Because evolution of sex-biased expression provides a resolution to sexual antagonism, the enrichment would manifest in a non-random genomic distribution of genes with sex-biased expression[48]. Empirical work confirms that the distribution of sex-biased genes deviates from random expectations. Male-biased genes expressed in somatic tissue of flies are significantly underrepresented on the X chromosome[8], which is consistent with the expectation for (at least partly) dominant mutations. The situation for gonads is more complex. An underrepresentation of male-biased genes on the X chromosome has also been reported for several other organisms[8,49–51]. However, gonadal tissue consists mainly of cells that are past early meiosis[51]. Assays that have specifically targeted genes expressed in pre-meiotic stem cells of spermatogenesis have instead found that male-biased genes are enriched on the mammalian X chromosome[50–52]; this is as would be expected for recessive mutations, but it is not obvious why the pattern would be different from that in somatic tissue. One possibility relates to meiotic X inactivation, which occurs during spermatogenesis in many species and would act as a strong impetus for the removal of male-specific genes that are active during meiosis from the X chromosome. However, the same force could result in an apparent enrichment of male-biased genes on the X chromosome, if selection favours the expression of male-specific genes to be concentrated at a time just before X inactivation.

The enrichment of male-biased genes on the mammalian X chromosome has important implications for human disorders related to sex determination, sexual development and reproduction. As would be expected from the density of male-biased genes, there is a relative excess of X-linked loci at which mutations lead to sex and reproductive disorders in human males[53]. For example, one-third of all disease-associated loci that map to the X chromosome have some phenotypic manifestation in sex or reproduction; the corresponding fraction for autosomal loci is only 10%. Interestingly, the region of the X chromosome that was added after the divergence of eutherians and marsupials (that is, the X added region (XAR)) shows the same enrichment of sex-related and reproduction-related loci as the rest of the X chromosome[53]. This suggests that X linkage is in itself what leads to the enrichment of sex-related genes and that this enrichment has occurred relatively rapidly over the course of mammalian evolution.

Retrogene
A gene duplicate that arose through reverse transcription of a cellular mRNA.

Subfunctionalization
The partitioning of multiple ancestral gene functions between gene copies following duplication.

Gene dosage and sex-biased expression

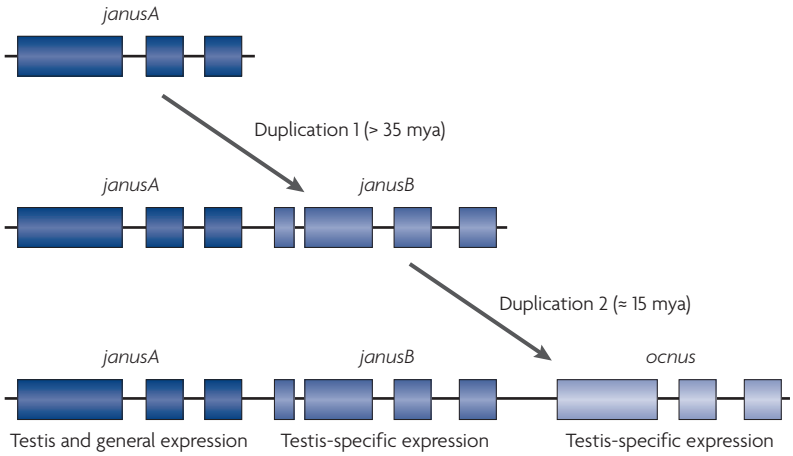
In the above discussion, sex-biased gene expression is understood as differential regulation of mRNA transcription between sexes. However, expression levels can also differ between sexes owing to differences in gene dose[54]. In organisms with sex chromosomes, sex-linked genes occur in a double dose in females (XY systems of male heterogamety) or in males (ZW systems of female heterogamety). Many organisms have evolved compensatory mechanisms to equalize sex-linked gene expression, an observation that is usually interpreted as an adaptation to the potentially detrimental effects of a large-scale imbalance in gene expression levels between sexes[55,56]. Thus, the evolution of dosage compensation and the evolution of sex-biased gene expression can be seen to represent two opposite processes.

Dosage compensation is an X-chromosome-wide phenomenon that is mediated by the triggering of silencing or hypertranscription by a non-coding RNA and epigenetic modification of the X chromosome, often in the form of histone and DNA methylation[57]; exactly how this is achieved differs between organisms. It is increasingly recognized that some sex-linked genes escape dosage compensation, which has the effect that the homogametic sex shows higher levels of gene expression; that is, there is sex-biased expression[58,59]. Although the effect is limited by the direction of bias, this could potentially be an adaptive means of ensuring sex-biased expression of sexually antagonistic genes. However, counter to this runs the observation that genes that escape dosage compensation are non-randomly distributed on the human X chromosome, apparently reflecting the evolutionary history of the mammalian sex chromosomes. Specifically, there is a higher proportion of genes with female-biased expression in those regions of the X that most recently ceased to recombine with the Y[60]. This suggests that genes acquire the ability to be dosage compensated in response to the decay of Y-linked homologues[61]. The absence of dosage compensation of sex-linked genes is, in many cases, therefore likely to be due to a lack of evolutionary time, rather than adaptation.

Intriguingly, it has recently been shown that birds apparently lack global dosage compensation of sex-linked genes[62]. In birds, females are the heterogametic sex (ZW) whereas males are homogametic (ZZ). As such, most Z-linked genes are expressed at higher levels in males than in females, although in most cases not at twofold-higher levels, as might be expected from the difference in gene dose alone. However, even in the absence of chromosome-wide dosage compensation, feedback regulation of biological networks should in many cases buffer differences in gene dose, so that sex differences in steady-state transcript levels become less than the sex difference in gene dose[54,63,64]. Still, what enables birds to cope with such a large-scale difference in levels of gene expression between the two sexes is most puzzling. The avian Z and W sex chromosomes started to differentiate (that is, they ceased to recombine) well before the radiation of extant bird orders more than 100 million years ago[65]. Thus, the lack of strict dosage compensation does not seem to be a consequence of a lack of evolutionary time.

Without dosage compensation, it is difficult to reconcile whether genes that have evolved sex-biased expression are non-randomly distributed in the avian genome. Although the observation of an excess of male-biased genes and a deficit of female-biased genes on the Z chromosome[66–68] agrees with the theoretical expectation for

Box 2 | **Gene duplication and the origin of new sex-biased genes**



The most common way for a new sex-biased gene to arise in the genome is through gene duplication. It is also possible for sex-biased genes to originate from previously non-coding sequences. Male-biased genes, in particular, seem to arise frequently through these mechanisms. Some examples from *Drosophila* are presented below.

Tandem duplication. This type of duplication typically includes the entire coding region of a gene, along with its flanking regulatory sequences. An example is the *janusA*, *janusB*, *ocnus* gene cluster of *Drosophila melanogaster*[79]. These three neighbouring genes arose through two separate duplication events. The inferred ancestral gene, *janusA*, uses alternative splicing to encode two slightly different proteins, one present in multiple tissues of both sexes and the other present only in sperm. Duplication of *janusA* created *janusB*, which then specialized to encode a sperm-specific protein. A subsequent duplication of *janusB* created *ocnus*, which also encodes a sperm-specific protein. All three of these genes have been maintained by selection over the past 15 million years, and molecular evolutionary analysis suggests that the three have diverged in function[80].

Retrotransposition. This type of duplication occurs when the mRNA of a parental gene is reverse transcribed and the resulting cDNA is inserted into a new genomic location. New genes that are generated by this mechanism, known as retrogenes, typically lack the regulatory sequences that controlled the expression of the original gene, so their expression pattern is determined by the sequence that flanks their random site of insertion. However, natural selection will favour the retention of retrogenes that happen to gain an expression pattern that is beneficial to the organism. Many retrogenes in *Drosophila* seem to be functional and show phylogenetic and/or population genetic evidence for selective maintenance[81]. Indeed, some have even gained functions that are essential for male fertility[82,83]. Interestingly, there is a large excess of autosomal retrogenes that are derived from X-linked parental genes and the vast majority of these new genes is expressed in the testes[81]. An example is the retrogene *Dntf-2r*, which is derived from the nuclear transport factor gene, *Dntf-2* (REF. 84). Although the parental gene is expressed in both sexes and in multiple tissues, the retrogene is expressed only in testes. The new gene arose within the past 3–15 million years and has since accumulated an excess of amino-acid replacements, indicative of positive selection.

De novo gene evolution. Sex-biased genes can also arise from DNA sequences that had no previous coding function. The availability of complete genome sequences from multiple closely related species now makes it possible to identify such genes and investigate their evolutionary history. The standard approach to finding *de novo* genes is to look for expressed sequences with intact reading frames that are present in one species (or a few closely related species) but absent in other near relatives and more distant species. In *Drosophila*, several *de novo* genes have been identified[85–88]. For example, a search for genes that are unique to the *D. melanogaster* genome uncovered five candidate *de novo* genes, all of which were expressed predominantly in the testis[88]. Molecular evolutionary analyses revealed evidence for adaptive evolution of at least three of these genes, indicating that they have evolved under positive selection since their formation 2–5 million years ago (mya).

at least partially dominant mutations, a failure of dosage compensation would also be a sufficient explanation.

Origin of sex-biased genes

What genetic and/or evolutionary mechanisms can lead to unequal expression of a gene between the sexes? Several possible scenarios for the origin of sex-biased genes are presented below. Further examples are provided in BOX 2.

Single-locus sexual antagonism. Consider an ancestral gene that is expressed equally in the two sexes. Mutations that increase (or decrease) expression can be beneficial to one sex, but harmful to the other (FIG. 1). If there is no sex-specific regulation of gene expression, then over evolutionary time expression is expected to reach an equilibrium that represents a compromise between the optima of the two sexes. However, because this compromise is suboptimal for each sex, selection will favour modifiers that increase or decrease expression in a sex-specific manner[46]. The selective fixation of such modifiers can optimize expression in each sex, resulting in a sex-biased gene.

Sexual antagonism plus gene duplication. This scenario is similar to that described above, but also involves a duplication of the ancestral gene. Following duplication, the two gene copies can specialize, each to a different sex. Thus, this represents a form of subfunctionalization. In the extreme case, the two copies can become sex-specific, with each being expressed exclusively in a different sex. This allows for further adaptations (for example, amino-acid replacements) that might previously have been prevented by their sexually antagonistic effects.

Duplication of sex-biased genes. Duplication of a previously existing sex-biased gene, along with its regulatory sequences, represents a simple way to generate a new sex-biased gene. Over time, the two copies will diverge by the accumulation of random mutations, some of which might lead to new functions (neofunctionalization) or to the division of ancestral functions between the two copies. The latter is a form of subfunctionalization that differs from the case described above, in that the ancestral sex bias in expression is retained by both copies. Male-biased genes in particular seem to increase in number through duplication and have a disproportionately high number of paralogues in both the worm and fly genomes[15,69].

Serendipity. Following gene duplication or some other form of genome rearrangement, a gene can acquire sex-biased expression purely by chance. If such sex-biased expression is beneficial to the organism, selection will maintain the gene and its new expression pattern in the species. This scenario might apply to many sex-biased gene duplicates that arose through retrotransposition, as the duplicates presumably lack the regulatory sequences that were associated with their parental genes and, instead, acquire them from the chromosomal region flanking their random site of insertion.

Neofunctionalization
A gain of a new function to a duplicated gene through mutation and selection.

Paralogues
Genes that arose from duplication of a common, ancestral gene. A gene that was duplicated before the divergence of two species is present as two paralogues, each of which has an orthologue in the other species.

Pleiotropy
The influence that a single gene has on multiple traits.

Epistatic selection
Selection for a certain combination of alleles at two or more loci.

Concluding remarks

The evolutionary dynamics of sexually antagonistic mutations is intimately connected with an adaptive pressure to limit their expression in either sex, through additional mutations. The underlying evolutionary forces that generate sexual conflicts should therefore also act as an engine behind sex-biased gene expression[3]. But how does sex-biased expression relate to other genetic processes for which regulation of transcription is important? One such aspect is the observation that the majority of genes are to some degree pleiotropic[70]. When a gene has either multiple functions or is expressed in multiple tissues, it already experiences considerable restrictions on adaptability and evolvability. Hypothetically, strong pleiotropic constraints would outweigh male-specific and female-specific selective pressures, and a pleiotropic gene would be forced to act sub-optimally in most contexts in order to balance all fitness criteria in both sexes. According to this hypothesis, one would expect pleiotropic genes to show less sex bias than more specialized genes. A recent study of chicken and mouse expression data suggests this to be the case[71].

Evolutionary theory predicts that recombination evolves in response to allelic association between loci (epistasis). It has also been suggested that epistatic selection can give rise to heterochiasmy, a difference in the rate of recombination between sexes, which is observed in many organisms[72]. Potentially, regional sex-biased recombination patterns could stem from sexual conflict, as sexually antagonistic selective pressures would favour the preservation of a set of linked sexually antagonistic genes in one sex, but the break up of that same linkage group in the other. This issue is worthy of detailed investigation and should include data on sex-specific recombination rates obtained from linkage analysis. Together with pleiotropy, this adds further dimensions to the complex nature of the evolution of gene expression in the two sexes.

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Competing interests statement
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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Dntf-2 | *Dntf-2r* | *janusA* | *janusB* | *ocnus*

FURTHER INFORMATION
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LMU — Evolutionary and functional genomics:
http://www.zi.biologie.uni-muenchen.de/evol/EvoGen.html

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