Review

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The faster-X effect: integrating theory and data

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Population genetics theory predicts that X (or Z) chromosomes could play disproportionate roles in speciation and evolutionary divergence, and recent genome-wide analyses have identified situations in which X or Z-linked divergence exceeds that on the autosomes (the so-called 'faster-X effect'). Here, we summarize the current state of both the theory and data surrounding the study of faster-X evolution. Our survey indicates that the faster-X effect is pervasive across a taxonomically diverse array of evolutionary lineages. These patterns could be informative of the dominance or recessivity of beneficial mutations and the nature of genetic variation acted upon by natural selection. We also identify several aspects of disagreement between these empirical results and the population genetic models used to interpret them. However, there are clearly delineated aspects of the problem for which additional modeling and collection of genomic data will address these discrepancies and provide novel insights into the population genetics of adaptation.

Motivations for studying faster-X evolution

The widespread availability of population and comparative genomic data has made it possible to estimate rates of molecular evolution and gene expression divergence in entire genomes, across broad swaths of the tree of life. These data, when considered within a statistical population genetic framework, can shed light on the biology of speciation, adaptation, and divergence, by permitting inferences about the processes contributing to evolutionary change [1–5]. The tools of evolutionary genomics produce the most useful insights when they can connect patterns of divergence with causal evolutionary processes, an objective that remains a considerable challenge.

Molecular evolutionary contrasts between the X (or Z) chromosome and autosomes are often motivated by such goals. Classical population genetics theory shows that the evolutionary dynamics of an allele depend, in part, on its mode of inheritance [6,7]. Under specific parameterizations of allelic dominance, selection in males versus females, mutation, recombination, and effective population size (N_e; see Glossary), X-linked genes can be more divergent between species compared with autosomal genes, a phenomenon known as the 'faster-X effect' [7–14]. Therefore, analyses of the relative divergence rates between X-linked and autosomal genes may provide insights into the population genetic basis of neutral and adaptive evolution, conditional on our ability to link pattern and process through evolutionary theory.

Although the past few years have witnessed considerable growth in theory and data on faster-X evolution, the fit between the two has become rather complicated. In this review, we emphasize the important assumptions and limitations of current theory, and reconsider the diverse array of published data within this theoretical foundation. Along the way, we outline several paths forward.

Theoretical background

Evolution proceeds by the fixation of neutral, slightly deleterious, and beneficial alleles. Although substitution rates (i.e., total divergence between species) reflect the cumulative fixation process of alleles of all three classes, most X versus autosome theory has emphasized, for two primary reasons, beneficial substitutions and the conditions leading to faster-X adaptive evolution. First, beneficial substitution rates on the X and autosomes are interesting for what they might tell us about the population genetics of adaptation [13]. Second, more elaborate

Glossary

α: proportion of substitutions that are fixed by positive selection, inferred within a MK test framework, $\alpha = 1 - (d_S p_N)/(d_N p_S)$.

Beneficial mutation and/or allele: genetic variant that confers increased fitness.

d_N: the number of nonsynonymous (amino acid-changing) substitutions standardized by the number of possible nonsynonymous mutations in a gene.

d_S: the number of synonymous (non-amino acid-changing) substitutions standardized by the number of possible synonymous mutations in a gene.

Effective population size (N_e): the size of an idealized population (with constant size, random mating, and no natural selection) that experiences a similar amount of genetic drift as a natural population.

Fixation probability: the probability that an allele that is present within a population eventually reaches a frequency of one within that population (i.e., it is eventually carried by every individual in the population).

Genetic drift: the process whereby allele frequencies change as a result of random sampling of genes within a population of finite size. The effect of genetic drift at a gene is inversely proportional to its N_e.

Heterogametic sex: the sex carrying only one copy of the X or the Z chromosome; the homogametic sex carries two copies of the X (as in *Drosophila* and mammals) or the Z (as in birds and moths).

McDonald–Kreitman (MK) test: statistical test comparing nonsynonymous (p_N) and synonymous (p_S) polymorphism and substitutions within a gene (d_N and d_S), often for the purpose of detecting a signature of historical adaptive evolution.

Neutral mutation and/or allele: genetic variant that does not affect fitness.

Slightly deleterious mutation and/or allele: genetic variant whose deleterious effect is below the level responsive to natural selection (typically $s < \frac{1}{2N}$).

Substitution rate: the rate at which genetic changes accumulate within an evolutionary lineage. Substitutions may become fixed by the action of natural selection or by random genetic drift.

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theory is required to characterize the evolutionary dynamics and genetics of adaptation, relative to the comparably simple theory of substitution by genetic drift. Although we emphasize the fixation of beneficial alleles in our outline of the theory of faster-X evolution, the predictions with respect to neutral and slightly deleterious substitutions are also discussed.

Charlesworth *et al.* [8] analyzed several models of substitution, including one for beneficial mutations, which is referenced in most molecular evolution studies that contrast the X and autosomes (Boxes 1 and 2). This model builds upon the pioneering work of Kimura and Ohta [15,16], which characterizes the substitution rate as the product of mutational input per generation and the fixation probability of each mutation. The simplest form of the model is based upon three conditions: (i) unique, beneficial mutations occur at a rate of u per gene copy, per generation; (ii) each beneficial mutation increases fitness by the amount sh in heterozygotes and s in hemi- and homozygotes ($1 \gg s > 0; 1 > h > 0$); and (iii) the N_e of the X is three-quarters that of the autosomes ($N_{eX}/N_{eA} = 3/4$). From these conditions, the relative rate of adaptive substitution of an autosomal gene (R_A) versus an X-linked gene (R_X) will be (Equation 1):

$$\frac{R_A}{R_X} = \frac{4h}{1+2h} \quad [1]$$

(see Equation 2a of Charlesworth *et al.* [8]). Faster-X evolution ($R_A/R_X < 1$) occurs when beneficial mutations are partially or completely recessive ($0 < h < 1/2$); otherwise, adaptive evolution is faster on the autosomes (Figure 1; Equation 1 corresponds to the black curve).

Equation 1 relies upon five simplifying assumptions, with violation of each altering the predicted relationship between dominance and R_A/R_X. These assumptions are as follows:

- Selection parameters are equal between males and females. The faster-X effect emerges due to selection on recessive beneficial mutations within the heterogametic (i.e., hemizygous) sex, in which there is no masking effect for X-linked alleles. However, the theoretical predictions of faster-X evolution change when mutations have asymmetric fitness effects between the sexes. Faster-X effects are slightly more pronounced when beneficial substitutions have stronger fitness effects in males than in females, and there is no predicted faster-X effect when selection only acts in females (Figure 1) [8].
- Mutation rates are equal between the sexes. The male germline often has more mitoses than the female germline, which can increase the mutation rate in males relative to females [17]. Because the X chromosome spends more time in the female germline, a higher male mutation rate will decrease the relative mutation rate of X-linked genes, which could decrease the rate of evolution of the X chromosome [10,18]. Nevertheless, the effects of sex-biased mutation on faster-X evolution can be controlled for by scaling gene substitution rates against the divergence rates at linked neutral sites [11] (as in Figure 1).
- The N_e ratio of the X to the autosomes is three-quarters ($N_{eX}/N_{eA} = 3/4$). N_e scales positively with the fixation probability of beneficial mutations [15,16]. Increasing the effective size of X-linked relative to autosomal genes (N_{eX} relative to N_{eA}) enhances opportunities for faster-X evolution [11] (the curves in Figure 1 shift down with $N_{eX}/N_{eA} > 3/4$); decreasing N_{eX}/N_{eA} reduces the faster-X parameter space. By estimating N_{eX}/N_{eA} from neutral diversity data, the effect of N_{eX}/N_{eA} on faster-X evolution can be disentangled from other contributing factors [12], although such corrections could prove misleading if selective sweeps are frequent [19].
- Substitution rates are limited by the fixation probabilities of individual beneficial mutations. Correlations between the adaptive substitution rate and fixation probabilities of unique mutants may be reduced or eliminated if (i) adaptation uses 'standing genetic variation' (i.e., it fixes segregating alleles that were neutral or deleterious before an environmental change), or (ii) mutations are recurrent [20]. Adaptation using standing genetic variation causes faster-autosome substitution, independent of the dominance of beneficial alleles, provided autosomal loci harbor greater amounts of genetic diversity [8,9,14]. Under recurrent mutation, dominance only influences R_A/R_X if multiple genes,

Box 1. X-linked and autosomal fixation probabilities of beneficial mutations

Theory for the adaptive substitution rate of X-linked versus autosomal genes is heavily influenced by the early work of J.B.S. Haldane [6], who was the first to characterize the fixation probabilities for beneficial mutations (i.e., the probability that individual mutations eventually reach a population frequency of one), and the population genetic dynamics associated with X-linkage, dominance, and sex differences in selection. Haldane showed that the fixation probability of a unique beneficial mutation is $\Pi \approx 2t$, where t represents the average fitness benefit provided to individuals that carry a single copy of the mutation. The model assumes that benefits are small and population size, $N = N_e$, is large [$\frac{1}{2N} \ll t \ll 1$; when $N \neq N_e$, $\Pi \approx 2t(N_e/N)$ [16]]. To incorporate sex differences in selection on a mutation [7], t can be replaced with the weighted averages of male and female fitness effects of a mutant. The fixation probability of a unique autosomal mutation is (Equation I):

$$\Pi_A \approx 2t_A \approx s_{mA}h_{mA} + s_{fA}h_{fA}, \quad [I]$$

where $s_{mA}h_{mA}$ and $s_{fA}h_{fA}$ represent the fitness effect of carrying a single autosomal copy of the mutation in males and females, respectively, and s_{mA} and s_{fA} represent the fitness effect of carrying two copies of the mutation (s_{jA} represents the autosomal selection coefficient in homozygotes, and h_{jA} is the dominance coefficient, which determines the fitness of heterozygotes relative to homozygotes; $j = \{m,f\}$). The probability of fixation for a unique X-linked mutation is (Equation II):

$$\Pi_X \approx 2t_X \approx 2(s_{mX} + 2s_{fX}h_{fX})/3. \quad [II]$$

Contrasts between Π_A and Π_X reveal two basic differences between the X and autosomes. First, because males are haploid, the fixation probability on the X is less sensitive to the dominance of a mutation (h_{mA}, h_{fA}) than is the autosomal fixation probability. Second, the relative importance of selection in males, versus selection in females, differs between chromosomes. For autosomal mutations, selection in males and females carries equal weight because inheritance is symmetric between the sexes (mothers and fathers transmit equally to offspring). X-linked transmission occurs twice as often through females than through males, which upwardly biases the importance of female selection on X-linked mutations (although the absence of dominance in males can counteract this asymmetry).

Box 2. Adaptive substitution rates of X-linked and autosomal genes

Here, we describe how the fixation probabilities of Box 1 relate to the adaptive substitution rates of individual genes (i.e., the tempo of nucleotide changes over time), as predicted from the influential theory of X versus autosome adaptive substitution developed by Charlesworth *et al.* [8]. The substitution rate of a gene is modeled as the product of the beneficial mutation rate of the population and the fixation probability of each mutation, which is assumed to be unique [7,8,15,16]. Autosomal genes mutate to a beneficial allele at rate u_A; with N individuals in the population, $2Nu_A$ mutations are expected to arise during each generation. The adaptive substitution rate for the autosomal gene is $R_A \approx 2Nu_A\Pi_A$, where $\Pi_A \approx 2t_A$ (Box 1). The adaptive substitution rate for an X-linked gene is $R_X \approx N_X u_X \Pi_X$, where N_X is the number of X chromosomes in the population ($N_X/N = 3/2$ is assumed [8]), u_X is the beneficial mutation rate at the X-linked gene, and $\Pi_X \approx 2t_X$ (Box 1). Given sex-specific beneficial mutation rates of u_f and u_m, $u_A = (u_m + u_f)/2$ and $u_X = (2u_f + u_m)/3$ [10].

Mutation and selection parameters are likely to be variable across genes, so that average rates of substitution among X-linked and autosomal genes will be $\langle R_A \rangle \approx 2N \langle u_A\Pi_A \rangle$ and $\langle R_X \rangle \approx N_X \langle u_X\Pi_X \rangle$, where $\langle\rangle$ denotes the expectation. The relative rate of adaptive substitution will be (Equation I)

$$\frac{\langle R_A \rangle}{\langle R_X \rangle} = (4/3)\frac{\langle u_A\Pi_A \rangle}{\langle u_X\Pi_X \rangle}, \quad [\mathrm{I}]$$

where $\langle u_j\Pi_j \rangle = \langle u_j \rangle \langle \Pi_j \rangle + cov(u_j,\Pi_j)$. To express $\langle R_A \rangle / \langle R_X \rangle$ as a simple function of the dominance coefficient, models of X versus autosome substitution must make assumptions about the distribution of beneficial mutation parameters and their covariances. This issue is usually sidestepped by assuming fixed parameter values (i.e., terms of u, s, and h are treated as constants), leading to (Equation II):

$$\frac{R_A}{R_X} = 2\left(\frac{u_A}{u_X}\right)\left(\frac{s_{mA}h_{mA} + s_{fA}h_{fA}}{s_{mX} + 2s_{fx}h_{fx}}\right). \quad [\mathrm{II}]$$

Equation 1 is obtained when parameters are identical between chromosomes ($u_A = u_X$, $h = h_{mA} = h_{fA} = h_{fX}$, and $s = s_{mA} = s_{mX} = s_{fA} = s_{fX}$). Effects of sex-biased mutation can be controlled by scaling the adaptive substitution rate against the neutral rate [11]. If v_m and v_f are the male and female mutation rates per silent site, then the neutral substitution rate on the X and autosomes will be $v_X = (2v_f + v_m)/3$ and $v_A = (v_f + v_m)/2$, respectively. Assuming that u_m, u_f, v_m, and v_f are constant, and that $u_m/u_f = v_m/v_f$, then the rescaled ratio of autosome to X-linked adaptive substitution will be (Equation III):

$$\frac{R_A/v_A}{R_X/v_X} = 2\left(\frac{s_{mA}h_{mA} + s_{fA}h_{fA}}{s_{mX} + 2s_{fX}h_{fX}}\right). \quad [\mathrm{III}]$$

In practice, this is accomplished by comparing d_N/d_S between X-linked and autosomal genes (see main text).

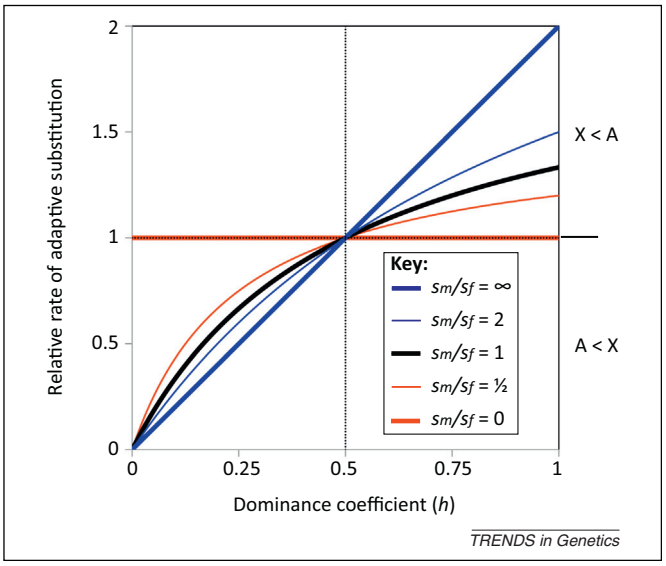

Figure 1. Dominance, sex differences in selection, and faster-X adaptive substitution. Curves show the theoretical predictions for the relative rates of adaptive substitutions at autosomal and X-linked genes, based on the model framework of Charlesworth *et al.* [8] (Box 2). The y-axis shows the autosome-to-X rate of adaptive evolution, $(R_A/v_A)/(R_X/v_X)$, which corrects for sex-biased mutation rates. The dominance coefficient of a beneficial mutation is assumed to be the same for males and females and for the X and autosomes (i.e., $h = h_{mA} = h_{fA} = h_{fX}$), and beneficial selection coefficients (s_m in males, and s_f in females) are treated as constants.

spread across the genome, compete to fix beneficial mutations during individual bouts of adaptation [14]. Experimental evolution experiments and genetic studies of natural populations indicate that individual bouts of adaptation are sometimes highly constrained, with beneficial substitutions recruited from a very small subset of genes [21,22]. Such scenarios of adaptation will tend to equalize the X and autosomal substitution rates over a wide range of dominance conditions [14].

- The distribution of mutant fitness effects (DMFE) is the same, on average, for X-linked and autosomal genes. This condition may be violated under plausible biological scenarios, and it is difficult to control for. For example, dosage compensation mechanisms, which vary between species [23], may systematically affect X-linked fitness effects. Opportunities for faster-X evolution are expected to decrease in species without dosage compensation [8] and increase in species with somatic X-inactivation (as in therian mammals), which generates haploid expression within individual female cells [12]. Gene content also differs between the X and autosomes [24], which can bias opportunities for adaptation between chromosomes. Finally, recent theory suggests that haploid versus diploid inheritance differentially shapes the DMFE [25], raising the possibility that X versus autosome differences are a fundamental property of ploidy differences between chromosomes.

Empirical tests of the faster-X effect

Tests of faster-X evolution typically fall into two categories. First, comparative genomic approaches test whether X-linked loci accumulate more substitutions than do autosomal loci. 'Faster-X divergence' is said to occur when d_N/d_S values for X-linked genes are greater than those of autosomal genes, where d_N is the rate of nonsynonymous (amino acid changing) substitutions and d_S is the rate of synonymous (silent or neutral) substitutions in a gene. Although d_N/d_S is useful for comparing X versus autosome divergence rates, it is important to note that d_N captures both nonadaptive (neutral and slightly deleterious) and adaptive substitutions. Therefore, this approach is ill equipped to differentiate between adaptive and nonadaptive causes of faster-X evolution. The second approach combines within-species polymorphisms and between-species divergence data to estimate adaptive substitution rates (i.e., within the analytical framework of the McDonald–Kreitman or 'MK' test [26–28]), which tests for 'faster-X adaptation'.

Faster-X divergence

Many of the earliest tests for faster-X divergence were performed in the genus *Drosophila* (Figure 2), where support for elevated d_N/d_S in X-linked genes was varied [1,29–34]. Of particular note were analyses that utilized natural autosome-to-X translocations to control for gene content effects (i.e., the *Drosophila pseudoobscura* and *Drosophila willistoni* neo-X chromosomes correspond to autosomes in *Drosophila melanogaster*), although these studies also failed to reach consensus on the faster-X effect [30–35] (Figure 2). The more recently arisen *Drosophila miranda* neo-X chromosome allows for an additional test of faster-X evolution by comparing genes that retain a Y-linked homolog (effectively diploid in males) and those that are hemizygous. Consistent with the predictions if beneficial mutations are recessive, hemizygous neo-X-linked protein-coding genes evolve faster than do diploid genes on the *D. miranda* neo-X [36] (Figure 2). Finally, *Drosophila* X-linked duplicated genes have elevated d_N/d_S relative to autosomal duplicates [37] (Figure 2), and the amount of chromosomal rearrangement divergence in many taxa, including *Drosophila* [38], is higher on the X chromosome [8].

Recent availability of high-quality genomes from the closely related species *D. melanogaster* and *Drosophila simulans* has allowed for tests of faster-X divergence at many different classes of site (Figure 2). Intriguingly, this comparison revealed that X-linked protein coding sites and many noncoding sites evolve faster than autosomal sites in the same functional class [1,39]. However, after using gene ontology classifications to control for gene content, d_N at X-linked coding sequences is no longer significantly elevated [39]. A signal of faster-X divergence remains among many classes of noncoding site [39], which could be driven by a higher mutation rate on the X chromosome or the adaptive fixation of recessive beneficial mutations that affect the transcription of nearby genes. In addition, the faster-X divergence of noncoding sites could be responsible for the faster-X evolution of gene expression (see below).

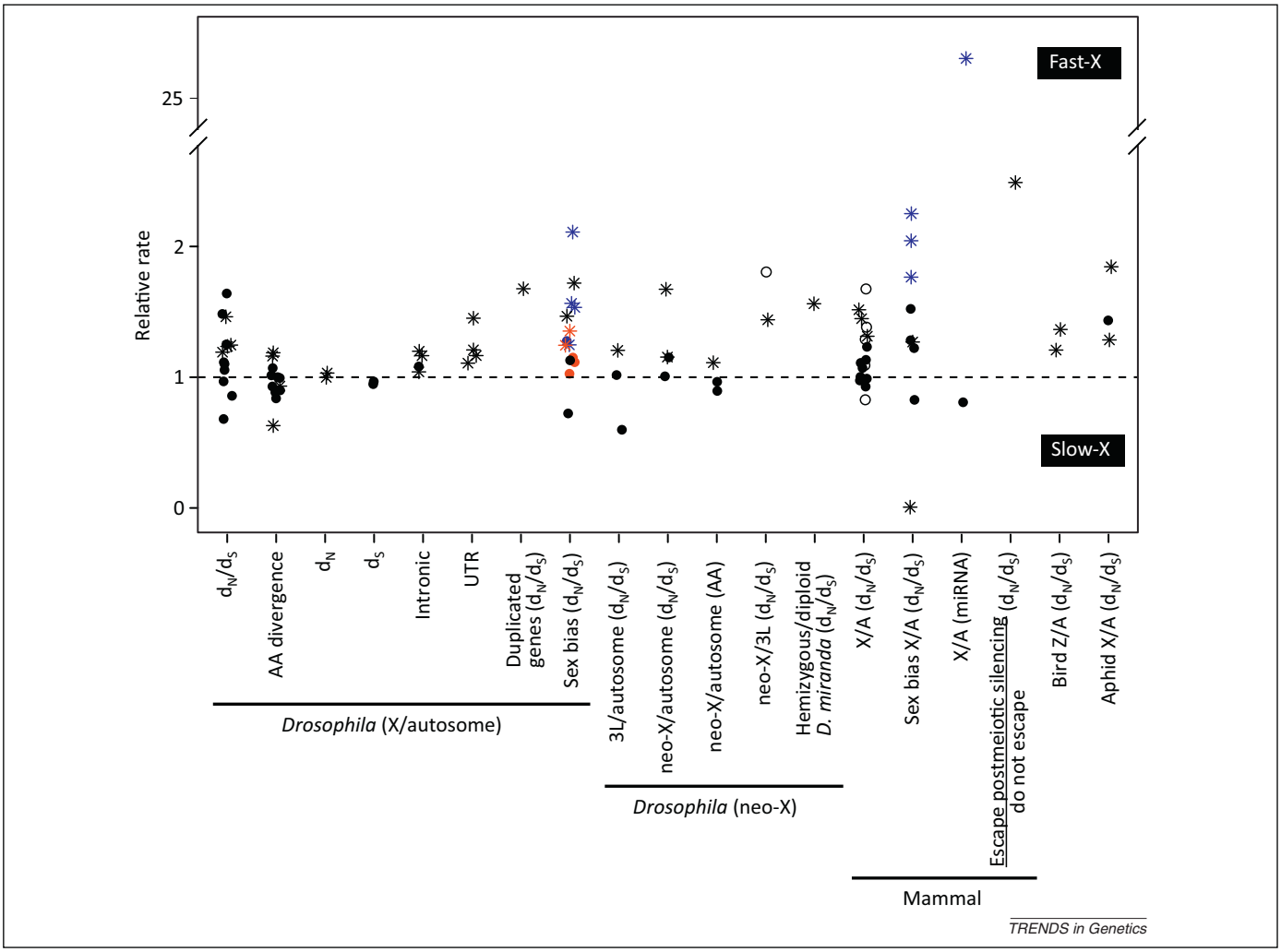

Figure 2. Tests for faster-X divergence. The relative rate of evolution is plotted for different classes of nucleotide site and chromosome in *Drosophila* [29–31,33,35–37,39], mammals [12,18,40,42,60,62,64,65], birds [48], and aphids [49]. The rate of evolution is measured as d_N/d_S, amino acid (AA) divergence, or nucleotide divergence at different classes of site (indicated on the x-axis). Relative rates are either X/autosome, *Drosophila melanogaster* chromosome arm 3L (homolog of the *Drosophila pseudoobscura* neo-X)/autosome, *D. pseudoobscura* neo-X/autosome, *D. pseudoobscura* neo-X/*D. melanogaster* 3L, hemizygous/diploid genes on the *Drosophila miranda* neo-X, or mammalian genes that escape postmeiotic silencing/those that do not escape. The expectation that X-linked and autosomal genes evolve at equal rates is represented by the broken line. Significant deviation from unity in the relative rate is indicated by an asterisk, whereas nonsignificant differences or studies in which significance was not reported are indicated by a black or white circle, respectively. In experiments where expression was measured (indicated by 'sex bias' in the x-axis label), the color of the point indicates the expression class of the gene (black, non-sex biased; blue, male biased; and red, female biased). Abbreviation: UTR, untranslated region.

Comparative genomic studies in other taxa reveal more consistent support for faster-X divergence (Figure 2). Mean d_N/d_S is higher for X-linked genes in comparisons between humans and chimpanzees [40–42] and in rodents [12,43]. In birds and moths, where females are the heterogametic sex (ZW), Z-linked genes have elevated d_N/d_S relative to autosomal genes [44–47]. However, faster-Z divergence in birds may not be due to positive selection [48], as described below. Aphids, which have XO males (i.e., no Y chromosome), also show evidence for faster-X divergence in d_N/d_S estimates [49].

Faster-X adaptation

Comparisons of polymorphism and divergence can be used to infer the proportion of substitutions that are fixed by positive selection (α) and the strength of selection [27,28]. Early implementations of the MK test, using subsets of the *D. melanogaster* and *D. simulans* genomes, provided mixed support for faster-X adaptation [50–53], with the strongest evidence among genes with male-biased expression [52] (see below). More recent, whole-genome analyses reveal robust evidence for elevated frequencies of adaptive substitution among *Drosophila* X-linked genes [4,54] (Figure 3). X-linked duplicated genes similarly accumulate more adaptive substitutions than do autosomal duplicates [55] (Figure 3). Although demographic events could differentially affect X-linked and autosomal genetic diversity, demographic history alone cannot explain the evidence for faster-X adaptation in *D. melanogaster* or the elevated divergence relative to polymorphism on the *D. melanogaster* X chromosome [54].

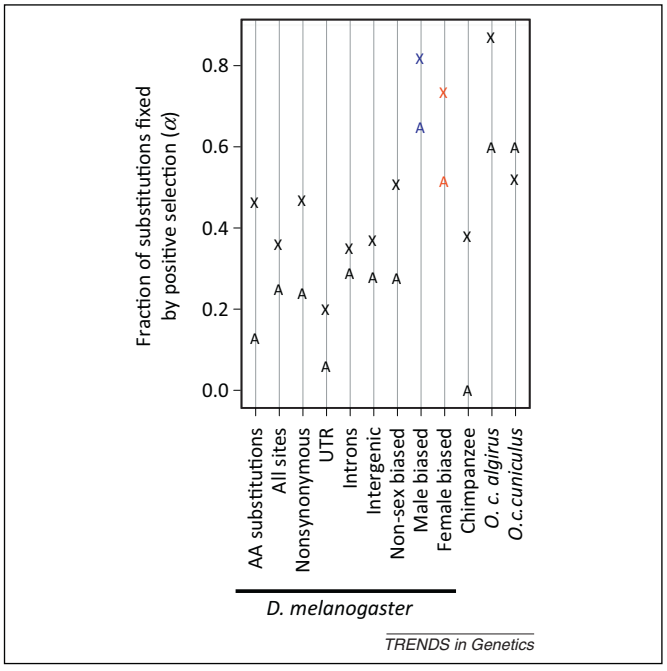

Figure 3. Tests for faster-X adaptation. The fraction of substitutions fixed by positive selection (α) is plotted for X-linked (X) and autosomal (A) loci. Estimates of α in the *Drosophila melanogaster* genome were calculated for amino acid (AA) substitutions [54]; all nucleotide sites, nonsynonymous sites, 3′ and 5′ untranslated regions (UTRs), introns, and intergenic regions separately [4]; genes with non-sex-biased, male-biased (blue), or female-biased (red) expression [59]. Estimates of α for the chimpanzee genome [42] and two subspecies of European rabbit (*Oryctolagus cuniculus algirus* and *Oryctolagus cuniculus cuniculus*) [56] reflect the fraction of amino acid substitutions fixed by positive selection.

Support for faster-X adaptation in vertebrate species is less clear than in *Drosophila*. Although X-linked genes in the human–chimpanzee comparison harbor more signatures of positive selection when compared with autosomal genes, based on d_N/d_S analysis [41], this has not, to our knowledge, been examined in the MK framework (possibly because of the relatively poor quality of DNA sequence polymorphism data for the human X chromosome). However, a recent MK-based, whole-genome analysis found evidence for faster-X adaptation within the chimpanzee lineage, following its split from the human lineage [42] (Figure 3). MK tests performed on wild mouse populations also yield support for faster-X adaptation [43] (A. Kousathanas *et al.*, unpublished). By contrast, support for faster-X adaptation in the European rabbit, *Oryctolagus cuniculus*, is limited to the subspecies with larger N_e, *O. c. algirus* [56] (Figure 3). In addition, there is evidence for faster-Z adaptation in silk moths [47], and reduced variation and excess divergence on the Z chromosome in flycatcher birds [44,57,58] are consistent with faster-Z adaptation. Although a similar pattern was initially observed for the chicken Z chromosome [45], subsequent work indicates that faster-Z divergence in the chicken lineage may be due to relaxed constraints rather than to adaptive evolution [48]. Overall, these results demonstrate that lineages with faster-X divergence do not necessarily exhibit faster-X adaptation, and vice versa. This may reflect differences among taxa in the role of neutral and adaptive causes of faster-X divergence [11,12], as described below.

Faster-X evolution of male reproductive genes

Several studies emphasize that faster-X effects should be most pronounced in genes with male-biased expression (i.e., primarily expressed in males) or male-limited functions [29,35,52,59], assuming that mutations in these genes have larger fitness effects in males than in females (e.g., Figure 1, blue curves). Consistent with this prediction, in both *Drosophila* and mammals, the strongest evidence for faster-X divergence and adaptation is observed in genes expressed primarily in male reproductive tissues [35,52,59–62] (Figures 2 and 3). Faster-X effects have also been observed in primate genes expressed in cancer and testis cells [63], miRNAs expressed in mammalian testis [64], and human genes that escape postmeiotic transcriptional silencing [65] (Figure 2).

Although the classical theory predicts a slight elevation in the magnitude of faster-X evolution for male-limited beneficial substitutions, relative to substitutions with similar effects on both sexes, this effect will not be nearly as great as the difference between substitutions beneficial to both sexes and those with female-limited effects (Figure 1). Intriguingly, although we do not expect faster-X divergence or adaptation among genes under selection only in females (Figure 1), there is evidence for faster-X evolution among *Drosophila* female-biased genes [35,59] (Figures 2 and 3). Genes with female-biased expression are, however, often expressed in males, and mutations in female-biased genes can have fitness effects in males [66]. Selection on recessive X-linked beneficial mutations in males may therefore drive the faster-X evolution of female-biased

genes. Alternatively, there could be something fundamentally different about the genetic basis of adaptation in genes from different functional or expression classes. For example, they might differentially utilize *de novo* mutations versus standing genetic variation, during adaptation, which can alter the influence of dominance on faster-X adaptation [9].

N_e and the faster-X effect

X-to-autosome substitution rates are a function of adaptive, neutral, and slightly deleterious substitutions, which each contribute to total divergence [8,11,12]. Differential accumulation of each substitution type between the X and autosomes can further complicate the interpretation of faster-X divergence patterns. Lineages with large N_e, such as *Drosophila*, should experience efficient positive and purifying selection, leading to a high proportion of substitutions driven by positive selection [2]. Conversely, small N_e will limit the accumulation of adaptively fixed mutations [5,15,16]. Relative divergence rates of X-linked and autosomal genes could similarly reflect the differential accumulation of adaptive, neutral, and deleterious substitutions among lineages with different N_e [12].

Factors such as mating system variation [67], recombinational differences between the X and autosomes [68,69], and genetic hitchhiking and background selection [68,70] can affect the N_{eX}/N_{eA} ratio. This could further affect the X-to-autosome divergence rates or the relative proportions of adaptive versus neutrally fixed substitutions. High variance in male reproductive success decreases N_{eZ}/N_{eA} in ZZ/ZW taxa, permits a higher rate of nearly neutral evolution of Z-linked genes, and contributes to faster-Z divergence in birds [48]. The N_{eX}/N_{eA} ratio is near one in *D. melanogaster*, but close to 3/4 in *D. pseudoobscura* [70]. If N_{eX}/N_{eA} is typically large across the *Drosophila* phylogeny, it could explain why there is robust evidence of faster-X adaptation in *Drosophila* [4,54] (Figure 3). However, the lack of conclusive evidence for faster-X divergence in *Drosophila* remains perplexing (Figure 2).

By contrast, larger populations are more polymorphic than are small ones. This can increase the probability of adaptation using standing genetic variation [71], which should reduce or eliminate opportunities for faster-X evolution [8,9,14]. The interaction between N_e and faster-X evolution is a particularly interesting research area, although it demands additional data. Readers interested in learning more about this topic should consult two recent, comprehensive treatments of the subject [11,12].

Faster-X evolution of gene expression

Recent work demonstrates that expression-level divergence is greater for X-linked than for autosomal genes, in both mammals and *Drosophila* [62,72–75], leading to a faster-X effect for gene expression. Because the expression of a gene is dependent on DNA sequences both at that locus (acting in *cis*) and elsewhere in the genome (acting in *trans*), elevated gene expression divergence in X-linked genes cannot entirely be attributed to rapid sequence evolution on the X chromosome. However, because *trans* factors should affect both X-linked and autosomal gene expression, whereas *cis* divergence should specifically affect expression divergence on a single chromosome, the faster-X divergence of gene expression is likely the result of faster-X evolution of *cis* regulatory sequences. This hypothesis is supported by the faster-X divergence of noncoding sequences [39]. The faster-X evolution of gene expression can inform our general understanding of expression evolution [72,73,75] and shed light on the nature of reproductive isolation between species [74].

Applying the MK test framework to gene expression, if faster-X expression evolution were the result of positive selection, we would not expect to see elevated expression polymorphism among X-linked genes [76]. There is no such elevation of gene expression polymorphism on the *D. melanogaster* X chromosome [73,75], suggesting that the faster-X divergence of gene expression in *Drosophila* is driven by faster-X adaptation in *cis* regulatory sequences that affect X-linked expression levels. This result further suggests that many mutations that affect gene expression have recessive fitness effects, and additional empirical and theoretical work is needed to examine this hypothesis. However, this conclusion comes with the caveat that applying an MK framework to gene expression evolution requires simplifying assumptions about *cis* and *trans* variation that may not be biologically realistic.

Lastly, although the faster-X divergence of gene expression in both mammals and *Drosophila* is detected across multiple tissue types and developmental stages [72,73,75], it is especially pronounced among genes expressed in male reproductive tissues [62,74,75], which is similar to faster-X effects in protein-coding genes (Figure 2). As with the protein-coding faster-X effect, it is unclear why genes with male-biased expression should represent the outlier gene category for faster-X divergence (Figure 1).

Future considerations

As in most areas of molecular evolution and population genetics, theory outpaced data during the early study of faster-X evolution. The genome-sequencing projects completed during the past decade have allowed for the first comprehensive tests of faster-X divergence and adaptation, but there still remains disagreement between theoretical predictions and empirical tests of the faster-X effect. For example, much of the theory contrasts rates of adaptive fixation on the X and autosomes, whereas most of the evidence for faster-X divergence combines both adaptive and nonadaptive substitutions. We therefore anticipate additional progress in this area by integrating divergence estimates (d_N) with calculations of the frequency of adaptive fixations (α) so that the rate of adaptive evolution relative to neutral substitutions (ω_a) [5] can be compared between X-linked and autosomal genes. Comparing rates of adaptive evolution will allow for a more coherent evaluation of empirical results within the framework of faster-X theory.

Although the increasing availability of population genomic data provides greater scope for testing hypotheses of faster-X adaptive evolution, it also introduces new challenges that must be overcome before inferences of faster-X adaptation can be accepted (e.g., those from Figure 3). MK-based approaches are useful for estimating the adaptively fixed component of amino acid substitutions, yet these

tests yield biased results under several plausible evolutionary scenarios, including: (i) population size changes; (ii) non-neutrality of nonsynonymous polymorphisms and/or synonymous mutations; and (iii) hitchhiking under recurrent selective sweeps [77–79]. The importance of these biases is likely to differ between the X and autosomes, potentially generating false signatures of faster-X adaptation [78]. Selection on synonymous mutations represents an important and well-studied bias of this sort within *Drosophila*, where codon usage bias is higher on the X [33]. This limits the utility of d_S as a mutation rate index in *Drosophila* because codon bias could disproportionately inflate X-linked relative to autosomal d_N/d_S values [34] and upwardly bias MK-based estimates of X-linked adaptation. Therefore, novel statistical approaches that can control for systematic biases between chromosomes should have great value for future faster-X studies.

We also foresee continued efforts to identify the consequences of faster-X molecular evolution on higher-level evolutionary process, such as intragenomic conflict, speciation, and phenotypic evolution [74,80]. Gene expression represents a particularly promising phenotype for study within the faster-X context, because gene and phenotype (mRNA transcription level) are coupled (provided that transcriptional changes are in *cis* [73,75]). By contrast, faster-X models are largely framed in terms of nucleotide substitution rates rather than tempos of phenotypic change. Therefore, adopting different theoretical frameworks for phenotypic evolution, including gene expression, may be warranted. These may include quantitative genetics models [8] or others that link genotype, phenotype, and fitness across distinct genetic systems [25].

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