Evolution on the X chromosome: unusual patterns and processes

Beatriz Vicoso and Brian Charlesworth

Abstract | Although the X chromosome is usually similar to the autosomes in size and cytogenetic appearance, theoretical models predict that its hemizygosity in males may cause unusual patterns of evolution. The sequencing of several genomes has indeed revealed differences between the X chromosome and the autosomes in the rates of gene divergence, patterns of gene expression and rates of gene movement between chromosomes. A better understanding of these patterns should provide valuable information on the evolution of genes located on the X chromosome. It could also suggest solutions to more general problems in molecular evolution, such as detecting selection and estimating mutational effects on fitness.

Haldane's rule
The disproportionate loss of fitness to the heterogametic sex in F1 hybrids between species.

Clade
A group of species which share a common ancestor.

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, UK.
Correspondence to B.C.
e-mail: Brian.Charlesworth@ed.ac.uk
doi:10.1038/nrg1914

Sex-chromosome systems have evolved independently many times, and have attracted much attention from evolutionary geneticists. This work has mainly focused on the steps leading to the initial evolution of sex chromosomes, and the genetic degeneration of Y and W chromosomes[1]. Here, we discuss the evolution of the X chromosome in long-established sex-chromosome systems, such as those of mammals and *Drosophila* species. The emphasis is on recent molecular evolutionary, genomic and gene-expression studies, especially as the whole-genome analysis of several *Drosophila*[2] and mammalian[3] species has provided estimates of divergence rates for both coding and non-coding regions of the sex chromosomes and the autosomes. In addition, several studies using microarray technology have revealed many genes that are expressed exclusively or preferentially in one sex in *Drosophila melanogaster*[4,5], mammals[6,7] and *Caenorhabditis elegans*[8], and have shown that these genes are unevenly distributed between the X chromosome and the autosomes.

The evolutionary properties of the X chromosome are also relevant to several interesting biological phenomena that occur above the molecular level. In the genus *Drosophila*, the X chromosome seems to be enriched in genes that cause reproductive isolation between species[9], helping to explain classic observations such as Haldane's rule[10]. Similarly, genes that affect brain function[11] and genes that control fertility[12] seem to be preferentially located on the human X chromosome. A better understanding of the general evolutionary properties of genes located on the X chromosome will help to determine the causes of these peculiarities. Furthermore, tests of the predictions of theoretical models of X-chromosome evolution will shed light on the assumptions on which the models are based, such as the degree of dominance of mutations and the existence of opposing forces of selection on males and females, leading to a better understanding of the forces that shape the evolution of eukaryotic genomes.

First, we examine DNA-sequence divergence to ask: is the X chromosome evolving at a different rate from the autosomes or Y chromosome, and what might cause such a difference? Second, we review evidence on the evolution of the expression patterns of X-linked genes, in particular discussing why so many of them exhibit sex-biased expression. Last, we examine the excess of gene movements from the X chromosome to the autosomes, found in both mammals and *D. melanogaster*.

In all the clades analysed, the X chromosome seems to be under more efficient selection and to accumulate new genes, or genes with new, sex-biased expression patterns, differently from the autosomes. However, differences between the extensively studied *D. melanogaster* and mammalian X chromosomes make it hard to explain all the current data, suggesting that more work is necessary to clarify the processes involved.

A different mutation rate on the X chromosome?

Male-driven evolution and the X chromosome. Most mutational changes in DNA are thought to occur through replication errors during cell division[13]. Consequently, the mutation rate per generation is expected to increase with the number of divisions in the germ line (only mutations in the germ line are transmitted to the next

Box 1 | Use of data on DNA-sequence evolution to estimate α

The rate of substitution, *K*, is defined as the number of mutations that become fixed in a population per unit of evolutionary time[18]. This value can be estimated from the degree of DNA-sequence divergence between two taxa with a known date of divergence by dividing the estimated proportion of nucleotide sites for which they differ by the time that separates them[18,19]. For neutral mutations (that is, mutations with no effect on fitness), *K* is equal to the mutation rate per site[18].

Assume that the only factor controlling the relative mutation rates of genes on the X chromosome, Y chromosome and autosomes is the time that they spend in females and males (male heterogamety is assumed). The ratio of male mutation rate (u_m) to female mutation rate (u_f) is α. The substitution rates for autosomal, X-linked and Y-linked mutations are K_A, K_X and K_Y, respectively. It is easily shown[16] that:

$$K_A = \frac{(u_f + u_m)}{2} = \frac{(\alpha + 1)\, u_f}{2} \quad (1)$$

$$K_X = \frac{(2u_f + u_m)}{3} = \frac{(\alpha + 2)\, u_f}{3} \quad (2)$$

$$K_Y = u_m = \alpha u_f \quad (3)$$

As u_f is common to all these expressions, it is simple to get two different estimates of α from ratios such as K_A/K_X and K_Y/K_X. For instance, for *Drosophila melanogaster* and *Drosophila simulans*, Bauer and Aquadro[22] found $K_A/K_X = 0.96$, giving an α estimate of approximately 1. In a human–chimpanzee comparison[23], on the other hand, K_A/K_X of 1.3 yields an α value of 5.6.

Similar expressions can be derived for female heterogamety[29].

generation)[14]. In species with separate sexes, males and females have different ways of making gametes, which may cause a difference in the number of cell divisions. In mammals, for instance, spermatogenesis requires more cell divisions than oogenesis, so that the mutation rate in the male germ line is likely to be higher than that in the female germ line[15,16]. This effect is sensitive to the average ages of males and females at reproduction, as the overall mutation rate for a given sex is the sum of mutations contributed by individuals from all reproductively active ages[17].

Genes on autosomes spend an equal amount of their time in males and females, so that their net mutation rate is the average of the male and female mutation rates. With male heterogamety, X-linked genes spend only one-third of their time in males and two-thirds of their time in females. If spermatogenesis is more mutagenic than oogenesis, the X chromosome is subjected to a lower mutation rate than the autosomes or the Y chromosome[15,16] (the reverse is true for Z-linked genes in taxa with female heterogamety). This results in corresponding differences in the rate of molecular sequence evolution, as the rate of neutral DNA sequence divergence between species is equal to the mutation rate[18,19] (BOX 1).

Assessing male-driven evolution in flies. Two complementary approaches have been used to detect such 'male-driven' evolution. The first uses comparative data on the number of cell divisions required for female and male gametogenesis[20,21]. The second estimates between-species divergence levels at silent nucleotide sites for autosomal, X-linked and Y-linked sequences; the differences among these yield estimates of α, the ratio of the male-to-female mutation rates[16] (BOX 1). If male-driven evolution is the sole cause of this difference, the estimate of α should be related to the ratio of the numbers of male and female germline divisions required to make a successful gamete, although the sensitivity of net mutation rates to demography[17] means that equality of the two estimates is not necessarily expected.

The two approaches have yielded consistent results for *D. melanogaster*: the mean number of divisions is estimated to be 35.5 for spermatogenesis and 34.5 for oogenesis[21]. Although silent divergence among *Drosophila simulans* and *D. melanogaster* is slightly higher for X-linked sites, this difference is not significant (that is, α is approximately 1) (REF. 22).

Assessing male-driven evolution in mammals. The results for mammals are less straightforward. The estimated mean number of cell divisions per generation is 401 divisions for human spermatogenesis and 31 for oogenesis[20]. A male-driven evolution effect was detected in a human–chimpanzee sequence comparison[23], where α was estimated to be approximately 3. Overall sequence divergence among humans and chimpanzees estimated from the genome sequences is highest for the Y chromosome and lowest for the X chromosome[3], yielding an α value of 2–6. This value is much smaller than the estimate from the cell-division data. By contrast, a comparison of X-chromosome and autosome mouse–rat silent divergence gave a much higher estimate of α than expected[24]. McVean and Hurst[24] suggested that this low level of X-chromosome divergence was caused by a local reduction in the mutation rate, which evolved by selection to avoid the expression of deleterious recessive mutations in hemizygous males. Their sample of genes was relatively small, however, and subsequent work with larger samples supports male-biased mutation as the main force reducing X-chromosome neutral divergence[25]. Malcom *et al.*[25] pointed out that, although there is great variation from chromosome to chromosome in human–mouse and rat–mouse comparisons[26], the X chromosome consistently shows the lowest divergence. The shorter generation time of rodents is expected to lead to a smaller α than in primates, making it more difficult to estimate (62 germ-cell divisions in males, assuming reproduction at 9 months, compared with 25 in females[20]).

It has also been argued that there are replication-independent mutational mechanisms, which could explain inconsistencies between the ratio of male-to-female gametogenesis divisions and α estimates[27]. Taylor *et al.*[28] analysed neutral divergence at X-linked and autosomal loci in a human–chimpanzee comparison, but separated mutations at CpG sites from the rest. These sites are known to be hot spots for mutations caused by deamination of methylated cytosines, a process that might be replication independent. Consistent with this, divergence at non-CpG sites showed a strong male bias, with α corresponding to the ratio of male-to-female germline divisions, whereas a much smaller effect was observed at CpG sites. Additional support for male-driven evolution in vertebrates comes from sequence comparisons of birds, the female heterogamety of which means that genes on the female-limited W chromosome should show

Male heterogamety
Describes the situation in which males carry two heteromorphic sex chromosomes (such as X and Y) and females carry two copies of the same chromosome (XX).

Neutral DNA
DNA that is not subject to selection.

Silent nucleotide sites
Nucleotides where mutations do not change protein sequences.

CpG sites
Adjacent cytosine and guanine bases in a DNA sequence.

Fitness
The expected contribution of an individual to the next generation.

lower rates of silent evolution than either the Z chromosome or autosomes, as is indeed observed[29–31]. This result cannot be explained by the hypothesis of McVean and Hurst[24].

In summary, the extent and effects of male-driven neutral evolution depend both on the life history of the species and on the molecular basis of mutation. Current work suggests that the mammalian X chromosome and bird W chromosome have lower mutation rates than the autosomes, resulting in lower levels of neutral divergence at X- and W-chromosome loci. In *Drosophila* species, on the other hand, no such effect has been detected, as expected from the similar number of cell divisions estimated for male and female gametogenesis.

Box 2 | **Selection on autosomal and X-linked mutations**

A simple model of the effects on fitness of a mutation is as follows, where s denotes the homozygous or hemizygous effect of a mutation, A_2, and *h* measures its degree of dominance.

	Females			Males		
Autosomal mutation						
Genotypes	A_1A_1	A_1A_2	A_2A_2	A_1A_1	A_1A_2	A_2A_2
Fitness	1	$1 + hs_f$	$1 + s_f$	1	$1 + hs_m$	$1 + s_m$
X-linked mutation						
Genotypes	A_1A_1	A_1A_2	A_2A_2	A_1	A_2	
Fitness	1	$1 + hs_f$	$1 + s_f$	1	$1 + s_m$	

The fate of a mutation is mainly determined by its rate of spread when rare, so we show the expressions for gene frequency change when A_2 is at a low frequency, *p*. Provided that selection is weak ($s << 1$), the change in frequency per generation of a rare autosomal mutation is:

$$\Delta p \approx \frac{ph(s_f + s_m)}{2} \quad (4)$$

The corresponding expression for an X-linked mutation is:

$$\Delta p \approx \frac{p(2hs_f + s_m)}{3} \quad (5)$$

A mutation will only spread in a very large population if Δp is positive; that is, there is a net selective advantage to the mutation over the wild type, A_1. In a finite population, it can spread by genetic drift even if $\Delta p < 0$; the probabilities that this happens for autosomal and X-linked mutations can be calculated[34], but will not be given here.

It is also of interest to know the rate of substitution (*K*) of mutations with fitness effects like A_2, as theoretical values of *K* can be compared with data on between-species DNA-sequence divergence (BOXES 1,4).

K for mutations that arise as unique copies in the population is equal to the expected number of mutations that enter the population times the probability that a mutation spreads through the population[18,34]. The former is given by the product of the mutation rate and the number of gene copies in the population ($2 \times$ the population size *N* for autosomal genes; $1.5 \times N$ for X-linked genes). With weak selection, the latter is determined by the ratio Δp:*p*.

To simplify the formulae, we express *K* relative to the product of 2*N* and the mutation rate. For beneficial autosomal mutations in a large population, we have:

$$K_A \approx 2h(s_f + s_m) \quad (6)$$

(provided that $s_f + s_m > 0$; otherwise $K_A = 0$).

The corresponding expression for X-linked mutations is:

$$K_X \approx (2hs_f + s_m) \quad (7)$$

(provided that $2hs_f + s_m > 0$; otherwise $K_X = 0$).

The ratio of *K* for X-linked and autosomal mutations (when both are > 0) is therefore:

$$R \approx \frac{(2hs_f + s_m)}{2h(s_f + s_m)} \quad (8)$$

If there are no sex differences in selection ($s_f = s_m$), then $R \approx (1 + 1/(2h))/2$; with selection on males only ($s_f = 0$), $R \approx 1/(2h)$; and with selection on females only ($s_m = 0$), $R \approx 1$.

Is selection more efficient for X-linked genes?

The fixation of beneficial and deleterious mutations. In randomly mating populations, newly arisen autosomal mutations are found mostly in heterozygotes, in which any recessive effects are masked by the ancestral allele and are therefore not exposed to selection[32]. If they arise on the X (or Z) chromosome, however, their effect on fitness is fully expressed in the hemizygous males (or females). Therefore, selection is expected to fix beneficial recessive, or partially recessive, mutations (and remove deleterious recessive mutations) more efficiently on the X or Z chromosomes than on the autosomes[33,34]. Theoretical predictions concerning the rates of molecular evolution for favourable mutations at X-linked and autosomal sites are shown in BOX 2.

Under some conditions, the X chromosome is expected to accumulate beneficial mutations at a faster rate than the autosomes, whereas weakly deleterious mutations are expected to accumulate by genetic drift at a higher rate on the autosomes[34]. This effect is especially strong for mutations that affect only males (BOX 2). Higher male mutation rates, on the other hand, reduce any tendency for faster evolution of beneficial mutations on the X chromosome, but have the reverse effect for Z chromosomes[35]. In addition, if adaptive evolution uses variants that have been maintained in the population by mutation pressure, rather than picking up new mutations, the relative rates of evolution for the X chromosome and autosomes can behave in the opposite way to these predictions[36].

If a substantial fraction of DNA-sequence divergence for non-synonymous mutations is driven by the fixation of beneficial mutations by natural selection (positive selection), as has been claimed for mammals[37] and some *Drosophila* species[38–40], we might see a higher rate of protein-sequence evolution for X-linked versus autosomal mutations. The reverse would be the case if protein evolution largely reflects the fixation of weakly deleterious, at least partly recessive mutations. The availability of large quantities of sequence data makes it possible to examine this question.

Testing the faster-X hypothesis in* Drosophila *species. The nature of selection that has shaped the between-species sequence divergence of a gene affects its K_a/K_s ratio (BOX 3). If positive selection is more effective at X-linked loci, these should have higher K_a/K_s ratios than autosomal loci; the reverse would the case if purifying selection against deleterious mutations is more effective. One way to test for this is to estimate average K_a and K_s values over large numbers of genes on the X chromosome and the autosomes. Betancourt *et al.*[41] found no difference between 51 X-linked and 202 autosomal loci in the

Genetic drift
Random fluctuation of allele frequencies in a population due to sampling effects (as only a subsample of the gametic pool is used in each generation).

Non-synonymous mutations
Mutations that change the protein sequence; these are likely to be under selection.

Fixation
Increase of an allele frequency to 1.

Positive selection
Spread of a mutation through a population, because of increased survival or reproduction of the individuals carrying it.

Purifying selection
Removal of mutations from the population, because of reduced survival or reproduction of the individuals carrying it.

D. melanogaster–D. simulans comparison. An even larger sample was provided by the release of the *Drosophila pseudoobscura* genome[2]. The values of K_a and K_s for alignable genes in this pair of species are similar for X-linked and autosomal loci[2]. Thornton and Long[42], on the other hand, studied duplicate gene pairs in the *D. melanogaster* genome, and observed that K_a/K_s values were significantly higher when both copies were located on the X chromosome than when one or both were located on an autosome. Subsequent population-genetics work detected more positive selection on X-linked duplicates[43].

These comparisons suffer from several problems, especially the fact that different sets of genes are often compared which might differ for reasons other than chromosomal location. This can be avoided by asking whether the same gene evolves faster when it is on the X chromosome than when it is on an autosome. In the *D. pseudoobscura* group, an autosomal arm (3L in *D. melanogaster*) has fused to the X chromosome. Counterman *et al.*[44] argued that if there is a faster-X effect then the genes on this new X-chromosome arm (XR) will evolve faster than their autosomal homologues. They compared rates of evolution in the *D. pseudoobscura* group and the *D. melanogaster* group and found that, for 3L/XR genes, there is an excess of genes evolving faster in the *D. pseudoobscura* group (where they are X-linked) than in the *D. melanogaster* group, in agreement with the faster-X hypothesis. However, a recent study in which the same approach was applied to a larger sample of genes suggested similar rates of evolution for X-linked and autosomal protein sequences[45].

These mixed results suggest that either some of the assumptions on which the model is based are incorrect, or that the fraction of mutations fixed by positive selection has been overestimated. There seems to be some evidence for the latter. The studies that detected a faster-X effect in *Drosophila* were biased towards fast-evolving genes. Counterman *et al.*[44] obtained part of their sample from a male-specific EST screen, thereby selecting genes that might be under stronger positive selection than is typical[46]. Similarly, newly duplicated genes[42] are likely either to evolve under strong positive selection or to decay into pseudogenes.

Testing the faster-X hypothesis in mammals. Recent studies also provide some indication of faster-X effects in mammals. Estimates of human–chimpanzee K_a and K_s values for many genes[3,47] show that X-linked genes have a statistically significantly higher mean K_a/K_s than autosomal genes. The values for X-linked genes are skewed towards the two extremes, giving further support to the idea that X-linked genes evolving mainly under negative selection are evolving more slowly, whereas genes subject to positive selection are evolving faster. Several studies have indicated that sperm proteins are under strong positive selection, and might therefore be a good target for faster-X evolution[48,49]. Furthermore, they are only expressed in males, which would enhance this effect (BOXES 2,4). In accordance with this prediction, X-linked sperm proteins in mammals evolve significantly faster than autosomal ones[50,51]. Similarly, Khaitovich *et al.*[52] analysed a large data set of tissue-specific genes and found that only testis-expressed X-linked genes have a higher K_a/K_i (K_i is the divergence for non-coding sequences).

Excess of codon bias on the X chromosome. Recent studies of codon bias suggest that purifying selection might be more efficient on the X chromosome. Although synonymous codons are often assumed to evolve neutrally (BOXES 1,3), there is evidence for selection favouring preferred codons in several organisms[53]. Hambuch and Parsch[54] and Singh *et al.*[55] estimated the levels of codon bias for X-linked and autosomal genes in *Drosophila* and *C. elegans* and found a stronger bias on the X chromosome. Lu and Wu[47] found a lower value of K_s for synonymous sites on the X chromosome in the human–chimpanzee genome-sequence comparison. This pattern suggests more effective weak purifying selection on the X chromosome, indicating that mutations affecting codon usage have partially recessive deleterious fitness effects[56].

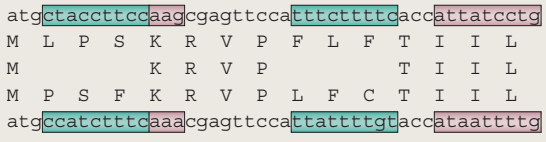
X-chromosome divergence within species. We have so far discussed the divergence of the X chromosome between species, but the same processes apply within a species. Both positive selection on new beneficial mutations and the continual removal of deleterious mutations reduce polymorphism levels at sites linked

Box 3 | K_a/K_s, an indicator of the nature of selection on coding DNA

We can classify differences at individual nucleotide sites in coding sequences as synonymous, if they do not affect the amino-acid sequence (pink codons in the alignment), or non-synonymous, if they affect the amino-acid sequence (green codons in the alignment). Mutations in non-coding sequences cannot change the amino-acid sequence; together with synonymous mutation, they constitute silent mutations.

Rates of sequence divergence can be estimated using comparisons of DNA-coding sequences from two species, as in the example shown below of the partial sequence of the *Acp33a* gene of two *Drosophila* species:

As synonymous mutations have no effect on amino-acid sequence, they are not under



strong selection, and the proportion of sites that differ with respect to synonymous mutations (expressed as a fraction of the total number of sites that can potentially produce silent mutations), K_s, can be used to provide an approximate estimate of the rate of neutral evolution (BOX 1). We use lower-case subscripts here, to distinguish the proportion of sites that differ between species from the rate of substitution.

On the other hand, K_a, the proportion of sites that differ for non-synonymous mutations (expressed as a fraction of the total number of sites that can potentially produce amino-acid mutations), is affected by both neutral evolution and selection.

The ratio K_a/K_s removes the effect of the neutral forces, and provides an estimate of the selective pressures constraining the evolution of the gene. If all amino acids in a coding region are evolving neutrally, then $K_a/K_s = 1$. Purifying selection eliminates mutations that change the amino-acid sequence, reducing K_a/K_s below 1. Positive selection, on the other hand, favours the fixation of non-synonymous mutations, and increases K_a/K_s above 1. As the same gene can experience purifying selection at some amino-acid sites and positive selection at others, it is rare to find $K_a/K_s > 1$ for a whole gene.

Box 4 | **Effects of sexual antagonism**

Sexual antagonism means that s_f and s_m in BOX 2 have opposite signs, where s denotes the homozygous or hemizygous effect of a mutation. The following conclusions can easily be obtained from the results in BOX 2.

Male advantage, female disadvantage

Let $s_m > 0$, $s_f = -ks_m$, where k is the ratio of fitness effects on females and males. For autosomal inheritance, a mutation will spread in a large population if $k < 1$. For X-linked inheritance, it will spread if $k < 1/(2h)$. The ratio of substitution rates for X-linked versus autosomal mutations (when both rates are > 0) is:

$$R \approx \frac{(1-2hk)}{2h(1-k)} \quad (9)$$

$R > 1$ if $h < 0.5$, and approaches infinity as h (the degree of dominance) tends to zero.

The conclusion is that some degree of recessivity ($h < 0.5$) of favourable fitness effects in males tends to lead to a higher rate of fixation of mutations on the X chromosome; dominance ($h > 0.5$) leads to a higher rate for the autosomes. This is true even if there are no deleterious effects in females ($k = 0$), but the effect increases with the value of k.

Female advantage, male disadvantage:

Let $s_f > 0$, $s_m = -ks_f$. For autosomal inheritance, a mutation will spread in a large population if $k < 1$; for X-linked inheritance, if $k < 2h$. The ratio of X-to-autosome substitution rates (when both are > 0) is:

$$R \approx \frac{(2h-k)}{2h(1-k)} \quad (10)$$

$R \geq 1$ if $h > k/2$, and approaches infinity as k tends to 1.

With favourable fitness effects in females, sexual antagonism leads to a higher rate of fixation of mutations on the X chromosome if there is some degree of dominance, and to a higher rate on the autosomes with recessivity; again, this effect increases with k.

We can also ask questions about the rates of evolution of sexually antagonistic mutations with a net selective disadvantage (Δp in BOX 2 is negative). The smaller the magnitude of Δp, the more likely it is that a deleterious mutation will be fixed by drift[34]. It is easy to see from the expressions for Δp that a partially recessive, X-linked mutation with $s_f < 0$ and $s_m > 0$ might have a lower selective disadvantage than a comparable autosomal mutation. The same is true for a dominant mutation with $s_f > 0$ and $s_m < 0$. It will have higher selective disadvantage with the opposite patterns of sex-specific selection coefficients.

to the genes in question[57]. If positive selection is more efficient on the X chromosome, we expect it to harbour less variability than the autosomes[58]. Although this pattern is not observed in African populations of *D. melanogaster* and *D. simulans*, the X chromosome is indeed less variable than the autosomes in non-African populations[59–63]. Because these species have recently spread from Africa into Europe and North America, they might have experienced new selection pressures, so that the lower levels of polymorphism on the X chromosome reflect a higher frequency of recent fixation of favourable mutations on this chromosome than on the autosomes. However, other demographic scenarios could account for this pattern[64], and more work is necessary to determine how much of the pattern is caused by selection[65].

Similarly, Wang *et al.*[66] detected an excess of linkage disequilibrium for X-linked loci in a large human polymorphism data set. This result could be caused either by reduced recombination or increased selection. Although the human X chromosome seems to have a lower recombination rate than the autosomes, it seems likely that the twofold difference in linkage disequilibrium is at least partially caused by more effective selection on X-linked loci[66].

Pseudogene
A gene that has accumulated mutations in its protein-coding sequence or regulatory region, so that it is no longer functional.

Codon bias
Preferred usage of some codons over others that code for the same amino acid, possibly as a result of selection for increased translation efficiency or accuracy.

Polymorphism
Genetic variation within a species or population.

Linkage disequilibrium
Non-independent associations of alleles at different loci in a population.

Summary: is there really a faster-X effect? Theoretical models predict that, if mutations are on average recessive, selection will be more efficient on the X chromosome. Between- and within-species DNA-divergence data are sometimes consistent with this prediction, both in *Drosophila* species and in mammals. Whether this corresponds to a faster or slower evolution of X-linked sites, however, depends on how much of the divergence is fixed by positive selection versus genetic drift. The fact that whole-genome comparisons among *Drosophila* species mostly yield similar rates of divergence for X chromosomes and autosomes, whereas studies that focus on genes under strong positive selection find a higher K_a/K_s at X-linked sites, indicates that positive selection is probably rarer than previously estimated. In human–chimpanzee comparisons, higher K_a/K_s is consistently observed for X-linked loci.

However, faster or slower X-chromosome evolution can arise in other ways, for example, if mutations have effects of opposite sign on the fitness of males and females; that is, they are sexually antagonistic (BOX 4). This means that no unambiguous conclusions concerning causality can be drawn simply from differences among X chromosome and autosomes in the distribution of K_a/K_s values.

Accumulation of sex-biased genes

The occurrence of sexual antagonism also implies that the X chromosome may preferentially accumulate genes with sex-biased fitness effects[33] (BOX 4). If an autosomal mutation with a significant fitness effect on heterozygous carriers is beneficial for females but deleterious for males, it will increase in frequency under positive selection only if the advantage to females is greater than the disadvantage to males (BOX 4). If a similar mutation occurs on the X chromosome, it will be subject to negative selection only one-third of the time, and therefore has a higher net selective advantage and probability of becoming fixed in the population. The X chromosome is then likely to accumulate genes that are expressed in females rather than males, at a faster rate than the autosomes (BOX 5).

But sexual antagonism involving alleles with recessive or partially recessive fitness effects leads to an accumulation of male-biased genes on the X chromosome rather than the autosomes[33]. New X-linked recessive mutations that are beneficial for males and deleterious for females can spread, as their beneficial effects are expressed in males, whereas at low frequencies their deleterious effects on females are masked (BOX 4). Depending on the level of dominance of the fitness effects of mutations, accumulation of either male- or female-biased genes on the X chromosome relative to the autosomes can occur.

***Results for* Drosophila *and* C. elegans.** Microarray data sets can be used to determine the patterns of expression of genes in relation to sex, allowing the distribution of female- and male-biased genes in the genome to be determined. Using this approach, an excess of female-biased genes on the X chromosome has been found in both *Drosophila* species and *C. elegans*[4,5,8] (TABLE 1),

Box 5 | **Paths for the evolution of sex-specific patterns of gene expression**

We assume here that the evolution of gene expression is driven primarily by positive selection, and that there is no movement of genes between the X chromosome and the autosomes. Step 1 is an essential starting point; step 2 represents distinct but non-exclusive possible ways to proceed.

Step 1: accumulation of sexually antagonistic mutations

Genes with sexually antagonistic fitness effects but with no initial sex differences in expression can evolve as a result of the fixation of coding-sequence mutations that have opposite effects on the fitness of males and females. The rate of accumulation of such mutations may vary according to their location on the X (or Z) chromosome versus the autosomes, according to the rules outlined in BOXES 2,4. This fixation pattern might lead to an uneven distribution of genes with sexually antagonistic fitness effects between the two types of chromosome.

Step 2: evolution of modifiers of gene expression

Given the presence of such sexually antagonistic genes, there will be selection for both *cis*- and *trans*-acting modifiers of their expression, either to ensure more gene product in the sex that they benefit, or less product in the sex that they harm. If there is already a non-random distribution of such genes between the X (or Z) chromosome and the autosomes, modifiers will lead to a non-random pattern of gene expression, even if only *trans*-acting modifiers are involved. Selection on *cis*-acting modifiers of gene expression might also contribute to non-random patterns of expression, even if the genes themselves are randomly distributed among the chromosomes. Several outcomes are possible, involving different types of mutation, which are again not mutually exclusive.

Cis-acting modifiers may arise that cause sexually antagonistic genes to be turned down in the sex that is harmed. In the heterogametic sex, such modifiers will tend to accumulate differentially on the X (or Z) chromosome if they are partially recessive, and on the autosomes if they are partially dominant (BOX 2).

Cis-acting modifiers that cause an increase in expression level only in the sex that is benefited will similarly obey the rules for favourable mutations with fitness effects confined to that sex.

Cis-acting modifiers that cause an increase in expression level in the sex that is benefited and a decrease in expression in the sex that is harmed will obey the rules for favourable mutations with effects on both sexes (BOX 2); that is, they will tend to accumulate differentially on the X (or Z) chromosome if they are partially recessive, and on the autosomes if they are partially dominant (BOX 2).

whereas genes with male-biased expression are under-represented on the X chromosome. Genes expressed in the gonads seem to show a particularly strong effect of this kind[4].

Different results for mammals. There has been some debate about whether there is evidence for an excess of female-biased genes on the X chromosome in mammals[6], but a recent study indicates that there is such an effect[7] (TABLE 1). Initial reports in rodents suggested that the X chromosome had an excess of male-biased genes[67]. The X chromosome is inactivated during meiosis in the male germ line, so that genes for which expression is required late in spermatogenesis must be located on the autosomes or Y chromosome[68]. This would prevent any accumulation of members of this subset of male-biased genes on the X chromosome. It has accordingly been suggested that the differences between the mouse and *C. elegans*/*D. melanogaster* results were mainly due to experimental design, as early spermatocytes were used in the rodent study. If this were the case, then the mammalian X chromosome should also show a deficit of late spermatogenesis genes, and the male-biased gene deficit on the *C. elegans*/*D. melanogaster* X chromosomes should be confined to spermatogenesis-related genes. The first prediction was confirmed by Khil *et al.*[7], who found that the rodent X chromosome was deficient in male-biased genes from mature-testis arrays (consisting mostly of mature spermatocytes), but enriched in male-biased genes from immature testes (where mature spermatocytes, with an inactive X chromosome, are absent or rare).

Oliver and Parisi[69] pointed out that somatically expressed male-biased genes in *D. melanogaster* are also scarce on the X chromosome, so that the second prediction is falsified. In particular, the accessory gland proteins are fertility-enhancing proteins that are produced by *Drosophila* males and transferred to females during mating. These are not expressed in spermatocytes, but are also present more rarely than expected on the X chromosome[70], suggesting that the deficit of this class of male-biased genes on the X chromosome is caused by evolutionary forces other than avoidance of X-chromosome inactivation.

Why the difference? There seems to be a real difference between the *Drosophila* species and mammalian results, once the effect of X-chromosome inactivation in spermatogenesis is removed. There is, however, no obvious reason why the dominance of the fitness effects of favourable mutations should be consistently different between these groups. Without direct evidence on the dominance effects of favourable mutations, it will be difficult to resolve this difficulty, and the interpretation of the patterns we have discussed remains speculative. One possibility is that differences in the mechanisms of X-chromosome dosage compensation could influence the evolution of the expression pattern at X-linked loci. In flies, nematodes and mammals, mechanisms are in place to ensure that haploid males and diploid females produce similar amounts of X-chromosome-derived mRNAs[71]. In *D. melanogaster*, this involves increasing the rate of expression of genes on the male X chromosome. It has been suggested[72] that male-biased genes evolve mostly by increases in the level of expression of existing genes in males; if this is the case, then higher expression levels could be harder to achieve on the already hyperactive X chromosome than on the autosomes, if the rate of mRNA transcription is limited.

It is interesting to note that a study of the distribution of sex-biased genes in the chicken genome has recently been completed (V. Kaiser and H. Ellegren, unpublished results). The results are similar to the *Drosophila* and *C. elegans* results, with a deficit of female brain and ovary genes on the Z chromosome, and an excess of male brain genes (TABLE 1). Studies in birds, in which the female is heterogametic, are useful, as they decouple the effects of sex and heterogamety. Not much is known, however, about the biology of the Z chromosome, making it difficult to evaluate the influence of other factors, such as dosage compensation, on the evolution of this chromosome.

It is important to note that the gene content of the X chromosome is very stable in both *Drosophila* species and mammals[73], so that the patterns we have described must overwhelmingly reflect evolutionary shifts in gene expression, and not physical movements of genes on and off the X chromosome. Various scenarios for this

Table 1 | **A summary of studies* on the genomic distribution of sex-biased genes**

Organism	Tissue/function	Genes on the X chromosome	
		Female	Male
Drosophila melanogaster	Gonads	+	–
	Whole adults	No effect	–
	Adult soma	No effect	–
Caenorhabditis elegans	Gametogenesis	–	–
	Soma	+	No effect
Mouse	Gonads	+	–
	Testis (*Spo11*$^{-/-}$)	NA	+
	Young testis	NA	+
Human	Prostate	NA	+
	Ovaries and mammary glands	No effect	NA
Chicken (females ZY)	Brain	–	+
	Gonads	–	No effect

*REFS 4,6–8; chicken data from V. Kaiser and H. Ellegren, personal communication. A plus sign is used to mark an excess of genes on the X chromosome, whereas a minus sign denotes a deficit. To disentangle the effects of meiotic inactivation and sexual antagonism in the distribution of male-biased genes in the mouse genome, Khil *et al.*[7] focused on genes involved in early spermatogenesis, before the X chromosome has been inactivated. To do so, they analysed testis expression data from young mice, as developing testes contain a higher proportion of cells in early spermatogenesis, and *Spo11*$^{-/-}$ mice, in which spermatogenesis is blocked in early meiosis. NA, not applicable.

are outlined in BOX 5. This high degree of stability of the gene content of the X chromosome casts doubt on the SAXI hypothesis of Wu and Xu[74], which appeals to gene movement rather than modification of expression to explain a lack of male-biased genes on the *Drosophila* X chromosome.

Gene movement between chromosomes

The availability of large amounts of genomic data has revealed that this stability is not absolute. With a single genome, the potential for analysis is limited, as the parental and derived copies of a gene usually cannot be distinguished. There is one exception: retroposons are genes derived from mRNA. RNA can be retrotranscribed into cDNA by viral retrotranscriptase and occasionally be integrated in the genome. These new genes harbour mRNA characteristics, such as lack of introns and presence of poly(A) tracks, which differentiate them from their parental copies. Retroposons have therefore become favourites for studying the direction of duplication when only one genome is available; the results are summarized in BOX 6.

Box 6 | **Retroposition from and to the X chromosome**

Excess of retroposition from the X chromosome to the autosomes

The study of retroposition in the *Drosophila melanogaster* genome[81] has revealed a statistically significant excess of autosomal duplicated genes that are derived from the X chromosome, suggesting that either retroposition from the X chromosome onto the autosomes is more frequent, or that the resulting newborn genes are preserved by lower deletion rates on the autosomes compared with the X chromosome and/or selection. A similar pattern also exists in the mouse and human X chromosomes[82,83].

If this excess were due to a mechanistic bias only, retroposed genes that have decayed into pseudogenes would follow the same trend. This is not the case, suggesting that selection influences this biased distribution.

One possible explanation for the excess is that meiotic X-chromosome inactivation causes a selective pressure for X-linked genes to migrate to the autosomes, as this will allow their expression in late spermatocytes, and therefore might be beneficial for males. Consistent with this model, most of the new genes that had escaped from the X chromosome were expressed in the testis in one study[82]. Although the experimental evidence is weak, it has been suggested that most retropositions occur in the male germ line[84]. This idea could also account for the observed enrichment in testis-specific expression, if a transposed gene is more likely to insert close to a regulatory sequence when it inserts into open chromatin.

Excess of retroposition onto the mammalian X chromosome

Emerson *et al.*[82] also found an excess of retropositions from the autosomes to the X chromosome in mammals, but not in *Drosophila*.

Duplicated sequences might be removed from the genome by ectopic recombination, to which the mammalian X chromosome is subjected less often than the autosomes, as it cannot recombine in the heterogametic male[85]. This could partially explain the bias (the opposite effect is expected in *Drosophila* species, with its lack of crossing over in males for all chromosomes[86]).

However, the bias is stronger for functional genes than for pseudogenes. Sexual antagonism or the faster-X effect working on newly replicated genes could accelerate their accumulation on the X chromosome.

Ectopic recombination
Recombination between homologous sequences that are located in different genomic locations. It can result in deletions and other types of chromosomal rearrangement.

What have we learnt from these patterns?

Although the sex chromosomes of mammals and *Drosophila* species have evolved independently, they are quite similar in their general properties, and their evolution seems to be shaped by similar evolutionary forces. However, we have highlighted several differences between them, which probably result from differences in the biology of insects and mammals.

The number of cell divisions is higher for spermatogenesis than for oogenesis in mammals, but not in the genus *Drosophila*. Probably as a result of this difference, silent site divergence for X-linked loci is lower than for autosomes in mammals, but is similar in *Drosophila* species. Recombination is lower for the X chromosome than the autosomes in humans; the opposite is true for *Drosophila*, which might account for the differences in patterns of accumulation of new, duplicate genes on the X chromosome (BOX 6).

The evidence regarding rates of protein-sequence evolution and codon-usage bias from both *Drosophila* species and mammals suggests that both positive and negative selection act more efficiently at X-linked loci. The classic explanation for faster protein-sequence evolution on the X chromosome invokes the faster accumulation of favourable recessive mutations[34]. As noted above and in BOX 4, there are other possible causes of this pattern. It will probably be necessary to relate differences in patterns of gene expression between the sexes to differences in evolutionary rates between X-linked and autosomal genes to answer questions of causation: for instance, genes that have been expressed only in one sex for a long period of evolutionary time are not likely to be subject to sexual antagonism.

The recessivity of beneficial mutations that is invoked by this model is, indeed, contrary to the expression data in *Drosophila*, for which the patterns of sex-biased genes are consistent with predictions for dominant mutations, with an accumulation of female-biased genes and a deficit of male-biased genes on the X chromosome[4]. As an excess of male-biased genes is observed for mammals[7], it is possible that other biological causes, such as differences in dosage-compensation mechanisms, are preventing male-biased expression patterns from evolving on the *Drosophila* X chromosome, but this needs to be further studied. A study of the evolution of patterns of gene expression in species such as *D. pseudoobscura*, in which a former autosome has been attached to the X chromosome for a long period of evolutionary time[44], would be illuminating in this regard.

Finally, both the faster-X effect and the accumulation of sex-biased genes on the X chromosome owing to sexual antagonism can account for the excess of brain- and testis-expressed genes detected on the human X chromosome, without involving female choice of more intelligent males, as proposed by Zechner *et al.*[75] Cognitive function and fertility are probably crucial for the evolution of mammalian lineages[76], and it is possible that the genes that influence them are especially subject to positive selection. X-linked loci in mammals might therefore have accumulated an excess of mutations that enhance these characteristics, making them more prone to mutations that impair them. Furthermore, behavioural patterns differ in the two sexes, and this might lead brain-expressed genes to accumulate on the X chromosome through sexual antagonism[77]. This is consistent with the higher expression level of X-chromosomal versus autosomal genes detected in the brain[78] (but not in other tissues), if sexual antagonism results in the evolution of increased gene expression in the beneficiary sex[72]. Analyses of gene expression in different mammalian tissues have shown that there is a correlation between the testes and the brain in patterns of gene expression, so that brain-expressed genes are to a certain extent also testis-expressed genes[79,80], which might further enhance their accumulation on the X chromosome.

We have described some ways in which evolutionary processes have acted in a distinctive way on the X (or Z) chromosome, leading to features that differentiate it from the autosomes. However, the exact nature of these processes remains to be elucidated, so that there is plenty of scope for future investigations of the chromosomal context of both sequence evolution and the evolution of patterns of gene expression. This should further our general understanding not just of sex chromosome evolution but also of genome evolution as a whole.

1. Charlesworth, D., Charlesworth, B. & Marais, G. Steps in the evolution of heteromorphic sex chromosomes. *Heredity* **95**, 118–128 (2005).
2. Richards, S. *et al.* Comparative genome sequencing of *Drosophila pseudoobscura*: chromosomal, gene, and *cis*-element evolution. *Genome Res.* **15**, 1–18 (2005).
3. The Chimpanzee Sequencing and Analysis Consortium. Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* **437**, 69–87 (2005).
4. Parisi, M. *et al.* Paucity of genes on the *Drosophila* X chromosome showing male-biased expression. *Science* **299**, 697–700 (2003).
A large-scale microarray analysis that reveals a deficit of male-biased genes on the X chromosome of *D. melanogaster*.
5. Ranz, J. M., Castillo-Davis, C. I., Meiklejohn, C. D. & Hartl, D. L. Sex-dependent gene expression and evolution of the *Drosophila* transcriptome. *Science* **300**, 1742–1745 (2003).
6. Lercher, M. J., Urrutia, A. O. & Hurst, L. D. Evidence that the human X chromosome is enriched for male-specific but not female-specific genes. *Mol. Biol. Evol.* **20**, 1113–1116 (2003).
7. Khil, P. P., Smirnova, N. A., Romanienko, P. J. & Camerini-Otero, R. D. The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. *Nature Genet.* **36**, 642–646 (2004).
The genomic distribution of early and late mouse spermatogenesis genes is analysed. This study provides evidence that the mouse X chromosome is indeed enriched in male-biased genes, once late spermatogenesis genes are excluded.
8. Reinke, V., Gil, I. S., Ward, S. & Kazmer, K. Genome-wide germline-enriched and sex-biased expression profiles in *Caenorhabditis elegans*. *Development* **131**, 311–323 (2004).
9. Tao, Y., Chen, S., Hartl, D. L. & Laurie, C. C. Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauritiana*. I. Differential accumulation of hybrid male sterility effects on the X and autosomes. *Genetics* **164**, 1383–1397 (2003).
10. Coyne, J. A. & Orr, H. A. *Speciation* (Sinauer Associates, Sunderland, 2004).
11. Skuse, D. H. X-linked genes and mental functioning. *Hum. Mol. Genet.* **14**, R27–R32 (2005).
12. Saifi, G. M. & Chandra, H. S. An apparent excess of sex- and reproduction-related genes on the human X chromosome. *Proc. Biol. Sci.* **266**, 203–209 (1999).
13. Drake, J. W., Charlesworth, B., Charlesworth, D. & Crow, J. F. Rates of spontaneous mutation. *Genetics* **148**, 1667–1686 (1998).
14. Keightley, P. D. & Eyre-Walker, A. Deleterious mutations and the evolution of sex. *Science* **290**, 331–333 (2000).
15. Haldane, J. The mutation rate of the gene for haemophilia, and its segregation ratios in males and females. *Ann. Eug.* **13**, 262–271 (1947).
16. Miyata, T., Hayashida, H., Kuma, K., Mitsuyasu, K. & Yasunaga, T. Male-driven molecular evolution: a model and nucleotide sequence analysis. *Cold Spring Harb. Symp. Quant. Biol.* **52**, 863–867 (1987).
17. Charlesworth, B. *Evolution in Age-Structured Populations* (Cambridge Univ. Press, Cambridge, 1994).
18. Kimura, M. Evolutionary rate at the molecular level. *Nature* **217**, 624–626 (1968).
19. Li, W.-H. *Molecular Evolution* (Sinauer Associates, Sunderland, 1997).

20. Drost, J. B. & Lee, W. R. Biological basis of germline mutation: comparisons of spontaneous germline mutation rates among *Drosophila*, mouse, and human. *Environ. Mol. Mutagen.* **25** (Suppl. 26), 48–64 (1995).
21. Drost, J. B. & Lee, W. R. The developmental basis for germline mosaicism in mouse and *Drosophila melanogaster*. *Genetica* **102/103**, 421–443 (1998).
22. Bauer, V. L. & Aquadro, C. F. Rates of DNA sequence evolution are not sex-biased in *Drosophila melanogaster* and *D. simulans*. *Mol. Biol. Evol.* **14**, 1252–1257 (1997).
23. Ebersberger, I., Metzler, D., Schwarz, C. & Paabo, S. Genomewide comparison of DNA sequences between humans and chimpanzees. *Am. J. Hum. Genet.* **70**, 1490–1497 (2002).
24. Mcvean, G. T. & Hurst, L. D. Evidence for a selectively favourable reduction in the mutation rate of the X chromosome. *Nature* **386**, 388–392 (1997).
25. Malcom, C. M., Wyckoff, G. J. & Lahn, B. T. Genic mutation rates in mammals: local similarity, chromosomal heterogeneity, and X-versus-autosome disparity. *Mol. Biol. Evol.* **20**, 1633–1641 (2004).
26. Lercher, M. J., Williams, E. J. B. & Hurst, L. D. Local similarity in evolutionary rates extends over whole chromosomes in human–rodent and mouse–rat comparisons: implications for understanding the mechanistic basis of the male mutation bias. *Mol. Biol. Evol.* **18**, 2032–2039 (2001).
27. Huttley, G. A., Jakobsen, I. B., Wilson, S. R. & Easteal, S. How important is DNA replication for mutagenesis? *Mol. Biol. Evol.* **17**, 929–937 (2000).
28. Taylor, J., Tyekucheva, S., Zody, M., Chiaromonte, F. & Makova, K. D. Strong and weak male mutation bias at different sites in the primate genomes: insights from the human–chimpanzee comparison. *Mol. Biol. Evol.* **23**, 565–573 (2005).
Shows that, in hominoids, male-driven evolution is the main cause of deceased neutral X-linked divergence when CpG sites are excluded.
29. Axelsson, E., Smith, N. G. C., Sundstrom, H., Berlin, S. & Ellegren, H. Male-biased mutation rate and divergence in autosomal, Z-linked and W-linked introns of chicken and turkey. *Mol. Biol. Evol.* **21**, 1538–1547 (2004).
30. Sundstrom, H., Webster, M. T. & Ellegren, H. Reduced variation on the chicken Z chromosome. *Genetics* **167**, 377–385 (2004).
31. Montell, H., Fridolfsson, A.-K. & Ellegren, H. Contrasting levels of nucleotide diversity on the avian Z and W sex chromosomes. *Mol. Biol. Evol.* **18**, 2010–2016 (2001).
32. Haldane, J. B. S. A mathematical theory of natural and artificial selection. Part I. *Trans. Camb. Philos. Soc.* **23**, 19–41 (1924).
33. Rice, W. R. Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**, 735–742 (1984).
This is the theoretical framework on which most of the work on the genomic distribution of sex-biased genes is based.
34. Charlesworth, B., Coyne, J. A. & Barton, N. H. The relative rates of evolution of sex-chromosomes and autosomes. *Am. Nat.* **130**, 113–146 (1987).
The rates of evolution at X-linked and autosomal sites are modelled and the conditions for faster X-chromosome evolution are determined.
35. Kirkpatrick, M. & Hall, D. W. Male-biased mutation, sex linkage, and the rate of adaptive evolution. *Evolution* **58**, 437–440 (2004).
36. Orr, H. A. & Betancourt, A. J. Haldane's sieve and adaptation from the standing genetic variation. *Genetics* **157**, 875–884 (2001).
37. Fay, J. C., Wyckoff, G. J. & Wu, C.-I. Positive and negative selection on the human genome. *Genetics* **158**, 1227–1234 (2001).
38. Smith, N. G. & Eyre-Walker, A. Adaptive protein evolution in *Drosophila*. *Nature* **415**, 1022–1024 (2002).
39. Bierne, N. & Eyre-Walker, A. The genomic rate of adaptive amino acid substitution in *Drosophila*. *Mol. Biol. Evol.* **21**, 1350–1360 (2004).
40. Sawyer, S. A., Kulathinal, R. J., Bustamante, C. D. & Hartl, D. L. Bayesian analysis suggests that most amino acid replacements in *Drosophila* are driven by positive selection. *J. Mol. Evol.* **57** (Suppl. 1), S154–S164 (2003).
41. Betancourt, A. J., Presgraves, D. C. & Swanson, W. J. A test for faster X evolution in *Drosophila*. *Mol. Biol. Evol.* **19**, 1816–1819 (2002).
42. Thornton, K. & Long, M. Rapid divergence of gene duplicates on the *Drosophila melanogaster* X chromosome. *Mol. Biol. Evol.* **19**, 918–925 (2002).
43. Thornton, K. & Long, M. Excess of amino acid substitutions relative to polymorphism between X-linked duplications in *Drosophila melanogaster*. *Mol. Biol. Evol.* **22**, 273–284 (2005).
44. Counterman, B. A., Ortiz-Barrientos, D. & Noor, M. A. Using comparative genomic data to test for fast-X evolution. *Int. J. Org. Evolution* **58**, 656–660 (2004).
45. Thornton, K., Bachtrog, D. & Andolfatto, P. X chromosomes and autosomes evolve at similar rates in *Drosophila*: no evidence for faster-X protein evolution. *Genome Res.* **16**, 498–504 (2006).
46. Zhang, Z., Hambuch, T. M. & Parsch, J. Molecular evolution of sex-biased genes in *Drosophila*. *Mol. Biol. Evol.* **21**, 2130–2139 (2004).
47. Lu, J. & Wu, C.-I. Weak selection revealed by the whole-genome comparison of the X chromosome and autosomes of human and chimpanzee. *Proc. Natl Acad. Sci. USA* **102**, 4063–4067 (2005).
By evaluating the rates of evolution for a large number of human–chimpanzee gene comparisons, this study provides evidence that both weak negative selection and positive selection are more efficient at X-linked loci.
48. Torgerson, D. G., Kulathinal, R. J. & Singh, R. S. Mammalian sperm proteins are rapidly evolving: evidence of positive selection in functionally diverse genes. *Mol. Biol. Evol.* **19**, 1973–1980 (2002).
49. Swanson, W. J., Nielsen, R. & Yang, Q. Pervasive adaptive evolution in mammalian fertilization proteins. *Mol. Biol. Evol.* **20**, 18–20 (2003).
50. Torgerson, D. G. & Singh, R. S. Sex-linked mammalian sperm proteins evolve faster than autosomal ones. *Mol. Biol. Evol.* **20**, 1705–1709 (2003).
51. Torgerson, D. G. & Singh, R. S. Enhanced adaptive evolution of sperm-expressed genes on the mammalian X chromosome. *Heredity* **96**, 39–44 (2006).
52. Khaitovich, P. *et al.* Parallel patterns of evolution in the genomes and transcriptomes of humans and chimpanzees. *Science* **309**, 1850–1854 (2005).
53. Powell, J. R. & Moriyama, E. N. Evolution of codon usage bias in *Drosophila*. *Proc. Natl Acad. Sci. USA* **94**, 7784–7790 (1997).
54. Hambuch, T. M. & Parsch, J. Patterns of synonymous codon usage in *Drosophila melanogaster* genes with sex-biased expression. *Genetics* **170**, 1691–1700 (2005).
55. Singh, N. D., Davis, J. C. & Petrov, D. A. X-linked genes evolve higher codon bias in *Drosophila* and *Caenorhabditis*. *Genetics* **171**, 145–155 (2005).
56. Mcvean, G. T. & Charlesworth, B. A population genetic model for the evolution of synonymous codon usage: patterns and predictions. *Genet. Res.* **74**, 145–158 (1999).
57. Gordo, I. & Charlesworth, B. Genetic linkage and molecular evolution. *Curr. Biol.* **11**, R684–R686 (2001).
58. Betancourt, A. J., Kim, Y. & Orr, H. A. A pseudohitchhiking model of X vs autosomal diversity. *Genetics* **168**, 2261–2269 (2004).
59. Begun, D. J. & Whitley, P. Reduced X-linked nucleotide polymorphism in *Drosophila simulans*. *Proc. Natl Acad. Sci. USA* **97**, 5960–5965 (2000).
60. Andolfatto, P. Contrasting patterns of X-linked and autosomal nucleotide variation in *Drosophila melanogaster* and *Drosophila simulans*. *Mol. Biol. Evol.* **18**, 279–290 (2001).
61. Kauer, M., Zangerl, B., Dieringer, D. & Schlotterer, C. Chromosomal patterns of microsatellite variability contrast sharply in African and non-African populations of *Drosophila melanogaster*. *Genetics* **160**, 247–256 (2002).
62. Mousset, S. & Derome, N. Molecular polymorphism in *Drosophila melanogaster* and *D. simulans*: what have we learned from recent studies? *Genetica* **120**, 79–86 (2004).
A review of the polymorphism data that have recently been collected in *Drosophila* species, with a discussion on the evolutionary forces that might cause the patterns observed for the X chromosome.
63. Schofl, G. & Schlotterer, C. Patterns of microsatellite variability among X chromosomes and autosomes indicate a high frequency of beneficial mutations in non-African *D. simulans*. *Mol. Biol. Evol.* **21**, 1384–1390 (2004).
64. Charlesworth, B. The effect of life-history and mode of inheritance on neutral genetic variability. *Genet. Res.* **77**, 153–166 (2001).
65. Haddrill, P. R., Thornton, K. R., Charlesworth, B. & Andolfatto, P. Multilocus patterns of nucleotide variability and the demographic and selection history of *Drosophila melanogaster* populations. *Genome Res.* **15**, 790–799 (2005).
66. Wang, E. T., Kodama, G., Baldi, P. & Moyzis, R. K. Global landscape of recent inferred Darwinian selection for *Homo sapiens*. *Proc. Natl Acad. Sci. USA* **103**, 135–140 (2006).
67. Wang, P. J., Mccarrey, J. R., Yang, F. & Page, D. C. An abundance of X-linked genes expressed in spermatogonia. *Nature Genet.* **27**, 422–426 (2001).
68. Lifschytz, E., Lindsley, D. L. The role of X-chromosome inactivation during spermatogenesis (*Drosophila*-allocycly-chromosome evolution-male sterility-dosage compensation). *Proc. Natl Acad. Sci. USA* **69**, 182–186 (1972).
69. Oliver, B. & Parisi, M. Battle of the Xs. *Bioessays* **26**, 543–548 (2004).
70. Mueller, J. L. *et al.* Cross-species comparison of *Drosophila* male accessory gland protein genes. *Genetics* **171**, 131–143 (2005).
71. Gupta, V. *et al.* Global analysis of X-chromosome dosage compensation. *J. Biol.* **5**, 3 (2006).
72. Connallon, T. & Knowles, L. L. Intergenomic conflict revealed by patterns of sex-biased gene expression. *Trends Genet.* **21**, 495–499 (2005).
73. Brudno, M. *et al.* Automated whole-genome multiple alignment of rat, mouse, and human. *Genome Res.* **14**, 685–692 (2004).
74. Wu, C. I. & Xu, E. Y. Sexual antagonism and X inactivation — the SAXI hypothesis. *Trends Genet.* **19**, 243–247 (2003).
75. Zechner, U. *et al.* A high density of X-linked genes for general cognitive ability: a run-away process shaping human evolution? *Trends Genet.* **17**, 697–701 (2001).
76. Wilda, M. *et al.* Do the constraints of human speciation cause expression of the same set of genes in brain, testis, and placenta? *Cytogenet. Cell. Genet.* **91**, 300–302 (2000).
77. Arnold, P. A. Sex chromosomes and brain gender. *Nature Rev. Neurosci.* **5**, 701–708 (2004).
78. Nguyen, D. K. & Disteche, C. M. Dosage compensation of the active X chromosome in mammals. *Nature Genet.* **38**, 47–53 (2006).
79. Guo, J. *et al.* *In silico* analysis indicates a similar gene expression pattern between human brain and testis. *Cytogenet. Genome Res.* **103**, 58–62 (2003).
80. Son, C. G. *et al.* Database of mRNA gene expression profiles of multiple human organs. *Genome Res.* **15**, 443–450 (2005).
81. Betrán, E., Thornton, K. & Long, M. Retroposed new genes out of the X in *Drosophila*. *Genome Res.* **12**, 1854–1859 (2002).
82. Emerson, J. J., Kaessmann, H., Betrán, E. & Long, M. Extensive gene traffic on the mammalian X chromosome. *Science* **303**, 537–540 (2004).
References 81 and 82 describe the patterns of retroposition to and from the X chromosome in *D. melanogaster* and mammals, and discuss possible causes.
83. Vinckenbosch, N., Dupanloup, I. & Kaessmann, H. Evolutionary fate of retroposed gene copies in the human genome. *Proc. Natl Acad. Sci. USA* **103**, 3220–3225 (2006).
84. Khil, P. P., Oliver, B. & Camerini-Otero, R. D. X for intersection: retrotransposition both on and off the X chromosome is more frequent. *Trends Genet.* **21**, 3–7 (2005).
85. Wichman, H. A., Van Den Bussche, R. A., Hamilton, M. J. & Baker, R. J. Transposable elements and the evolution of genome organization in mammals. *Genetica* **86**, 287–293 (1992).
86. Langley, C. H., Montgomery, E. A., Hudson, R. H., Kaplan, N. L. & Charlesworth, B. On the role of unequal exchange in the containment of transposable element copy number. *Genet. Res.* **52**, 223–235 (1988).

Acknowledgements
We thank H. Ellegren and V. Kaiser for providing us with their unpublished manuscript. B.V. is supported by a postgraduate fellowship from the Fundação de Ciência e Tecnologia of Portugal, and B.C. by the Royal Society (UK). We thank two anonymous reviewers for their comments, which helped to improve the manuscript.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Institute of Evolutionary Biology, University of Edinburgh: http://www.biology.ed.ac.uk/research/institutes/evolution
Access to this links box is available online.